PREDICTIVE MAPPING OF THE RESTORATION-AGE DYNAMICS OF TAIGA FORESTS ON THE BASIS OF REMOTE SENSING DATA AND GEOGRAPHICAL KNOWLEDGE

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Abstract. We examine some theoretical and applied issues related to mapping taiga forests using high-resolution remote sensing data and mathematical models. New technologies are suggested for processing space-acquired images. Some results from implementing this technique for the northern Irkutsk region (Ust-Ilimsk district, East Siberia) are provided in the form of a sequence of predictive maps.

Keywords: *Taiga forests, restoration-age dynamics, predictive mapping, GIS, remote sensing, East Siberia*

Introduction

Estimating biological parameters of vegetation cover is an important element of the study on forest ecosystems. Phytoindication methods used in remote sensing are based on identifying the linkage between spectral characteristics of the image and biological parameters (such as the reserve of phytomass, and species composition) parameters of forest stands and seasonal variability of the spectral radiance coefficient.

Multispectral and multitemporal satellite data have played a primary role in characterizing land cover change and deforestation rates (Lu et al. 2004), but are fast becoming a fundamental component of conservation planning and biodiversity assessment (Sesnie et al. 2008, Stickler and Southworth, 2008). In determining bioparameters and identifying terrestrial surface structures, high-resolution multispectral satellite data are considered most appropriate. It is a matter of common knowledge that spectral curves of natural objects are determined by three spectral regions: green, red, and near infrared. Existing methods for processing multispectral images are represented by linear combinations of spectral bands with coefficients obtained from field measurements, and with ratio indexes of spectral band brightnesses which are usually referred to as vegetation indexes (Vladimirov, Sorokovoy 2011).

To map and model the restoration-age dynamics of taiga forests we used different-quality information derived from space-based research, cartographic data, ground-level research findings, archival data, and GIS databases. The entire data set includes 1) high resolution satellite images (Landsat TM, Landsat ETM+, and ASTER/TERRA); 2) topographic maps at a scale of 1:100 000, and 1:200 000; 3) data of national forest reserve inventories, and forest assessment descriptions; and 4) field route survey materials.

The ground-based observations were made as part of the route surveys, with visual, descriptive and photographic fixing of landscapes to verify image interpretation results.

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The data obtained were processed using different methods with the purpose of comparing the image processing results with field investigations to generate the landscape map, the map of forest types, and predictive maps of the dynamics.

Material and methods

Computer-aided interpretation of images does not take proper account of the geographical principles of investigations: the territoriality, integrated character, multifactoriality, ambiguity, uniqueness, concreteness, individuality, account for local conditions, etc. The image is more frequently regarded as a whole, rather than as a territorial system of heterogeneous objects. Consequently, it is necessary to switch over to the local analysis of geoimages where not the methods of statistical processes but of mathematical analysis are mostly used, including numerical methods of analysis capturing he individual character of geographical reality.

The interpretation procedure is customarily divided into sequential logical stages, the main of which are recognition, interpretation, and decision-making. At the recognition stage, an analysis is made of the interpretation attributes to solve the problem of establishing the depicted objects, phenomena or their properties. This, most easily formalizable, stage has received wide acceptance in raster image processing programs. Thematic interpretation is performed upon completion of the recognition stage and involves constructing a model of factors influencing the state and classification position of interpretation objects. An object is assigned to a particular classification group using a set of rules that not necessarily follow from the properties and characteristics of the remotely obtained image. Interpretation uses logical categories based on correlative links between geosystems components. Decision-making in thematic interpretation is mainly associated with the procedure of graphically identifying a current object. The apparent simplicity of the decision-making stage in practice involves one of the most tedious and non-technological procedures of estimating the degree of reliability of the interpretation reference attributes for a current object.

In the process of interpreting satellite images, the following problems are solved: recognizing the boundaries of natural objects depicted on images, establishing interrelationships between individual objects and characteristic properties of their spatial location, and recognizing and recording dynamical natural processes and phenomena that occur and develop over the territory encompassed by satellite imagery.

Analysis of geoimages in delineating the ecosystems is based on representing it as a system of data, i.e. such a set of qualitative and quantitative characteristics for the territory, each of which is uniquely inferable from the others using one-type relations. In this case, a study of the system’s properties reveals a great deal of new fundamental possibilities that are commonly referred to as identifiability. In an abstracting-theoretical treatment, the notion of identifiability (parametric at least) is a particular case of observability (a possibility of indirectly determining the quantities, based on measuring some other quantities and using a priori information). Parametric identifability implies a possibility of determining the parameters of a mathematical model or a process from observations spanning some time interval.

Study Area

The study area is located about 30 kilometers north of the city of Ust-Illimsk (59°49′ -59°44′ N Lat., 102°44′ -102°57′ E Long.). This is part of the Irkutsk Oblast located partly in the Angara river basin, in East Siberia north-west of the Baikal lake, see *Figure 1*. The landscape is rolling and rises between 185 and 946 meters above sea level (Baltic sea elevation system).

Figure 1. *Location map of the study area in the Angara river basin, in East Siberia*

Jacobi's determinant

Good results from processing multiband images are provided if Jacobi's determinant, the Jacobian, is used (Vladimirov 2007). There exist some invariant properties of the ecosystem that retain its value within a natural contour, and within this contour brightness characteristics of satellite images are related by a definite functional correlation that varies on the boundary. If the brightness characteristics of such images are specified parametrically $x_i(x, y, t)$, where (x, y, t) are spatial coordinates of a pixel, and the observation time, then Jacobi's determinant *D*, which is decomposed into minors, is 0, is the characteristics $x_i(x, y, t)$ are interrelated.

$$D = \begin{vmatrix} \frac{\partial x_1}{\partial x} & \frac{\partial x_1}{\partial y} & \frac{\partial x_1}{\partial t} \\ \frac{\partial x_2}{\partial x} & \frac{\partial x_2}{\partial y} & \frac{\partial x_2}{\partial t} \\ \frac{\partial x_3}{\partial x} & \frac{\partial x_3}{\partial y} & \frac{\partial x_3}{\partial t} \end{vmatrix} = 0,$$

$$D = \begin{vmatrix} \frac{\partial x_1}{\partial x} & \frac{\partial x_1}{\partial y} \\ \frac{\partial x_2}{\partial x} & \frac{\partial x_2}{\partial y} \end{vmatrix} \frac{\partial x_3}{\partial t} + \begin{vmatrix} \frac{\partial x_1}{\partial x} & \frac{\partial x_1}{\partial y} \\ \frac{\partial x_3}{\partial x} & \frac{\partial x_3}{\partial y} \end{vmatrix} \frac{\partial x_2}{\partial t} + \begin{vmatrix} \frac{\partial x_2}{\partial x} & \frac{\partial x_2}{\partial y} \\ \frac{\partial x_3}{\partial x} & \frac{\partial x_3}{\partial y} \end{vmatrix} \frac{\partial x_1}{\partial t} = 0$$

More importantly, the way in which (or in terms of which particular type of models) this correlation holds for all models of the description of objects does not matter. *D* induces the presence or absence of such correlation. There appears an objective criterion for identifying homogeneous D=0 and heterogeneous $D \neq 0$ ecosystem areas. The proximity of *D* to 0 determines the degree of homogeneity (spatial homogeneity) of ecosystems.

The Normalized Difference Vegetation Index

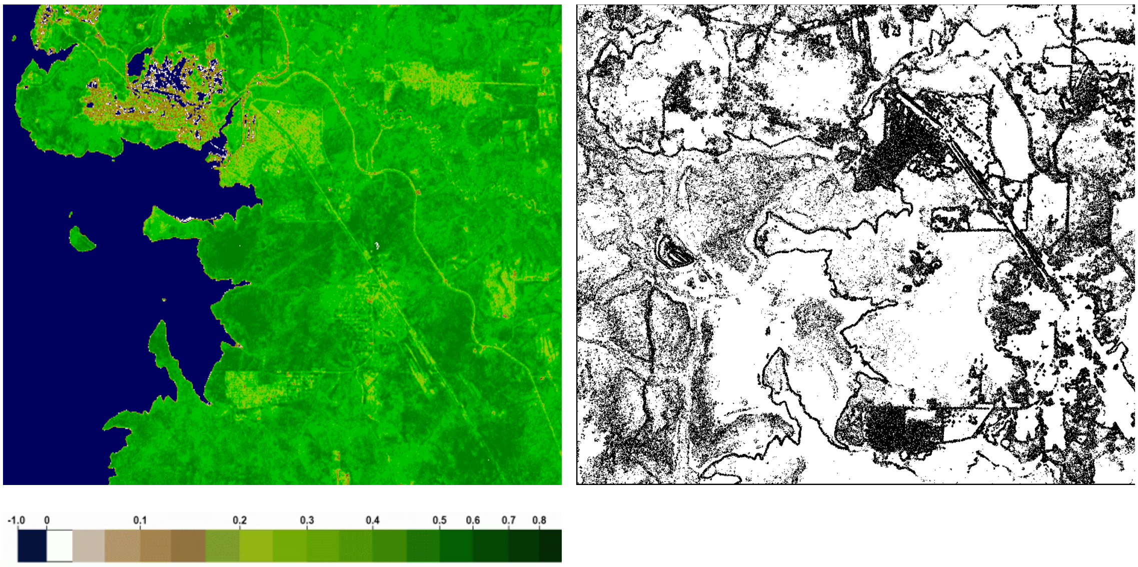
Physiological state of vegetation is largely determined by the content of chlorophyll and moisture level of the green fractions of woody vegetation. Direct determination of absolute values of these parameters from remote sensing data is difficult to date and requires additional ground-based measurements. In this connection it is advisable to use relative indices, obtained on the ground of spectral indices, which correlate with the level of chlorophyll and moisture supply to plants. The normalized difference vegetation index (NDVI) can serve in this respect.

The vegetation index is an indicator that is calculated as a result of procedures with different spectral ranges of remote sensing data, and that is related to the vegetation parameters in a given pixel of an image. The main assumption on the use of vegetation indices is that some mathematical operations with different bands of remote sensing data can provide useful information about vegetation. This is confirmed by multiple empirical data. The second assumption is an idea that the open ground in an image will form a straight line (the so-called soil line) in the spectral space. Almost all of the common vegetation indices use only the correlation of red – near-infrared bands, suggesting that in the near-infrared region there is a line of open soil. It is understood that this line means a zero amount of vegetation.

The Normalized Difference Vegetation Index (NDVI; Tucker and Sellers 1986) is one of the most well-known indices; it is simple to calculate, and has a wide dynamic range and better sensitivity to changes in vegetation cover. It is moderately sensitive to changes in soil and atmospheric background.

Calculation of the NDVI is based on the two most stable (not dependent on other factors) sections of the spectral reflectance curve of vascular plants: the red spectral region (band 3 of Landsat 7 ETM + -0.63-0.69 µm), which is a region of the maximum absorption of solar radiation by chlorophyll, and the near-infrared region (band 4 of Landsat 7 ETM + -0.78-0.90 µm), which is a region of maximum reflection of cellular structures of a leaf, i.e. high photosynthetic activity leads to less reflection in the red spectral region and greater reflection in the infrared region. The NDVI is calculated as the ratio of the measured values of spectral brightness in the red (RED) and near-infrared regions (NIR) of spectrum by the following formula: $NDVI = (NIR - RED)/(NIR + RED)$.

Comparison of route survey results with the findings from interpreting satellite images (NDVI, and identification of the boundaries of forest ecosystems using Jacobi's determinant (*Fig. 2*) in order to delineate the natural boundaries suggest that the automatically identified boundaries (by Jacobi's criterion, the existence of a functional dependence) correspond mainly to regions of relief line inflection (edges, watersheds, lower parts of the slopes), as well as indicating an abrupt change in the structure of biogeocoenoses. For each ecological-geographical situation, which on images is recorded as an individual of the ecosystem, typically has its own linkage system of parameters of the tree stand, surface cover, and soil, influencing the single-type character of the dependence of image characteristics in different survey channels, and this is actually recorded using the criterion selected.



a *b*

Figure 2. *Results from processing space-acquired images. a) NDVI (Landsat ETM+), b) Ecosystem boundaries delineated using Jacobi's determinant (ASTER/TERRA).*

Generating a landscape map

At the first stage of the thematic analysis of images the calculation of the vegetation index NDVI and the classification of results are carried out. A set of classes, distinguished in the course of the analysis of satellite data, should provide a separation of forested areas from areas without forest cover, as well as a subdivision of forested areas into coniferous, deciduous and mixed stands. As a source of auxiliary data for image classification we used forest management materials, reflecting the spatial distribution of the region's forests and their species composition, as well as the data of field research in key areas.

Digital multispectral satellite information reflects diverse factors of landscape environment and can be interpreted as the coordinate space of complex factors of such an environment. This makes it possible to explore the regularities of the pixels distribution within the patches in the given factor space of ordination. Each patch has its own frequency distribution of pixels according to the index values (histogram), and in the ordination space a patch is represented by a set of points, for a specific definition of which a modal value (the point of optimum) is identified. The remaining index values are considered as the set of admissible (typical) deviations from it, beyond which unusual states of this location are situated.

The NDVI values varied within individual phytocenoses ambiguously, which was determined, on the one hand, by the predominance in them of plants of various life forms and species, and, on the other hand, by the accumulation of biomass reserve during the growing period. The largest values of the NDVI in the key areas in the period of maximum vegetation development are characteristic for patches with tree layer continuum, represented by light-coniferous forests (0.20-0.50); slightly lower index values correspond to small-leaved forests (0.10-0.20), and the values 0 to 0.10 correspond to burned areas, cutover areas, and open stands. The proximity of patches in ordination space is not always due to their typological similarity in the composition of the stand or type of location. For example, it is often difficult to distinguish between Siberian stone pine and fir forests, pine and larch forests, etc. In disputable regions a patch is assigned a type, which manifests itself to the maximum (according to the number of pixels) within the boundaries of the contour in an image.

To explain the objective identification of the locations and of their subsequent standardization, a digital elevation model was constructed. The 1:25 000 landscape map at the level of facies was compiled on its basis from field observation results using space-acquired information and results from its automatic processing with the use of Jacobi's determinant (*Fig. 3*).

The dynamical aspect was reflected in the legend to the map: the name of landscape facies is followed (in brackets) by its dynamical state: (N) - native, (IN) – imaginary native, (S) – serial, and (SL) – stable long-derivative, transformed (disturbed), of different variability. The map shows also the geotechnical systems, and the character of anthropogenic transformations: anthropogenically transformed, and anthropogenically disturbed. The former, upon cessation of the anthropogenic impact, can revert to a state close to the original state, and, on the other hand, the changes have a long or irreversible character.

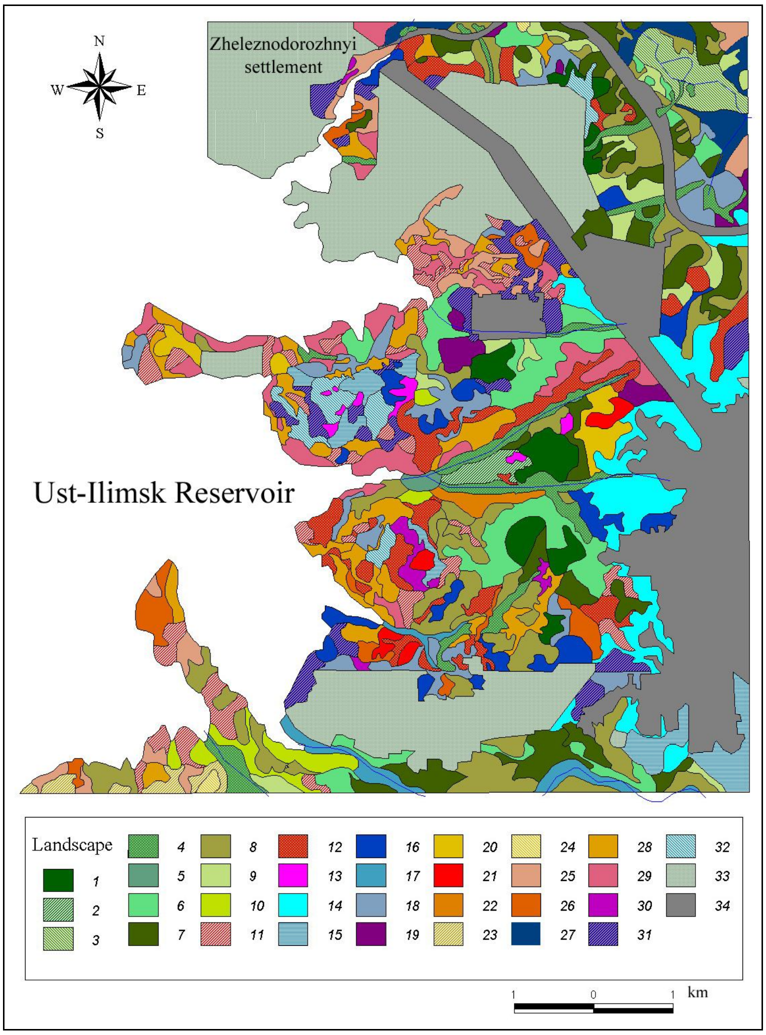


Figure 3. *Landscape map for the key area.*

Legend of the landscape map:

A. ARCTIC-BOREAL NORTH-ASIAN.

A_1- SUBAREAL PLAINS-UPLAND TAIGA-FOREST SHARPLY CONTINENTAL MODERATELY WET AND DIFFERENT THERMAL CONDITIONS (MIDDLE-SIBERIAN)

A_1I. Plains southern-taiga

A_1I_1. Dark-coniferous denudation plateau-plains

1. Interfluve of elevated plains, fir-stone-pine with undergrowth of honey-sucke and juniper, grass-green moss, on soddy-taiga soils (N); 2. Dome-shaped tops of watersheds, fir-spruce-larch, with undergrowth of honey-suckle, grass-green-moss, on soddy-taiga soils (IN); 3. Valley and floodplain spruce-fir large-grass on humus and humus-peaty soils combined with alluvial soddy (S); 4. Bottoms of creek valleys and narrow river valleys, fir (with stone pine and spruce), large-grass, on humus and humus-peaty soils, combined with alluvial soddy (S); 5. Bottoms of creek valleys and narrow river valleys, fir-spruce, fir, on humus and humus-peaty soils combined with alluvial soddy (S); 6. Slope footing, fir-stone pine with the involvement of larch, with mixed undergrowth, forbs, with patches of green mosses, on soddy-taiga soils (IN); 7. Gently sloping weakly dissected surfaces, dark-coniferous grass-green moss, on soddy-taiga soils (IN); 8. Slopes of moderate steepness. Fir-spruce-larch grass-green-moss, on soddy-taiga soils (IN); 9. Slopes of moderate steepness dark-coniferous with the inclusion of larch, grass-shrubs, with patches of green mosses, with mixed undergrowth, on soddy-taiga soils (IN); 10. Steep slopes dark-coniferous with the inclusion of larch, grass-shrubs, with patches of green mosses, with mixed undergrowth, on soddy-taiga soils (IN);

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Anthropogenically-disturbed

11. Gently sloping weakly dissected surfaces pine with the inclusion of fir, forbs on soddy forest soils (SL); 12. Slopes of moderate steepness pine with the involvement of dark-coniferous species, forbs, with undergrowth of spiraea and mountain-ash, on soddy-taiga soils (SL);

Anthropogenically modified

13. Leveled areas of watersheds aspen progressive series (with larch and stone pine as undergrowth) withmountain ash and alder as undergrowth, sedge-forbs, on soddy-taiga soils; 14. Flat weakly dissected surfaces birch progressive series (with fir and stone pine as undergrowth) shrub forbs on soddy forest soils; 15. Flat weakly dissected surfaces birch progressive series (with spruce and fir as undergrowth) forbs, on soddy forest low-thickness loamy and light-loamy soils; 16. Gently-sloping weakly dissected surfaces birch progressive series with the inclusion of dark-coniferous species shrubs forbs on soddy forest soils; 17. Bottoms of small creek and river valleys, birch (with stone pine and fir as undergrowth) forbs on soddy-forest soils combined with alluvial soddy; 18. Slopes of moderate steepness, birch progressive series (with fir and spruce as undergrowth) shrubs forbs on soddy forest soils; 19. Slopes of moderate steepness aspen progressive series (with fir and stone pine as undergrowth) with honey-suckle in undergrowth sedge-forbs on soddy forest soils;

A_1I_2*. Light-coniferous-taiga denudation-erosion plateau-plains*

20. Leveled areas of watersheds pine foxberry-forbs with mixed undergrowth on soddy-taiga soils (N); 21. Lowerings of watersheds and gentle near-watershed slopes larch with spruce and fir, grass-shrub, with patches of green mosses on soddy-taiga soils (IN); 22. Gentle near-valley slopes light-coniferous with spruce and stone pine, grass-green moss, on heavy-loamy soddy-forest soils (IN); 23. Gently sloping of weakly dissected surfaces larch with the inclusion of pine, grass-moss, on soddy-taiga soils (IN); 24. Gently sloping of weakly dissected surfaces larch shrub-moss on soddy-taiga soils (IN); 25. Slopes of moderate steepness pine sedge-forbs with mixed undergrowth on soddy taiga soils (I);

A_2*. SUBBOREAL MOUNTAIN AND MOUNTAIN-VALLEY TAIGA OF WET AND CONTRAST THERMAL CONDITIONS OF INLAND MIDDLE MOUNTAINS AND HIGH PLATEAUX*

A_2I*. Mountain-taiga light-coniferous southern-Siberia type*

A_2I_1*. Piedmont elevations light-coniferous of optimal development*

26. Gently-sloping weakly dissected surfaces pine fox-berry-forbs with sparse undergrowth of dog rose and alder on slightly loamy soddy grey forest low-humic soils (N); 27. Floodplain and terrace birch grass with patches of green mosses on floodplain-layered low-thickness soils (S); 28. Slopes of moderate steepness pine with larch with mixed undergrowth, forbs., on soddy grey forest soils (IN); 29. Slopes of moderate steepness pine foxberry-forbs on soddy-taiga soils (S);

Anthropogenically modified

30. Gently-sloping weakly dissected surfaces, aspen progressive series with the inclusion of pine with undergrowth of alder and honey-suckle, forbs, on soddy forest soils.; 31. Gently-sloping weakly dissected surfaces birch progressive series with the inclusion of light-coniferous, forbs, on soddy forest soils; 32. Flat weakly dissected surfaces birch progressive series (with spruce and larch as undergrowth), forbs-grass, on soddy forest thick loamy and slightly loamy soils;

B. GEOTECHNICAL SYSTEMS

33. Residential; 34. Transport-technical.

Geographical analysis of forest ecosystems

A definite natural regime of functioning and development corresponds to each facies. Within a facies, forest restoration in cut-over and burned-over areas follows a certain sequence of biocoenoses succession to form a climax coenosis (restoration-age series, succession). A change of states embodies time-different manifestations of the changes in ecosystems caused, in particular, by meteo-energetic factors, and by the succession-age dynamics of the biota, both natural and associated with human activity (Krauklis 1975, Sochava 1978, Vladimirov 2009).

As is known, different ecosystems are characterized by different impacts of naturally-occurring destructions on the taiga and its different sensitivity to natural and anthropogenic effects, as well as by a different course of its restoration. Geographical patterns of the dynamics of these processes deserve study in the interests of rational utilization and conservation, improvement, and build-up of the region's taiga forests. On the other hand, an understanding of the dynamics, disturbances and restoration, succession and age changes of the forest in regard to key types of landscape units and in different landscape regions is of great scientific interest, specifically for developing a theoretically well-grounded and practicable ecological-geographical classification of taiga lands, estimating the coefficients for predictive models of the taiga forest dynamics with due regard for landscape structure.

Important implications for the geographical analysis of forest ecosystems come from the restoration-age dynamics that determines the short-term changes in forest cover, and the changes introduced by natural and anthropogenic destructive impacts which make it possible to explore and assess the potential possibilities that the forest cover would recover, its future properties and economic significance, and the changes associated with geosystems transformation processes that are responsible for the possible formation of biogeocoenoses in the new evolving environmental conditions. The first two types of changes lead to restoration and stabilization of forest cover, and the third type of dynamics is characterized as a transforming one.

Results

Interrelationship between forest-typological and landscape-geographical units

The idea of a reconciled classification of the vegetation cover from the landscape-geographical standpoint was put forward by V.B. Sochava (1957). Forest biogeocoenoses, according to the character of forest-vegetation conditions, correspond to elementary individuals of a particular landscape facies.

The study of the restoration-age dynamics of communities has a long history and received a rather clear justification in forest biogeocoenology in connection with the development of the genetic approach to forest typology by B.P. Kolesnikov (1956, 1958). Also, he formulated the concept of the forest-forming process as a specialized version of the general and historical concept that reflects, under present conditions, the characteristics of emergence, formation, destruction and transformation of forest cover, as well as the changes in forest-vegetation conditions and of the entire system of interrelationships of the natural complex. The forest type is regarded as a certain temporal stage of the forest-forming process characteristic for a particular type of landscape conditions. Forest types of a genetic classification reflect to a different extent the spatial-orographic differentiation of forest-vegetation conditions (the conditions of growth location, the landscape structure of the territory), and the changes in growing vegetation associated with the process of settlement, emergence and formation of forest communities, and their functioning across time, destruction, and subsequent restoration. Early in stage, the dynamical aspect reflected a combination, in a single forest type, of native and derivative (potentially native) communities of the demutation series but led subsequently to a need for a special study of the restoration-age dynamics or assessment of forest communities in terms of their dynamical state.

Territorial characteristics of restoration-age dynamics of taiga ecosystems can be revealed by inferring the changes in the structure of tree stands through diversity patterns of their composition. The above approach to identifying the dynamics is justified by the fact that the distribution of vegetation cover of the succession or demutation series in space can correspond to their sequential changes across time (Clements 1928), and by relevant concepts suggested by N.V. Tretiakov (1927) that in studying the course of growth it is advisable to combine different-age stands, having a similar history (of the same forest type), into a single natural-genetic series of development.

The dynamics of forest communities over time is also revealed through a mathematical-statistical analysis of the assessment data on the forest reserves. For this purpose, assessment descriptions of the areas must be classified according to forest types by averaging assessment characteristics over age classes. However, geographical interpretation requires comparing classification categories of silvics (phytocoenosis, forest community, biogeocoenoses, assessment area, forest type, and the type of restoration conditions) and landscape studies (elementary individual, facies).

Geographical analysis of forest ecosystems

The processes of restoration-age dynamics, observed in the Irkutsk region, have been brought about mostly by forest fires and, to a significant extent, by continuous felling (Vladimirov, 2009). The other factors that destroy tree stands or introduce dramatic changes into the environmental conditions in them, have a very infrequent occurrence over this territory.

The trend of restoration-age dynamics of forests is determined by the territory's landscape structural features (forest vegetation conditions). In small areas, the differences of this process depend on soils and position relative to relief elements. And, to a lesser extent, this process is also influenced by climatic factors; therefore, in different parts of the Irkutsk region, even at places with similar soils and relief, the restoration-age dynamics of the forests is proceeding in a different fashion.

In the Irkutsk region, the following progressive series of forest types are rather clearly identified according to L.V.Popov (1982) (*Fig. 4*).

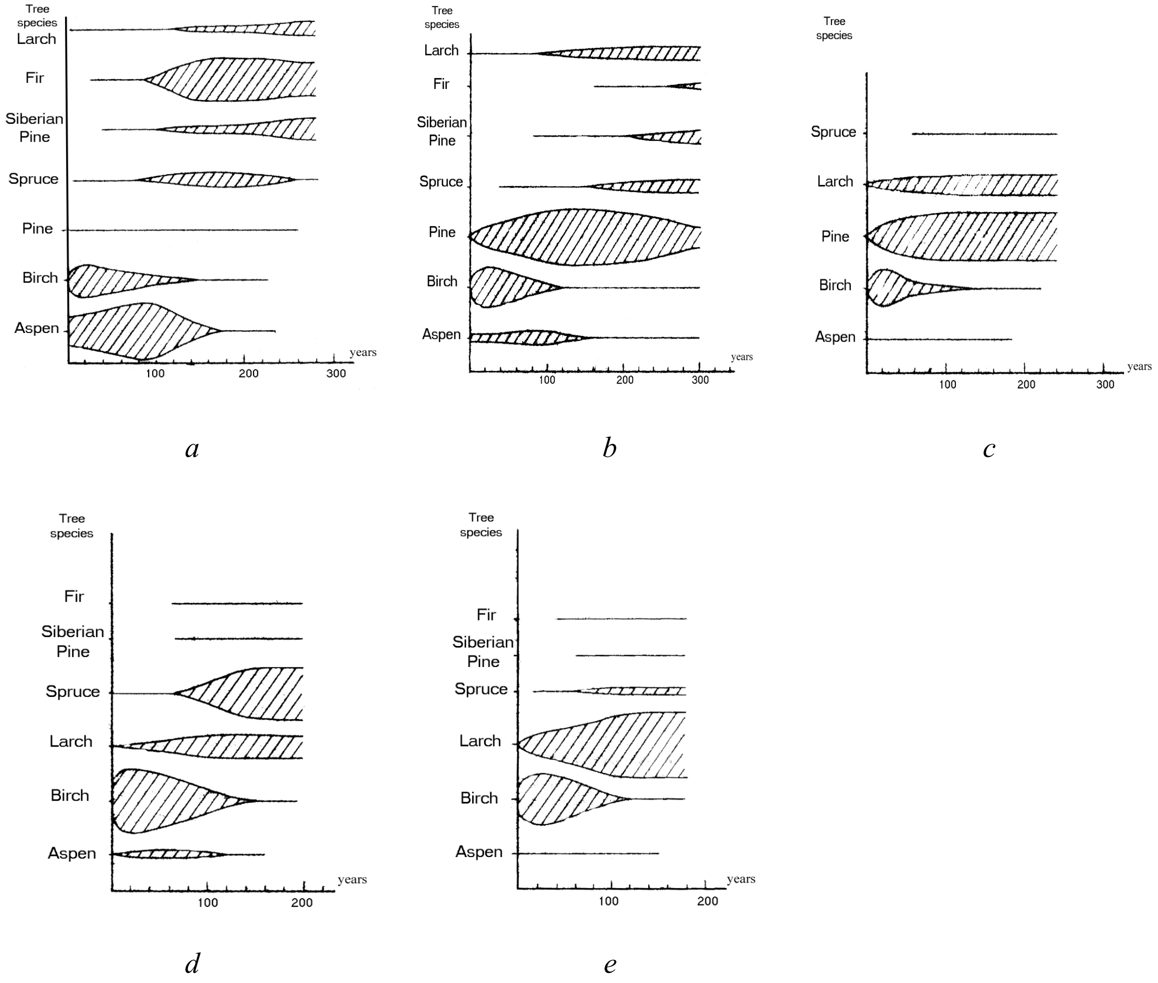

Figure 4. *Dynamics diagrams of species composition of tree stands for progressive series: (a) II, (b) III, (c) VIII, (d) IX, and (e) X.*

I. Series of dark-coniferous taiga on soddy-podzolic and soddy-forest ferruginous loamy soils of drained watersheds and slopes.

II. Series of dark-coniferous taiga on soddy-podzolic and soddy-forest ferruginous loamy and clayey soils of flat watersheds and wet slopes.

III. Series of pine stands on soddy-podzolic and soddy-forest ferruginous soils with intermittent fires.

IV. Series of pine stands on soddy-podzolic and podzolic soils of light mechanical composition.

V. Series of pine stands on sandy podzolic soils of the watersheds and upper terraces of river valleys with intermittent ground fires.

VI. Series of light-coniferous forests on soddy-calcareous soils with long-lasting periods between fires.

VII. Series of light-coniferous forests on soddy-calcareous soils with frequent sweeping ground fires.

VIII. Series of pine stands on soddy-podzolic and soddy-forest ferruginous and clayey soils of the northern part with intermittent fires.

IX. Series of spruce stands of the lower part of the hillsides with soddy-podzolic and soddy-forest ferruginous soils.

X. Series of larch stands on the hillsides with soddy-podzolic and soddy-forest ferruginous loamy and clayey soils.

XI. Series of stone pine stands in low-lying watersheds with soddy-podzolic soils.

A classification of progressive series as suggested by L.V. Popov (1982) for the subzone of the Southern taiga of the Middle Siberia is largely similar to a classification of landscapes for this territory as identified by V.B. Sochava (1978). For instance, the series of dark-coniferous taiga on medium-podzolic and soddy-forest ferruginous loamy soils of the drained watersheds and slopes is realized within the boundaries of the southern-taiga dark-coniferous-forest class of facies of fixed elevations. The existing links of the facial structures of the territory with the dynamics of tree stands permit, on the one hand, landscapes on a terrain to be more easily identified, and, on the other, the forest dynamics to be inferred at a particular point of space from landscape mapping results.

Comparison of the landscape structure and progressive series of the forest type

Within the boundaries of each facies, the simplest variant of directional or fluctuational dynamics is realized. From start to finish, this process is evolving unidirectionally, and all elements involved fully reach their final state corresponding to a given facies.

In this case, each of the dynamics stages characterizes the formation time of the community during which its composition is dominated by a definite forest-producer or a generation of a tree stand that has an edificatory and regulatory effect on the intracoenotic environment, and on biogeocoenotic processes (Popov, 1982).

Based on our research results, it was established that definite progressive series of the forest type correspond to different facies and progressive series of the key area (*Table 1*).

Table 1. *Title of the table*

Landscape facies	Progressive series
Interfluve of elevated plains, fir-stone-pine with undergrowth of honey-sucke and juniper, grass-green moss, on soddy-taiga soils nterfluve of elevated plains	II
Dome-shaped tops of watersheds, fir-spruce-larch, with undergrowth of honey-suckle, grass-green-moss, on soddy-taiga soils	II
Valley and floodplain spruce-fir large-grass on humus and humus-peaty soils combined with alluvial soddy	IX
Bottoms of creek valleys and narrow river valleys, fir (with stone pine and spruce), large-grass, on humus and humus-peaty soils, combined with alluvial soddy	IX
Bottoms of creek valleys and narrow river valleys, fir-spruce, fir, on humus and humus-peaty soils combined with alluvial soddy	IX
Slope footing, fir-stone pine with the involvement of larch, with mixed undergrowth, forbs, with patches of green mosses, on soddy-taiga soils	II
Gently sloping weakly dissected surfaces, dark-coniferous grass-green moss, on soddy-taiga soils	II
Slopes of moderate steepness, fir-spruce-larch grass-green-moss, on soddy-taiga soils	II
Slopes of moderate steepness dark-coniferous with the inclusion of larch, grass-shrubs, with patches of green mosses, with mixed undergrowth, on soddy-taiga soils	III

Steep slopes dark-coniferous with the inclusion of larch, grass-shrubs, with patches of green mosses, with mixed undergrowth, on soddy-taiga soils	III
Gently sloping weakly dissected surfaces pine with the inclusion of fir, forbs on soddy forest soils	III
Slopes of moderate steepness pine with the involvement of dark-coniferous species, forbs, with undergrowth of spiraea and mountain-ash, on soddy-taiga soils	III
Leveled areas of watersheds aspen progressive series (with larch and stone pine as undergrowth) withmountain ash and alder as undergrowth, sedge-forbs, on soddy-taiga soils	II
Flat weakly dissected surfaces birch progressive series (with fir and stone pine as undergrowth) shrub forbs on soddy forest soils	IX
Flat weakly dissected surfaces birch progressive series (with spruce and fir as undergrowth) large-grass, on soddy forest thick loamy and light-loamy soils	IX
Gently-sloping weakly dissected surfaces birch progressive series with the inclusion of dark-coniferous species shrubs forbs on soddy forest soils	IX
Bottoms of small creek and river valleys, birch (with stone pine and fir as undergrowth) forbs on soddy-forest soils combined with alluvial soddy	IX
Slopes of moderate steepness, birch progressive series (with fir and spruce as undergrowth) shrubs forbs on soddy forest soils	IX
Slopes of moderate steepness aspen progressive series (with fir and stone pine as undergrowth) with honey-suckle in undergrowth sedge-forbs on soddy forest soils	II
Leveled areas of watersheds pine foxberry-forbs with mixed undergrowth on soddy-taiga soils	VIII
Lowerings of watersheds and gentle near-watershed slopes larch with spruce and fir, grass-shrub, with patches of green mosses on soddy-taiga soils	X
Gentle near-valley slopes light-coniferous with spruce and stone pine, grass-green moss, on heavy-loamy soddy-forest soils	VIII
Gently sloping of weakly dissected surfaces larch with the inclusion of pine, grass-moss, on soddy-taiga soils	VII
Gently sloping of weakly dissected surfaces larch shrub-moss on soddy-taiga soils	X
Slopes of moderate steepness pine sedge-forbs with mixed undergrowth on soddy taiga soils	VIII
Gently-sloping weakly dissected surfaces, pine foxberry-forbs, with sparse undergrowth of dog rose and alder, on light-loamy soddy grey forest low-humic soils	VIII
Floodplain and terrace birch grass with patches of green mosses on floodplain-layered low-thickness soils	IX
Slopes of moderate steepness pine with larch with mixed undergrowth, forbs, on soddy grey forest soils	VIII
Slopes of moderate steepness pine foxberry-forbs on soddy-taiga soils	VIII
Gently-sloping weakly dissected surfaces, aspen progressive series with the inclusion of pine with undergrowth of alder and honey-suckle, forbs, on soddy forest soils.	III
Gently-sloping weakly dissected surfaces birch progressive series with the inclusion of light-coniferous, forbs, on soddy forest soils	X
Flat weakly dissected surfaces birch progressive series (with spruce and larch as undergrowth), forbs-grass, on soddy forest thick loamy and slightly loamy soils	X

Prognostic-dynamical maps of forest types

Based on the data for progressive series of forest types and on comparing them with the facial structure (see *Table 1*), the landscape map (see *Fig. 3*), and forest assessment data, it was possible to create the map for the present state of forest types (*Fig. 5*), and the prognostic-dynamical maps for forest types for a period of 50 and 100 years (*Figs. 6 and 7*).

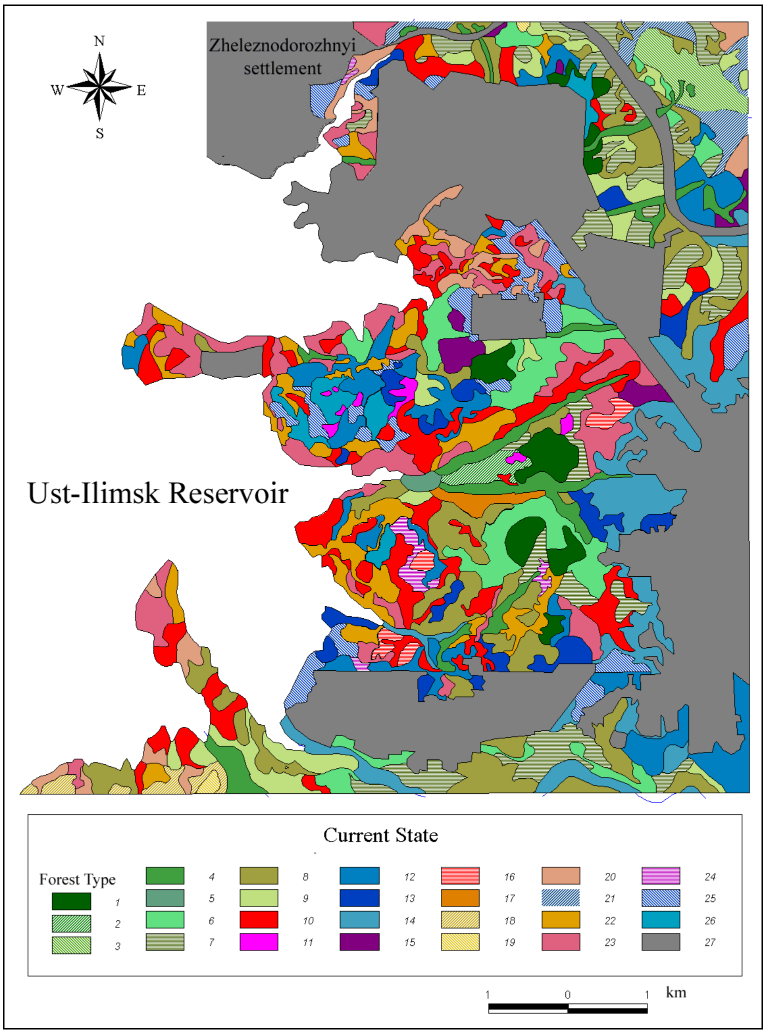

Figure 5. *Map for forest types (present state)*

1. Stone pine-fir stands with undergrowth of honey-suckle and juniper, grass-green moss; 2. Fir-spruce-larch with undergrowth of honey-suckle, grass-green moss; 3. Spruce-fir, large grass; 4. Fir (with stone pine and spruce), large-grass; 5. Fir-spruce, fern; 6. Stone pine-fir with the involvement of larch with mixed undergrowth, forbs with patches of green mosses; 7. Dark-coniferous, grass-green moss; 8. Fir-spruce-larch, grass-green moss; 9. Dark coniferous with the inclusion of larch, grass-dwarf shrub, with patches of green mosses; 10. Pine stands with the involvement of dark-coniferous species, forbs; 11. Aspen-tree stands (with larch and stone pine as undergrowth) with mountain ash and alder as undergrowth, sedge-forbs; 12. Birch stands with the inclusion of dark-coniferous species, shrubs-forbs; 13. Birch stands (with stone pine and fir a undergrowth), forbs; 14. Birch stands (with fir and spruce as undergrowth), shrubs-forbs; 15. Aspen-tree stands (with fir and stone pine as undergrowth) with mountain ash as undergrowth, sedge-forbs; 16. Larch stands with spruce and fir, grass-shrubs, with patches of green mosses; 17. Light-coniferous with spruce and stone pine, grass-green moss; 18. Larch stands with the inclusion of pine, grass-moss; 19. Larch stands, shrubs-moss; 20. Pine stands, sedge-forbs, with mixed undergrowth; 21. Birch stands, grass, with patches of green mosses; 22. Pine stands with larch with mixed undergrowth, forbs; 23. Pine stands, foxberry-forbs; 24. Aspen tree stands with the inclusion of pine with undergrowth of alder and honey-suckle, forbs; 25. Birch stands with the inclusion of light-coniferous species, forbs; 26. Birch stands progressive series (with spruce and larch as undergrowth), forbs-grass; 27. Anthropogenic structures.

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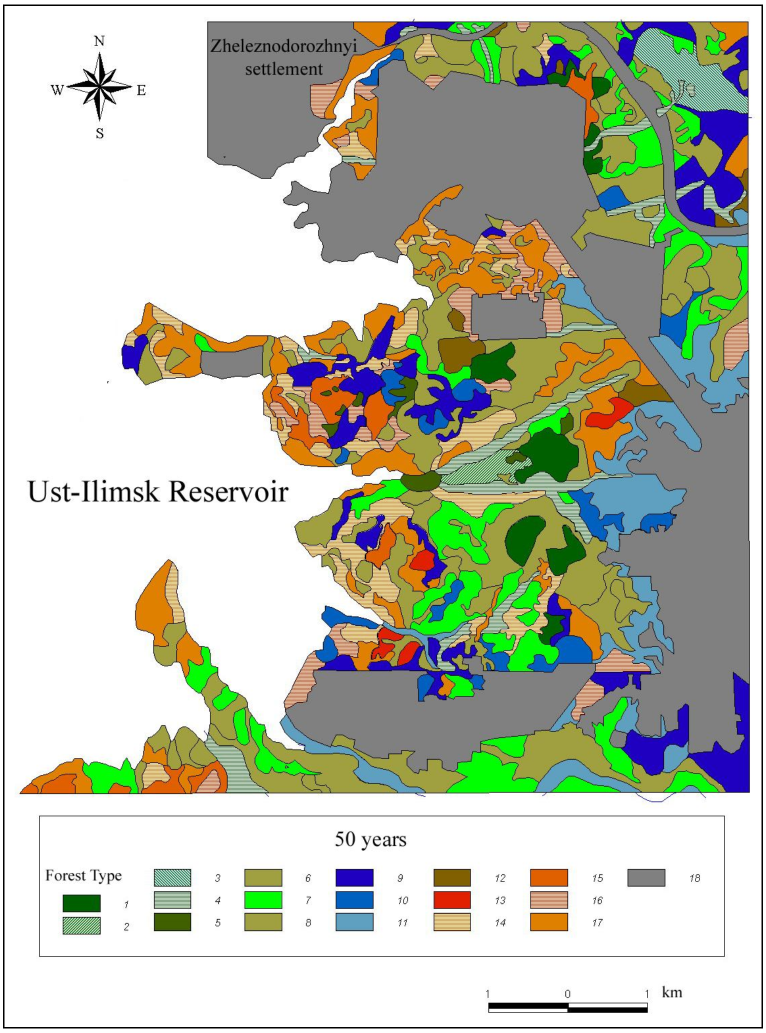

Figure 6. *Prognostic-dynamical map for forest types (the state within 50 years)*

1. Fir-stone pine with the involvement of larch, grass-green moss; 2. Fir-larch stands with the involvement of spruce, grass-green moss; 3. Fir-spruce, large grass; 4. Spruce-fir wit the inclusion of stone pine, large grass; 5. Larch-stone pine with the involvement of fir, forbs with patches of green mosses; 6. Stone pine-fir with the involvement of larch, forbs-green mass; 7. Spruce-larch, grass-green moss; 8. Spruce-stone pine-pine, forbs; 9. Birch stands with the inclusion of spruce and larch, green moss-large grass; 10. Birch stands with the inclusion of fir, green moss-large grass; 11. Birch stands with the inclusion of spruce and fir, green moss-large grass; 12. Stone pine-fir with the involvement of larch, forbs; 13. Larch stands with the inclusion of spruce and fir, grass-shrubs with patches of green mosses; 14. Pine stands with the involvement of larch, foxberry-forbs; 15. Larch stands with the inclusion of dark-coniferous species, green moss; 16. Larch stands, green moss; 17. Pine stands, foxberry-green moss; 18. Anthropogenic structures.

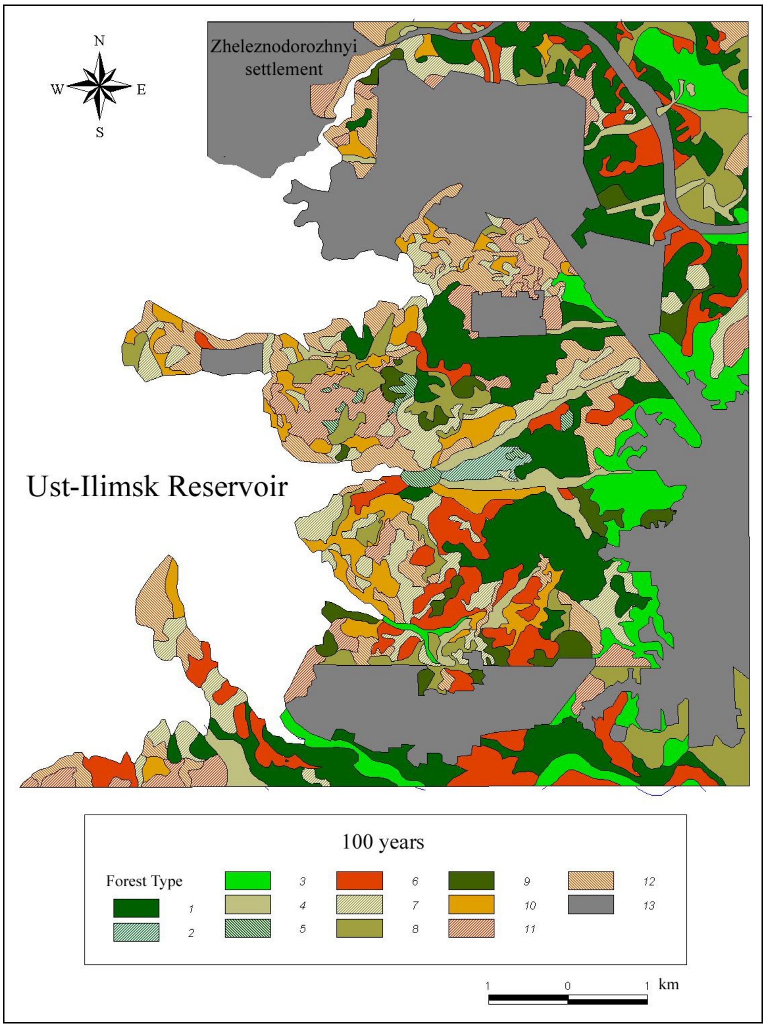

Figure 7. *Prognostic-dynamical map for forest types (the state within 100 years)*

1. Larch-fir-stone pine, grass-green moss; 2. Fir-larch, grass-green moss; 3. Fir-spruce with the involvement of larch, large grass green moss; 4. Spruce stands with the inclusion of stone pine and larch, large grass with patches of green mosses; 5. Larch-stone pine, forbs-green moss; 6. Larch stands with the participation of spruce, taiga moss; 7. Spruce-stone pine-pine stands with the inclusion of larch herbaceous; 8. Spruce stands with the inclusion of birch and larch, green moss-large grass; 9. Stone pine-fir stands with the inclusion of larch, green moss-large grass; 10. Pine stands with the involvement of larch, foxberry; 11. Larch stands with the inclusion of dark-coniferous species, green moss; 12. Pine stands, foxberry; 13. Anthropogenic structures.

Discussion

The comparison of the landscape structure and progressive series of forest types reported here does of course not reflect all possible variants of restoration-age dynamics of forests. The characteristic property of the last of each facies is determined by ecological conditions of environment, and by the biological properties of the main and accompanying species composing the communities and maintaining a complicated interspecies linkage over the course of the entire cycle of progressive successions. The trend of the dynamics is often determined also by the intensity and recurrence of forest fires that are responsible for the duration of the stages, species composition, and for the appearance of the living surface cover. On the other hand, it has been established that in the process of formation, within the boundaries of each facies the vegetation goes through numerous, morphologically similar, restoration stages of the native community.

The theoretical framework for the creation of prognostic-dynamical maps for forest types is becoming interpretation mapping where the landscape-typological map is used as a contour map and as a classification framework for developing derivative cartographic documents (Mikheyev et al. 1996). This approach is best realized within the framework of structural-dynamical landscape studies and genetic silvics, because it is with this approach that it is possible to manage to identify a spatial individual (a facies, and a biogeocoenoses), the regime inherent in it, the intensity and sequence of dynamics, temporal and spatial diversity, economic utilization, etc. (Konovalova et al. 2005).

Conclussion

The algorithm for creating prognostic-dynamical maps for forest types is as follows. 1) Field investigations of the facial structure and restoration series of forest types for the study area; 2) Creation of the landscape-typological map on the basis of remotely sensed data; 3) Creation of dynamics diagrams for species composition of tree stands of progressive series of forest types; 4) Referencing of forest assessment data to the landscape-typological map; 5) Comparison of the facial structure and progressive series of the forest type, based on the data obtained as part of the analysis of forest assessment information and field observations; 6) Construction of the prediction of forest development on the basis of the landscape-typological map, and dynamics diagrams for natural composition of tree stands, and entering it into the database; and 7) Visualization of the prediction database in the GIS environment.

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EVALUATION OF A GENERAL ECOSYSTEM STATE INDICATOR BASED ON FARMLAND BIRDS

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Abstract. The comparison analysis and assessment of certain landscapes and landscape units is an effective tool for the decision makers for meeting appropriate land use decision. In our analysis we compared various groups of indices applicable for ecological landscape assessment applying them on three lowland landscape units. The assessed indices characterize the landscape from the point of view of vegetation (vegetation-based natural capital index) and birds (diversity of birds species of agricultural areas). The vegetation-based natural capital index values have been derived from the MÉTA database collected between 2003 and 2008. The ornithological survey was carried out in the spring of 2011 by modified Danish point counting system. Our sample areas were the geographical micro-regions Csepeli-sík, Hortobágy and Nagyberek, which are landscapes dominated by agricultural lands and grasslands. The major question was whether different naturalness indicators characterize basically similar but differently used landscape areas in a similar way. We found that while a higher NCI value corresponds to a higher abundance of birds, it also corresponds to a lower diversity of bird species. This contraintuitive effect partially might be explained by the variance of the large-scale landscape structure of the assessed landscape areas.

Keywords: *biodiversity index, bird indices, habitat quality, vegetation-based natural capital index, naturalness*

Introduction

One of the most serious environmental problems of our time is the loss of biodiversity caused by human activities which is associated by the continuous deterioration of the state of health of ecosystems (MEA, 2005; Rockström et al., 2009). The greatest difficulties in monitoring of biodiversity are the immense complexity of ecosystems, lack of financial resources, time, and lack of experts and appropriate technical conditions (Gregory and van Strien, 2010; Rodrigues and Brooks, 2007). To overcome these difficulties, various types of indicators are used to substitute for several parameters (Eglington et al., 2012; Gregory et al., 2005). These indicators aim at characterizing not only the compositional part of biodiversity, but the state of ecosystems in an integrated way (Noss, 1990; Niemeijer and de Groot, 2008). In addition to appropriately reflecting the condition of an ecosystem, such biodiversity indicators should also respond quickly to changes in the environment, and be easily monitored with sufficient information available about them (Dale and Beyeler, 2001). The most important goal of the development of such indicators is to measure the state

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and trends of ecosystems and the ecological processes occurring in them (EEA, 2007; UNEP; 1992; van Strien et al., 2009). For the evaluation of the ecoystems and biodiversity states, as well as for monitoring of trends, different proxies are used, such as plants (Croft and Chow-Fraser, 2007) and birds (Bradford et al., 1998; Canterbury et al., 2000).

Currently, one of the key sectors in land use is agriculture. This kind of human activity is the most responsible for the disappearance and transformation of natural areas. In the beginning, traditional extensive farming did not result in diminishing diversity, however, by the second half of the 20th century the significant population growth has had an increasing negative effect on the ecosystems with the use of machinery and various chemicals (crop protection products, fertilizers, etc.) (Reidsma et al., 2006). Diversity of agricultural landscapes was significantly reduced by the intensification of agriculture, starting from the 1600s in the wake of the industrial revolution and further accelerated during the 19th century (Donald et al., 2001). In case of several groups of organisms continuous population decrease were detected, particularly at birds. Since 1970 the population of farmland birds has decreased drastically, in many cases by 50% but the Grey Partrodge (*Perdix perdix*), Eurasian Sparrow (*Passer montanus*) Corn Bunting (*Emberiza calandra*) decreased by more than 80% (Siriwardena et al., 1998). It is clearly associated with the intensification of agriculture, changes of land use and plowing of former grassland (Aebisher et al., 2000; Gregory et al., 2005), which is especially in Western Europe and North America prominent (Herkert, 1994; Schifferli, 2000; Vickery et al., 2001), while in Central and Eastern Europe the negative effects are more nuanced (Verhulst et al., 2004). Agro-environmental programmes were launched in the Member States of the European Union in order to reduce these harmful effects, promoting environmentally sound extensive farming methods (Ángyán, 2008).

In the light of these processes it is not at all surprising that most ecological indicators were developed for agrarian regions on a national and global scale (EEA, 2005; OECD, 1999). Most of the decision on the use of land is made locally, therefore the need to support decision making is most intense on the local and regional level. In order to adapt to this demand numerous international and regional indicators have been elaborated in recent years (Csorba, 2006; Dumortier et al., 2006), each of these indicators have a ‘special approach' to landscape.

One of the most often used multi-species bird indicators was elaborated in the United Kingdom and Europe, which is the Farmland Bird Index, which describes the loss of biodiversity associated with agricultural intensification. The Farmland Bird Index (FBI) was set up in the United Kingdom to collectively monitor the 19 species of birds related primarily to agricultural areas (Gregory et al., 2005). The survey of abundance of bird species related to this index is based on the monitoring of the biodiversity of the easily observable and identified species (the birds) which reflects the ecologic state of the landscape. FBI is recognized as an official biodiversity indicator by the Central Statistical Office in Great-Britain and, in a modified form, also by EUROSTAT, the statistical office of the EU (Gregory et al., 2005).

Another promising indicator, which observes the landscape even in a more local way, is the vegetation-based natural capital index, elaborated in Hungary (Czúcz et al.; 2008). This indicator provides a flexible evaluation and comparable measurements for the general characterization of the natural status, based on plant (vegetation) data (Czúcz et al.; 2012). The concept of the Hungarian vegetation-based natural capital index is based on an indicator of similar name (Natural Capital Index, NCI) developed in the Netherlands at the end of the 1990s (ten Brink, 2000). The original formula expresses the differences between former and actual natural conditions of a complex landscape using numerical data:

$$\text{NCI} = \text{ecosystem quality} \times \text{ecosystem quantity}$$

In the current study two different sets of biodiversity indicators (indicators based on bird data and indicators based on plant/vegetation data) with high potential in ecological landscape evaluation were compared on the example of three Hungarian lowland landscape units. Our main question was exploring the relation between the vegetation based natural capital and the main characteristics of the bird asemblages (abundance, species number, inverse Simpson-diversity, Shannon-diversity) observed at the same sites. We hypothesized that we will get the highest abundance and diversity of birds at sites with a relatively undisturbed close-to-natural state vegetation (high NCI value); and vice versa.

Materials and methods

In the year 2011 we selected three lowland geographical micro-regions with more or less of the same geographical characteristics based on the book entitled „Cadastre of landscape character units of Hungary" (Dövényi 2010): Csepeli-sík, Hortobágy and Nagyberek (*Figure 1).* Main characteristic of these micro-regions are high proportion of agricultural areas with agricultural plots covered by various vegetation cultures combined with dry and wet grassland. Csepeli-sík and Hortobágy are essentially characterized by large pannonic salt and pannonic loess steppes with marshlands wedged between them. Agricultural areas are typically divided into smaller lands with extensive cultivation. The northern and western parts of the Csepeli-sík are the exceptions, as they include large monocultures, a vast number of small lots as well and smaller and larger mine lakes. Because of the floodplains of the river Tisza and the related vegetation, the northern part of the Hortobágy is fundamentally different from the above described structure. Because of these characteristic differences of vegetation in case of lowland Csepeli-sík and Hortobágy we limited the survey for habitat mosaics and these were considered as landscape units. So we excluded from the survey the areas north to the road No. 51 and road Kiskunlacháza-Bugyi in case of lowland Csepeli-sík and areas north to the road No. 37 in Hortobágy. Similar to the above mentioned landscapes extensive agriculture dominates in Nagyberek, however we can find monocultural plots of greater scale as well. Previously water-covered habitats were featureless and the lower areas were covered by moorland and mud land. The vegetation of the three landscape units have their regional characteristics, but the composition of bird species settled over time is similar so they can be compared with each other.

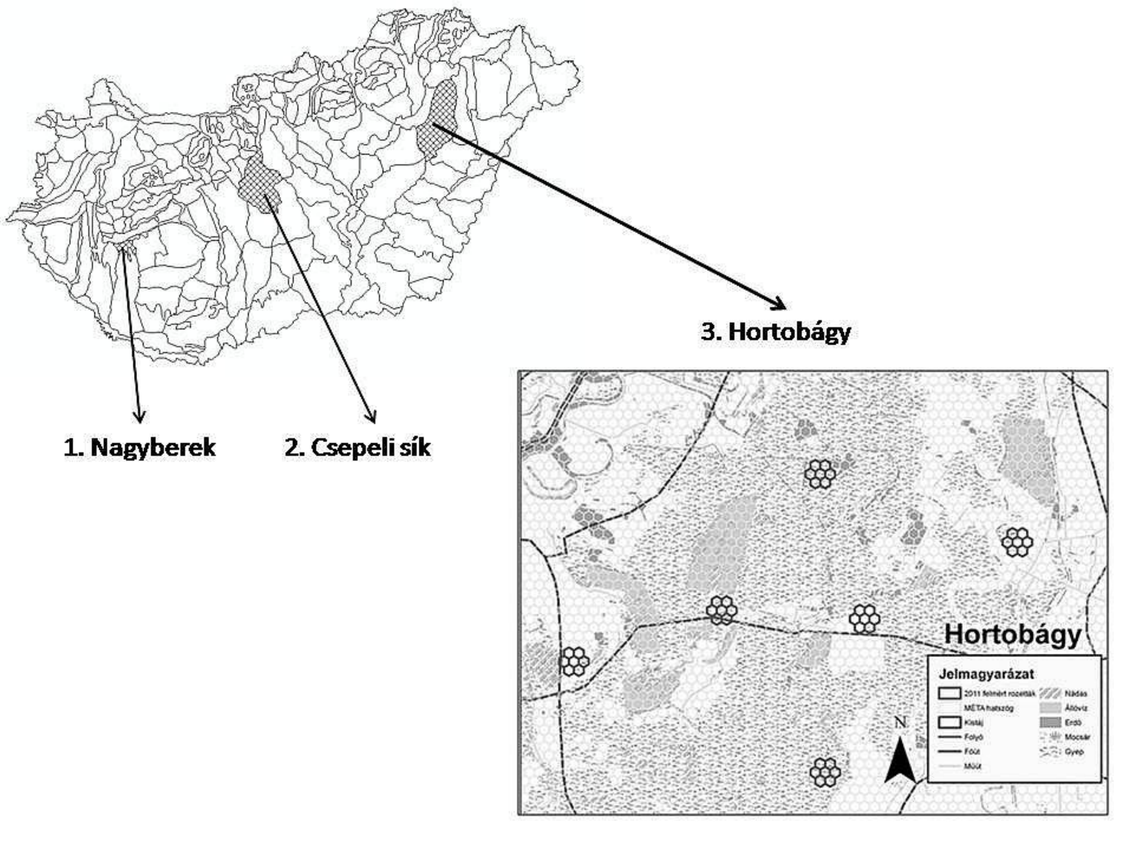

Figure 1. The location of the three plots in Hungary and the six selected rosette pattern in the Hortobágy

Six rosettes have been selected in each geographical micro-regions, each of which is part of one central and six surrounding MÉTA hexagons (these units of 7 hexagons can be approximated by a circle with a radius of ~880m and a ground area of 245 hectares) (Molnár et al., 2007). At the beginning of the designation process each landscape unit was divided into six parts, the center of each area was used as the point of origin for the search. We defined three designation criteria: the central point of the rosette could be accessed relatively easily; 80% of all the ground area of the seven MÉTA hexagons constituting the rosette could be accessible; there should be at least 70% open area within the rosette, cropland and/or grassland (based on the webpage www.pannongyep.hu and Google Earth). If the designation criteria were not met then heading east and moving along a spiral in clockwise direction we checked the subsequent potential center points until a suitable was found. In Nagyberek, the selection has been influenced mostly by closed private areas and conservation restrictions at the Csepeli-sík, as we were only allowed to enter areas for purposes of ornithological recording that were exempt from these restrictions. Our data are derived from seven hexagons in each of the six rosettes, i.e. 42 in each lowland landscape units and 126 hexagons in total (survey points). The determination of vegetation-based natural capital index based on the MÉTA mapping has been carried out between 2003 and 2008, *Figure 2* shows the methodology (Czúcz et al., 2008; Czúcz et al., 2012).

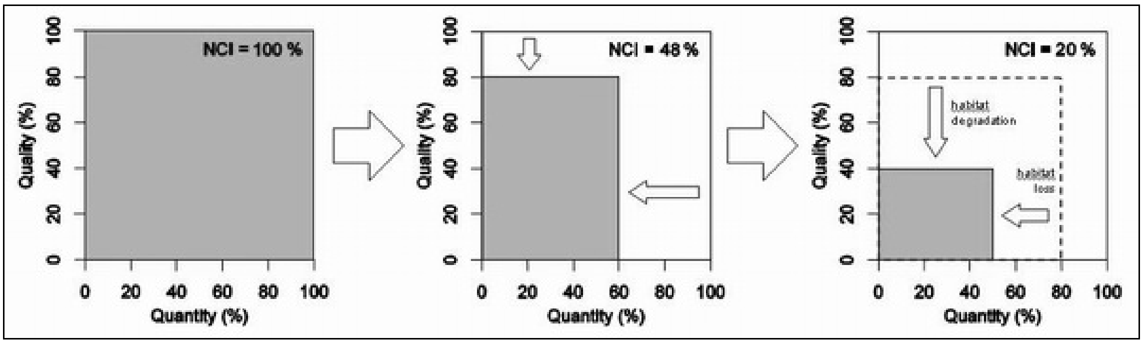

Figure 2. *Demonstrating the calculation of NCI: the NCI value of an area equals the product of the quality and quantity of the remaining natural and semi-natural areas, which falls into the interval [0,1]. For example, if the half of the habitats is destroyed in an area and the naturalness of the remaining ones is reduced to 40%, that means only the 20% of the original natural capital remains in the area (Czúcz et al., 2008; Czúcz et al., 2012).*

The ornithological survey has been carried out between 20 April and 15 June 2011. All rosettes were surveyed in one occasion between 5am to 10 am of the same day. We applied the modified Danish point counting method (Báldi et al., 1997). The center points of each hexagon served as survey points, around which the species heard and sighted within a circle of 100 m were registered. During the counting we also registered other species which were using the territory in other activities mostly for feeding and resting. For some species (e.g. great egret, *Egretta alba*) which startle and leave or avoid the survey area upon our approach, individuals observed within 5 minutes before and after the observation were also recorded and included in the sampling The data evaluation was focused on the population of bird species included into the EU Farmland Bird Index (FBI species). We analyzed the abundance values of the bird species according the Farmland Bird Index (*Table 1*, Gregory et al., 2005; http://www.rspb.org.uk/ourwork/farming/whyfarming/whyfarming/fbi/).

Table 1. *Bird species belonging to the Farmland Bird Index (Gregory et al., 2005; http://www.rspb.org.uk/ourwork/farming/whyfarming/whyfarming/fbi/).*

No.	*English name*	*Scientific name*
1.	Grey Partridge	Perdix perdix
2.	Common Kestrel	Falco tinnunculus
3.	Northern Lapwing	Vanellus vanellus
4.	Rock Dove	Columba livia
5.	Common Wood Pigeon	Columba palumbus
6.	European Turtle Dove	Streptopelia turtur
7.	Eurasian Skylark	Alauda arvensis
8.	Western Yellow Wagtail	Motacilla flava
9.	Common Whitethroat	Sylvia communis
10.	Western Jackdaw	Corvus monedula
11.	Rook	Corvus frugilegus
12.	Common Starling	Sturnus vulgaris
13.	Eurasian Tree Sparrow	Passer montanus
14.	Yellowhammer	Emberiza citrinella
15.	Common Reed Bunting	Emberiza schoeniclus
16.	European Goldfinch	Carduelis carduelis
17.	European Greenfinch	Carduelis chloris
18.	Common Linnet	Carduelis cannabina
19.	Corn Bunting	Emberiza calandra

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To characterize the diversity of bird associations the number of species, the total abundance, the inverse Simpson-index, and the Shannon-index were used; for comparing the similarity of the set of species the Jaccard-index was applied. To evaluate the similarity of perceptions provided by various indices on variant areas we used linear models with various bird-indices (number of species, inverse Simpson and Shannon indices and total abundance) as dependent variables, while the NCI value of each rosette was used as the independent variable and the three micro-regions were the categorical variables. As the diversity indices are not necessarily normally distributed square-root variance-stabilizing transformations were applied to the number of species and the inverse Simpson-index. The analysis was carried out with the R statistical software package (R Development Core Team 2011).

Results

The values of Natural Capital Index based on six-six sample areas are the following: Nagyberek 11%, Csepeli-sík 30% and Hortobágy 45%. Number of FBI species is the following: the highest number of species has been found on Csepeli-sík, the second highest value has been at Nagyberek and the third has been on the Hortobágy. We excluded the flocking rook (*Corvus frugilegus*) from the records as we considered that to reliably capture the relatively rare occurrences of the large flocks of these birds would demand much higher survey efforts and different sampling approaches. Therefore we have found the highest bird density in Hortobágy and in Csepeli-sík. Nagyberek region counted significantly less bird population. With respect of the nearly identical values received for Hortobágy and Csepeli-sík it should be noted that the number of individual (269) in the former lowland area was distributed across 11 species, while in the latter case the 256 birds seen or heard accounted for 15 different species (*Table 2*).

Table 2. *Some parameters of farmland bird species in the three lowland geographical micro-regions (* the number of individual is equal the number of territories; ** except of Rook Corvus frugilegus).*

	Nagyberek	Csepeli-sík	Hortobágy
Natural Capital Index	11%	30%	45%
Number of species	14	16	12
Number of individuals*	172**	256**	269**
Shannon-diversity index	2,2722	2,0432	1,4264

The values of Jaccard-index used to analyse the similarity of species was 76% in comparison of Csepeli-sík and Nagyberek, 75% in comparison of Csepeli-sík and Hortobágy and 53% in comparison of Nagyberek and Hortobágy. The survey of the five most frequent species refers clearly to a large degree of similarity between Csepeli-sík and Hortobágy (*Table 3*).

Table 3. *The most frequent farmland bird species in the three lowland geographical micro-regions and their number of individual (the number of individual is equal the number of territories, except Rook* Corvus frugilegus*).*

Nagyberek	Csepeli-sík	Hortobágy
Sturnus vulgaris (47)	*Alauda arvensis* (90)	*Corvus frugilegus* (280)

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Sylvia communis (31)	*Corvus frugilegus* (89)	*Alauda arvensis* (130)
Motacilla flava (26)	*Vanellus vanellus* (46)	*Motacilla flava* (64)
Corvus frugilegus (15)	*Emberiza calandra* (32)	*Emberiza calandra* (32)
Alauda arvensis (12)	*Motacilla flava* (29)	*Sturnus vulgaris* (15)

The linear models showed that the diversity of the bird species has significant correlation with the vegetation-based natural capital index (NCI) of the area. Nevertheless the direction of this correlation was surprising: the studied community diversity indices (inverse Simpson and Shannon-diversity) reflect a decline with the increase of NCI (*Table 4*). The correlation between the vegetation-based natural capital index and the number of species was not significant. The total abundance exhibits a marginally significant positive correlation with the NCI, reverse to the diversity indices: the greater the NCI of a rosette the greater the total number of observed birds is. The effect of landscape units (geographical micro-regions) and the interaction between the landscape unit and vegetation-based natural capital index was not significant in any case (*Table 4*).

Table 4. *Interaction between vegetation-based natural capital index (NCI), lowland geographical micro-regions, number of species, inverz Simpson-index, Shannon-diversity index and total abundance (p. = significance level, p < 0,05).*

	NCI sign	p. NCI	p. Lowland geographical micro-region	p. Interaction
Number of species	-1	0,13489515	0,3960755	0,9219733
Inverz Simpson-index	-1	0,02068213*	0,3418034	0,778328
Shannon-diversity index	-1	0,02842609*	0,296472	0,861669
Total abundance	1	0,07805489	0,1605993	0,146789

Discussion

Verhulst et al. (2004) compared bird assemblages of extensively and intensively used grasslands and found that density and diversity were highest in abandoned areas. On the contrary, in our investigation the diversity indices were the lowest in the two most naturally reserved areas (Csepeli-sík and Hortobágy). We have to mention that in the study of Verhulst at al. (2004) a high number of species is not connected to the grassland areas. On the whole the naturalness of landscape parts are ordered differently by the vegetation-based natural capital index and the diversity indices of bird species since we observed a decrease in the inverse Simpson and Shannon-indices with the increase of NCI. The human landscape transformation and the large mosaicity of some of the landscape units might be the background of this phenomenon. Hortobágy, which is dominated by vast homogenous grassland areas, can support fewer FBI bird species, which are connected to agricultural areas, then Nagyberek, which is of a more complex mosaic structure. The prevalence of hedges and shelter belts made possible the settlement of forest bird species like common whitethroat (*Sylvia communis*) and common starling (*Sturnus vulgaris*) which contribute significantly to the increase of biodiversity values. The degree of human influence on the Csepeli-sík, sprinkled by alluvial forest fragments of the Danube valley, is between that of the two other sites, which is also indicated by its NCI values. Its set of species is highly identical to that of the Nagyberek, however the survey of the five most common bird species established a definite similarity with the Hortobágy.

The total set of results indicates that the examined ecological status indicators perceive and evaluate the landscapes differently, and there can be many differences of the details, meanwhile they are suitable for quick and superficial comparisons and for in-depth analysis as well. NCI, for example, is not suitable for handling local peak values and cannot express the large-scale naturalness of the landscape (Czúcz et al., 2012). This limitation can be partly eliminated by using bird indicators. Birds, as vagile species on the top of food chain are – with some limits –suitable to characterize the ecological status of the entire landscape at a larger scale than plants alone. Birds are highly sensitive to environmental changes, but the interpretation of changes is made difficult by many other factors e.g. the effects on migratory birds outside nesting time or factors influencing the quantity of prey animals (Gregory et al., 2005).

Bradford et al. (1998) compared areas grazed with various intensity using indicators characterizing bird communities, which provided results similar to ours: birds give a poor indication on the status of ecosystems. According to the results of Eglington et al. (2012) birds poorly indicate the wealth of other taxonomic groups in temperate grassland areas, on the other hand they give a much better indication on agricultural areas of more characteristic mosaic structure. Contrary to this, they function well as indicators in case of forest ecosystems (Canterbury et al., 2000). These statements direct the attention on the deviation of landscape assessment carried out with the help of plants and birds.

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APPLYING ARTIFICIAL MYCORRHIZAE IN PLANTING URBAN TREES

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Abstract. Urban habitats are unique and harsh environments for established plant communities, basically due to the increased stress (disturbance, pollution, drought etc.). However, ever increasing is the need for extending the green areas of the cities. There are several possibilities for urban afforestation, yet most of them require the use of chemicals that burden the soil. A feasible solution is applying artificially modified microbiota to enhance the growth and stress tolerance of the ornamental species. Mycorrhiza is mutualistic interaction between plant roots and fungal hyphae that increases the ecological fitness of both partners, especially among harsh environmental conditions. As a consequence, exploiting the advantages of this symbiosis can be a proper and sustainable strategy for establishing artificial plant communities in urban areas. In the present article we review the most important questions of urban afforestation and describe the most important general features of mycorrhizae that are related to their extended use by the humankind. Afterwards, we sum up the actual knowledge on mycorrhizae of plants living in urban areas. Then we report about the Bavarian research project "Urban Green 2021". Finally, we introduce an extensive ongoing study aiming to gain new results on this topic among Hungarian conditions.

Keywords: *urban trees, afforestation, ectomycorrhiza, endomycorrhiza, inocula*

Introduction

A brief historical review of Hungarian allées/tree lines

In the ancient times and the Middle Ages, allées were not mentioned in the European cities. Afterwards, the signs of allées can be discovered on the works of Dutch painters in the 17th century. Later, the people disengaged from the castles, so the urban afforestation began (Cérer 1928). The first allées in Hungarian horticulture were planted in the Visegrád Garden, from that time linden was the most frequently favoured tree of the country. In the Primacy Garden of Pozsony, Ágost Keresztély established a French garden (parterre, parkland and a linden tree line) on the site of a previous renaissance garden (in 1735). In the 18th century, the allées were appreciated much more than in any other centuries, therefore, further beautiful linden lines were planted (Rapaics 1932). In the 19th century several species were introduced into urban afforestation, like white mulberry (*Morus alba*), Japanese pagoda tree (*Sophora*

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japonica or rather lately *Styphnolobium japonicum*), green or red ash (*Fraxinus pennsylvanica*) and the black locust (*Robinia pseudoacacia*) from North America.

Epoches of favoured genera and species used in Hungary (Schmidt & Honfi 2006):

- Early 1900's – the epoch of sycamore (*Platanus*) and horse chestnut (*Aesculus*);
- From 1950 until the early 1960's – the epoch of white linden (*Tilia tomentosa*) and sorb species (*Sorbus*);
- From the end of the 1900's to nowadays - species of hackberry (*Celtis*) and ash (*Fraxinus*);
- The spread of the American species like Indian bean tree (*Catalpa bignonioides*), honey locust (*Gleditsia triacanthos*);
- In the following years several other species extended in the allée from East Asia, e.g. Empress tree or royal paulownia (*Paulownia tomentosa*), China tree or golden rain tree (*Koelreuteria paniculata*), maidenhair tree (*Ginkgo biloba*) and the pear (*Pyrus* species) in the present epoch (Schmidt 2003, Vajna 2012).

The first urban allée in Budapest was established near the VIGADÓ, consisting of unidentified species was watered by prisoners (1789). According to Rapaics (1934) its trees were lindens and black locusts. The second urban allée was founded near the Chain Bridge from the bridgehead of Pest (1856). This was the first urban tree line by Radó's opinion (1981), but copper engravings seem to contradict this opinion. The planted trees were *Robinia pseudoacacia* and one individual of these locusts remained even till today.

Criteria for choosing species and cultivars in brief

There are two most important viewpoints that should be taken into consideration being in close correlation with each other: the planted area and the planned plants.

Important properties of the planted area are:

- location, exposure;
- climate: particularly the effects of frost or heatwave;
- soil: pH, groundwater;
- size of planted area in view of the size of root system and the crown;
- degree to which area is built up: buildings, sidewalks, overhead wires and subsurface wires;
- air contamination;
- pavements.

Important features of the planned plants are:

- height and width of the crown;
- stability (storm injury, wind tunnel);
- tolerance against drought and the high level of groundwater;
- resistance against pests and causative agents.

Once upon a time carriage drivers thought that the only purpose of planting urban trees was decoration, giving shade without disturbing the traffic. Then a list of requirements rose, like stress tolerance, ornamental value, central leader, trunk remains straight up through the crown, optimal crown, sturdy branch system (not fragile) and even that the tree has no fruits or if it has only little ones and thermo radiation, high concentration of salt and metal finally the capability of long leafy or late defoliation.

Of course, there are no such species or cultivars that fulfils all these requirements. The principal feature should be decided by the user's group (Biza 2006). Nowadays, in the mass of asphalt and concrete the most important need is that the plant or the tree should survive the urban environment.

Overview of mycorrhizal interactions

According to the most detailed definition (Brundrett 2004), mycorrhizae are intimate mutualistic interactions between modified absorptive organs (chiefly roots) of a plant (photobiont) and the hyphae of a fungus (mycobiont) with the main purpose of nutrient transfer between the organisms. Though this broadest accepted terminology refers also for symbiotic interactions of thallic plants (mosses or prothallia of pteridiophytes), sometimes called mycothalli or paramycorrhizae (Strullu-Derrien and Strullu 2007), for the purpose of urban plantations much more important are the eumycorrhizae, hosted by vascular plants.

Based on the anatomical structure, two main types of mycorrhizae have been distinguished since as early as the first description of the interaction in the 19th century (Trappe 2005). In the evolutionarily oldest type, called endomycorrhiza (Selosse and Le Tacon 1998, Brundrett 2002), the hyphae penetrates through the plant cell wall and form intracellular structures by invaginating the cell membrane (Smith and Read 2009). In the root cells the following structures may be present: *hyphal coil*s and/or multiply branching *arbuscule*s for the nutrient transfer between the mutualistic partners and *vesicle*s for the storage of lipids (Bonfante and Genre 2008). From the root surface, extraradical hyphae run far into the neighbouring soil areas. The now described so-called vesicular-arbuscular mycorrhiza (VAM) is the most widespread mycorrhiza type (Wang and Qui 2006) occurring on the roots of more than 67 % of angiospermous (Trappe 1987) and over 80% of all terrestrial plants (Smith and Read 2009), Though the spectrum of host plants is extremely wide (Harley and Harley 1987, Wang and Qui 2006) the fungal partners belong to a single phylum, Glomeromycota (Schüßler et al. 2001, Brundrett 2002). Due to its frequent occurrence in natural ecosystems, VAM interactions have been quite intensely studied for the last decades (Koide and Mosse 2004, Triparthi and Khare 2012), including its potential application for forestry and agriculture (Peterson et al. 1984, Azcón-Aguilar and Barea 1997, Gosling et al. 2006, Gianinazzi et al. 2010).

The other large group, ectomycorrhizae (EM) are characterised by an obvious mycelial mantle covering the fine roots, from which hyphae grows into the root forming an almost exclusively extracellular interface (Hartig-net) enwrapping the cortical cells (Bonfante 2001, Smith and Read 2009). From the mantle, different emanating elements (cystidia, hyphae, rhizomorphs) derive (Agerer 1999). Though merely 4-5% of vascular plants form ectomycorrhizae (Brundrett 2002), the most important forest trees of all climatic zones are amongst them (Wang and Qui 2006), which reveals a fundamental ecological significance of the symbiosis. Mycobionts of the interaction are either ascomycetes (Maia et al. 1996) or basidiomycetes, with an estimated number of 5-6000 species (Molina et al. 1992). The list of documented EM-forming fungi was compiled by several authors (De Román et al. 2005, Agerer 2006), but the number is continuously increasing.

In addition to the above mentioned mycorrhizal types, further categories are distinguished based on special anatomical features. A transitional state between ecto- and endomycorrhizae is the ectendomycorrhiza bearing fungal mantle together with intracellular hyphal structures (Yu et al. 2001). Some mycorrhiza types of unique anatomy are restricted to certain plant taxa, such as the ericoid (Straker 1996), arbutoid, monotropoid (Zelmer et al. 1996) and orchid (Rasmussen 2002, Dearnaley 2007) mycorrhizae. In addition to the structure-based classification, mycorrhizae can be categorised on the grounds of the quality of the ecological relationship between the fungus and its host (Brundrett 2004, Egger and Hibbett 2004). In this system fungus-plant relationships form a continuum from the parasitic interactions formed by non-green mycorrhizal plants explointing their fungal partners (Taylor et al. 2002, Selosse et al. 2006) through commensalisms (represented by endophytes – Jumpponen 2001, Newsham et al. 2009) right to the clearly mutualistic mycorrhizae.

The reciprocal benefit of the mutualistic partners is manifested at different levels (Johnson et al. 2006). The basic advantage is the nutrient transfer via the interface: the mycobiont gets organic molecules from the plant (Rosling et al. 2004, Grunze et al. 2004), while owing to their extrametrical mycelia, the fungi represent an enlarged water and mineral absorptive surface for the plant (Bucher 2007, Lee et al. 2013) together with the ability to degrade and so transport the of the stored nutrients of dead organic materials (Smith and Read 2009). Furthermore, the presence of mycorrhizae protects either directly or indirectly against different forms of stresses (Nadeem et al. 2014) including both abiotic factors (drought – e.g. Di Pietro et al. 2007, Wu et al. 2013, heavy metals – e.g. Ott et al. 2002, Hildebrandt et al. 2007 or cold – Tibbett et al. 2002, Wu and Zou 2010) and pathogens (e.g. Pozo and Azcón-Aguilar 2007, Cameron et al. 2013). As a consequence, on the population level, the benefit for the partners is the increased overall fitness (Johnson et al. 2006).

The advantageous relationship between the fungal and plant communities of a certain area is hidden in the diversity of species and ecological functions (Buscot et al. 2000, Feddermann et al. 2010). In natural assemblages poor in plant species may bear their diversity potential belowground in their fungal (often mycorrhizal) microbiota (Dahlberg 2001). Mycorrhizae may be sorted into different functional groups, even within a single anatomical category, based on the nutrients mobilised (Feddermann et al. 2010) or the way how they explore their habitat (Agerer 2001). The high species diversity causing redundancy in ecological functions may stabilise the community of formed by mycorrhizal plants. Besides, high species richness enables the development of diverse ecological relationships at a certain habitat. Since the mycelium of a fungus may form mycorrhizae with roots of several individuals (Lian et al. 2006) and on the root system of a single plant may host mycorrhizae of several fungal species at a certain time, and the mycelia of certain fungi may anastomose with each other (Jakobsen 2004), plants are interconnected into a physiologically continuous system (‘common mycelial network’, CMN) (Selosse et al. 2006). The experimentally proven ecological consequences of CMN are manifold, from the nursing of suppressed (e.g. shaded) plants right to such indirect parasitic interactions as those of myco-heterotrophic and myxotrophic plants gaining organic molecules or nutrients without an equal compensation (Selosse et al. 2006, Simard et al. 2012).

As members of ecosystems, mycorrhiza and mycorrhizal fungi have profound impacts on the abiotic environment. The role of fungal mycelia (including emanating hyphae of mycorrhizae) on soil characteristics is studied by the field of geomycology (Burford et al. 2003, Gadd 2007). Mycorrhizal fungi contribute significantly to soil development (Johnson et al. 2006) by improving the soil structure via adhering soil particles (Moreno-Espindola et al. 2007, Miransari et al. 2009), mobilising insoluble nutrients from minerals (Landeweert et al. 2001, Winkelmann 2007) or dead organic matter (Smith and Read 2009) as well as taking part in the biogeochemical cycles of the elements (Millard et al. 2007, Paul et al. 2007, Veresoglou et al. 2012). Beside their effect on the soil, mycorrhizal interactions have profound consequences on the resilience of natural ecosystems against the components of the global climatic change (reviewed e.g. by Mohan et al. 2014).

Owing to their multi-level beneficial effects, mycorrhizae are utilised in a manifold way by the humankind. By preserving stress caused damages and increasing growth rate and thus the commercial yield and quality, artificial inoculation is applied by agriculture (Stützer et al. 2008, Linderman 2008, Takács et al. 2008, Gianinazzi et al. 2010), for micropropagated plants in orchards (Balla et al. 2008) and in forestry (Peterson et al. 1984, Haselwandter and Bowen 1996) as means of sustainable development (Rooney et al. 2009). Being biological agents, their application can substitute for different chemical treatments, first of all this is the reason why they can be related to sustainable development, but mycorrhizae are even more directly applied by nature conservation for recultivation (Estaún et al. 2008), as well as for *in situ* (Turrini and Giovannetti 2012) and *ex situ* plant protection (Bothe et al. 2010) and as a way of bioremediation (Khan et al. 2000).

Mycorrhizae in urban environments

Fungi of urban environments belong to different ecological functional groups living in manifold interactions with plants and animals. In addition to spores, indoor fungi (living in buildings), parasites and fungi of the phylloplane – similarly to the native habitats – significiant is the role of mycorrhizal fungi in these habitats (Newbound et al. 2010). Although the mycorrhizal status of the most important ornamental plants is included in general reviews compiling the known literature on mycorrhizal plants (e.g. Harley and Harley 1987, Wang and Qiu 2006), some publications focus straight on the colonisation of urban samples (e.g. Gáper 1989, Baxter et al. 1999, Timmonen and Kauppinen 2008, Bainard et al. 2011, Karpati et al. 2011, Karlinski et al. 2014).

The comparison of the mycobiota of rural and urban habitats revealed admirable differences between the natural and disturbed areas. In general, the decrease of species diversity and shift in species composition was observed on the level of macrofungi (Newbound et al. 2010, Barrico et al. 2012) as well as when comparing the soil microbiota using high throughput sequencing methods (Jumpponen et al., 2010). For endomycorhhizal glomeromycetes never form sporocarps, studying the EM community seems to be easier due to the presence of their sporocarps. However, it has been accepted already for decades that above-ground view cannot be exactly related to the below-ground composition, either in case of EM fungi (Gardes and Bruns 1996, Dahlberg et al. 1997, Horton and Bruns 2001). Consequently, works based on mycorrhiza samples reveal the real effects of urban disturbances. Nevertheless, trends similar to the fruitbody-based studies were gained from the mycorrhiza based studies. Almost each result indicated decreased species richness yet unchanged EM colonisation level at low disturbance (Baxter et al. 1999, Gebhardt et al. 2007, Bainard et al. 2011, Karpati et al. 2011, Tyburska et al. 2013). Interestingly, EM communities of the disturbed (i.e. urban) sites included a generally shared species group (‘Urban EMF suite’ – Del Tredici 2010 cit. in Karpati et al. 2011, Timmonen and Kauppinen 2008) in addition to the ‘site specific’ species (Gebhardt et al. 2007). From the disturbed sites, the EM species of the late successional stages were missing (Karpati et al. 2011).

The level of endomycorrhizal colonisation was found to be decreased (Bainard et al. 2011, Tyburska et al. 2013) or just slightly affected by the urban environment (Karlinski et al. 2014), while the composition of VAM-community became impoverished to different levels depending on the current land-use and land-use history (Stabler et al. 2001, Cousins et al. 2003). A general view is that EM fungi are more seriously affected in urban habitats, and they are suppressed by increasing saprotroph dominance (Newbound et al. 2010) thus VAM might be a more important symbiosis in disturbed urban areas (Bainard et al. 2011). However, it is important to note that occurrences of different fungal functional groups have a seasonal alteration throughout the year (Jumpponen et al. 2010).

Characteristic changes of the environmental factors caused by the humankind lie in the background of the observed changes in community traits. Urban sites are intensely polluted, their soil has high nitrogen depositions and it suffer regular, often serious mechanical disturbances (Baxter et al. 1999, Cairney and Meharg 1999, Egerton-Warburton and Allen 2000, Khan 2001, Polanco et al. 2008) each bearing a proven negative effect both on the root parameters (Karlinski et al. 2014) and on the fungal communities (Bernatzky 1974, Stabler et al. 2001, Bainard et al. 2011, Jumpponen and Jones 2010, Jumpponen et al. 2010, Vauramo and Setala 2010). Although mycorrhizae of natural sites also have to face different disturbances (e.g. Gehring and Whitham 1992, Byrd et al. 2000, Jones et al. 2003), their effect is less deleterious, just like in urban sites in a state close to natural (Lothamer et al. 2014). Therefore, keeping urban stands in a state similar to the native ones may be more resistant to the humankind caused stresses. A certain aspect of this is keeping the soil mycobiota as intact as possible, which can be enhanced by close natural stands that can serve as resources for fungal propagules (Cousins et al. 2003, Schaefer 2011) and from where mutualist fungi can immigrate via wind-dispersal or any other transporting agents (Stabler et al. 2001).

Beside harsh environmental conditions, a further problem of establishing urban ecosystem is the fact that quite frequently non-native species are introduced. The ability of introduced species to utilise native mycorrhizal community is proven for both EM (Tedersoo et al. 2007) and VAM (Cousins et al. 2003) hosts. Nevertheless, introduced non-native stands always had less diverse fungal communities, as it was revealed by both sporocarp (O'Hanlon et al. 2013) and mycorrhizal surveys (Cousins et al. 2003, Dickie et al. 2010, O'Hanlon and Harrington 2012). While native hosts live in mutualism with rare and more exclusive EM taxa (Lothamer et al. 2014), the mycorrhizae of introduced plants are chiefly cosmopolitan and wide-distributed fungi (Trocha et al. 2012). Nevertheless, among certain environmental conditions similar studies led to inconsistent results, for instance, non-native pine trees had higher EM sporocarp and root tip diversity compared to a native *Pinus* (Trocha et al. 2012). The decreased mycorrhizal diversity of introduced species may be traced back to diferent facts: non-native plants alter the soil microbiota (Bahram et al. 2013, Kohout et al. 2011), these plants (especially the invasive ones) might be less dependent on mycorrhizae (Pringle et al. 2009) and planting aliens means a co-invasion of their endophytic (Knapp et al. 2012) and mycorrhizal fungi at the same time (Cousins et al. 2003, Tedersoo et al. 2007, Vellinga et al. 2009, Dickie et al. 2010) that may overcompete the native species. Unfortunately, the invasion biology of mycorrhizal fungi is just rarely discussed (Vellinga et al. 2009). Besides, since urban sites are mostly dominated by non-mycorrhizal or facultatively mycorrhizal plants (Jumpponen and Egerton-Wahrburton 2005 cit. in Karpati et al. 2011), the low proportion of mycorrhizal plant species may also decrease the mycorrhizal diversity of the soil (Bainard et al. 2011). Moreover, according to some authors (e.g. Trocha et al. 2012) native trees had a longer evolutionary time to adapt to the local community. At the same time, introduced ornamental species both use the native fungal community for their survival (Cullings et al. 2000, Bahram et al. 2013), as well as their co-introduced mycorrhizae (see above). A possible solution might be if native hosts are preserved among the introduced ones, because this way a considerably high proportion of the native fungal community can be preserved (Lothamer et al. 2014), and these 'refugial' plants aid to keep up a kind of 'ecological memory' of the soil microbiota (Schaefer 2011).

In order to enhance the establishment of urban plantations, another rational idea, i.e. the use of artificial mycorrhizae, rose as early as in the 1980's (Kuhns 1980). Despite these early thoughts, even at the beginning of the third millennium this solution was only a 'rare practice' (Pauleit et al. 2002), although a conceptual decision model for predicting its success had been elaborated by then (Findlay and Kendle 2001). The background of this method is the importance of mycorrhizae in plants' resistance against urban soil disturbances (Polanco et al. 2008) and the low level of available fungal inocula in disturbed soils (Bainard et al. 2011). (However, some results debate this latter observation – Tarvainen et al. 2011.) Knowing that introduced plants may adapt to native microbiota, the mostly recommended technique is using the native fungi in the process (Chan and Griffith 1991, Fini et al. 2011). The positive effect of mycorrhization was found to be even more significant when co-inoculation of rhizobacteria was applied (Rao et al. 2006).

Although nowadays commercial products with impressive predicted effect are available on the market, and their flexibility allows the inclusion of the mycorrhizal technology to integrated plant production systems and recently has an economically growing market (Feldmann 2008). Some of them are proven to be advantageous for the ornamental hosts (Lanthier 2009), the results of studies using them are inconsistent (Gilman 2001, Ferrini and Nicese 2002, Weissenhorn and Külling 2008). The use of AMF inoculum is recently facing a highly diverse host plant spectrum and diverse substrates for specific uses at the front-end in the market. Recently, more and more positive cost/benefit relationships of mycorrhiza use lead to the development of bioproducts containing AMF (Schneider and Feldmann, 2007; Young, 2005; Gianninazzi et al, 2002) or combination of AMF and bacteria (Ravenskov and Jakobsen, 1999).

Inoculation may be accomplished either in the nurseries or in the target site. When inoculated in nurseries, the lowest mycorrhizal diversity is observed here (Rao et al. 2006), then these fungi are outcompeted by the micorrhizosphere of the urban environment for nursery mycorrhizae are not adapted to street conditions (Timmonen and Kauppinen 2008). In the long run, the most characteristic change of the mycorrhizal community of previously inoculated plants is the increasing proportion of EM of long-distance exploration type (Fini et al. 2011). When inoculating with artificial methods, it should also be born in mind that the target site itself may have the potential for infecting the trees (Appleton et al. 2003), so in such habitats, this procedure would be unnecessary (Findlay and Kendle 2001), depending on the amount of available inocula.

The exact strategy how inoculation is carried out is of admirable importance. Ortaş et al. (2004) tested several techniques to develop suitable inoculum strategies. Ortas (2008) found in the Eastern Mediterranean Horticultural Region that sometimes if mycorrhizal inoculated seedlings are not well infected with mycorrhizae spores, it may be necessary to re inoculate them during transplantation to the soil. Most of the time re inoculation seems to give a better response. But it is important to produce well inoculated seedlings. If seedlings are not infected with mycorrhiza, usually there is high mortality.

Fortunately, today we are already aware of the results of long-term monitoring studies on the level of mycorrhization and its result on physiological parameters following artificial inoculation (Ferrini and Fini 2011). These reveal that host species and site characteristics affect the speed of colonisation. When the physiological, growth and nutrition parameters were measured after artificial inoculation, all were found to be improved, in general (Stabler et al. 2001, Zandavalli et al. 2004; Kapoor et al. 2008, Ferrini and Fini 2011, Garcia et al. 2011). Pre-mycorrhization helped some tree species to establish after transplantation, and grew faster during their first season in the field. Mycorrhizal inoculated *Acer platanoides, Acer saccharinum, Celtis occidentalis, Fraxinus pennsylvanica* and *Gleditsia triacanthos* species were observed by Chapdelaine et al (2008) and after two month in the field, non-inoculated control plants generally had smaller trunk diameters than pre-mycorrhized trees and their levels of AM colonization remained lower.

As it was proven, sometimes not the growth itself was enhanced by the artificial inoculation, but physiological traits related to drought stress were ameliorated (Fini et al. 2011). The application of AMF inoculums were examined in semi-arid regions, under greenhouse conditions in Israel for a variety of ornamental crops (herbaceous plants) AMF inoculation can retain plant growth and allow overcoming potential stresses under horticulture practices (Koltai et al 2008). Some authors state that better growth and nutrient response of host plants are not explained by high mycorrhizal colonisation but by the changes in ectomycorrhizal diversity in artificially inoculated hosts (Baxter and Dighton 2001).

As a general conclusion, we can state that mycorrhizal fungi could play an essential role in soil reclamation because they help in recovering biological activity, improving physical properties of soil and mobilizing accumulated minerals (Calvet et al. 2001). Moreover, using artificial inocula is a cost-effective solution for establishing urban plantations of both native and non-native species. Since the use of excess water and fertilisers can be substituted this way, this method means less load on the environment, too (Tschirner et al 2008, Fini et al. 2011) and so applying mycorrhizae can be integrated into sustainable strategies of plant production (Varghese 2000).

Testing stress-tolerant tree species in the Bavarian research project „Urban Green 2021“

Roadside trees have to constantly face a great number of devitalizing stress factors as they are situated in a highly unnatural environment with greatly confined tree pits. During summer months roadside trees often suffer from drought and heat stress, especially at night, owing to the radiation reflection of buildings and paved surfaces. Climate change causing rising temperatures and increased drought stress during summer (see for example 2003, 2006 and 2010) as well as more frequent weather extremes is further aggravating this stress situation. There is some evidence that in our latitudes some of the traditional urban tree species will not always be able to meet future requirements (Roloff et al. 2009), as they won't fit aesthetic standards any more (e.g. *Cameraria ohridella* on *Aesculus hippocastanum*), are becoming increasingly a safety risk (e.g. *Massaria* on the London plane) or will fail completely (e.g. ash dieback disease on native *Fraxinus* species).

To broaden the presently limited repertoire of urban tree species and to find out, which species or cultivars are able to tolerate the expected future climate conditions in our cities, a long-term project has been started, testing 20 promising tree species in three Bavarian cities with different local microclimates from 2010 to 2021 (*Table 1*). When selecting the various tree species their natural habitat requirements were considered as well as their drought and frost resistance and susceptibility for diseases and pests. Also urbanistic aspects as habit and crown shape of the trees were selection criteria.

***Table 1** Test tree species/ cultivars in research project "Urban Green 2021", their associated mycorrhiza-types (AM =arbuscular mycorrhiza, EM=ectomycorrhiza) and evaluation of their winter-hardiness (1- very high frost tolerance, 2 – high f.t., 3 – limited f.t., 4 – no f.t., ? – unknown f.t.)*

Test tree species/cultivars	**Mycorrhiza-type**	**Frost tolerance "Urban Green 2021"**	**Winter hardiness Roloff et al. 2009**
Acer buergerianum	AM	3	1
Acer monspessulanum	AM	1	2
Alnus x spaethii	EM	2	1
Carpinus betulus 'Frans Fontaine'	EM	2	1
Celtis australis	AM	4	3
Fraxinus ornus	AM/ EM	1	4
Fraxinus pennsylvanica 'Summit'	AM/ EM	1	1
Ginkgo biloba (male selection)	AM	2	2
Gleditsia triacanthos 'Skyline'	AM	1	2
Liquidambar styraciflua	AM	1	3
Magnolia kobus	AM	1	2
Ostrya carpinifolia	EM	2	1
Parrotia persica	AM/EM	2	?
Quercus cerris	EM	1	2
Quercus frainetto 'Trump'	EM	?	2
Quercus x hispanica 'Wageningen'	EM	?	?
Sophora japonica 'Regent'	AM	3	2
Tilia tomentosa 'Brabant'	AM/ EM	3	2
Ulmus 'Lobel'	AM/EM	1	1
Zelkova serrata 'Green Vase'	AM/ EM	3	2

In the different cities, each tree species was planted 8-fold (in some cases only 6-fold for lack of space), altogether summing up to 460 test trees. The different study sites in Bavaria, Germany are located at Würzburg (a city with above average summer temperatures and drought periods (viticultural climate), apt to test the trees for drought and heat resistance), Hof/ Münchberg (the coldest test site influenced by continental climate with the highest number of ice days, suited to test the frost tolerance of the trees), and Kempten (at the foothills of the Alps, with a moderate climate and a high precipitation pattern). At all sites standardized soil substrates with high load bearing properties were used that are in accordance with the recommendations of the Research

Society for Landscape Developing Landscape Construction. These substrates are one-layered to prevent superficial root growth and have a high air capacity as well as a high usable water capacity. The high pore volume guarantees good root penetration into great depth. The tree pits have a standardized size of $8m^3$ and a fixed depth of 1,50m. Planting and maintenance procedures were specified according to current practice.

Increasingly, mycorrhizal fungi products are used for tree maintenance, especially when old trees are restored. There are a number of positive reports (Kutscheidt 2006), however there are hardly any experimental data available on the effectiveness of such products (but see Ferrini & Fini 2011b). Yet, considering the acknowledged significance of mycorrhizal fungi in stressful habitats (Raman & Mahadevan 1996, Feldmann 2008), the application of mycorrhizal fungi products might be particularly effective at urban sites. In this project a controlled application of species-appropriate mycorrhizal fungi preparations to half of the experimental trees was carried out during planting at all sites (*Table 1*). This should supply data to answer the question if the application of mycorrhizal fungi can alleviate the “plant shock” and promote growth and health of roadside trees. At the planting, samples of fine roots of the trees, of the soil of the tree balls, and of the pit substrate were taken to determine the initial mycorrhizal status of the nursery trees as well as the fungal spore content of the pit substrate. At one study site the mycorrhizal status as well as the mycorrhiza species composition is followed up over the years for several experimental tree species. Mycorrhiza composition is determined by morphotyping and by molecular analysis. First results are described by Herrmann et al. (2011, 2013).

Twice a year, during late spring and early fall, a screening of the experimental trees is carried out where frost and drought damages, crown vitality and health status are scored and various growth parameters are measured. In addition, assisted by the municipal garden offices of the partner cities, the phenology of the different tree species are monitored at the different sites, i.e. the calendar week of bud burst, leaf coloring and leaf fall are recorded. This allows not only to assess the risk of late frost damage but also to determine the length of the vegetation period (no. of weeks between bud burst and leaf coloring) of the different experimental tree species at the different sites.

As roadside trees have to be regularly watered during their initial growth phase after planting, data on the drought resistance of the experimental trees are not yet available. However, the pronounced winter of the past years allow to present preliminary results on the frost tolerance of the different tree species which compared to the winter hardiness evaluation of urban tree species by Roloff et al. (2009; *Table 1*). Our results led to a more negative rating of these species/cultivars. However, the evaluation of Roloff et al. (2009) is based on literature of tree species, not specific cultivars, and it does not consider frost splitting risks in its ratings. In contrast, *Acer monspessulanum, Fraxinus ornus, Liquidambar styraciflua, Magnolia kobus* and *Quercus cerris* exhibited a greater frost hardiness in this project than described by Roloff et al. More detailed results are found in Böll et al. (2014).

Introduction to the first extensive case-study on urban trees in Hungary

In Hungary tree species applicable for urban afforestation are continuously searched by improvers, maintainers, gardeners, as well as tutorial institutions. Year by year new cultivars (trademark or registered trademark) were introduced into nursery gardens, but there seems to be so many unfavourable conditions of urban environments that no species or cultivar have been found so far that fulfil all the requirements and can be applied among all environmental conditions.

In the last few years, several projects were carried out in Europe with the aim of enlarging the spectrum of tree species and cultivars applicable in urban environments. In some of these projects, several tree species were tested under urban conditions, and a set of sample plants were artificially colonised with with mycorrhizal fungi (one of such projects was introduced in the previous part). However, similar experiments on ornamental trees used in urban afforestation were not carried out in Hungary. We started a new Hungarian study in the March of 2014 with the following nine woody species, all of which may have potential roles in Hungarian urban afforestation: *Acer buergerianum, Carpinus betulus, Fraxinus ornus, Ginkgo biloba, Magnolia kobus, Platanus sp., Sorbus borbasii, Tilia tomentosa, Ulmus pumila*. The main criterion for the selection was the proper tolerance against polluted urban condition. We used one-year-old seedlings obtained from different nurseries and forestry gardens. In our study, mycorrhizal products available in Hungary (licensed or not yet) are tested in a half-year-long run on 75 specimens of taxa inoculated with ectomycorrhiza and 120 specimens with either only endomycorrhiza or both with ecto- and endomycorrhiza.

The main question of the study is the effect of mycorrhizal treatment on different parameters of the species examined. Three and six months after inoculations, the degree of colonization in different experimental lines and different species will be examined. We will evaluate the degree of rooting, sprouting/shooting and compare the phenological features of different treatments. In our observation we will take root samples of examined taxa from nursery gardens, forestry gardens and old trees in urban conditions and the aim of our experiments is to determine changing quality and diversity of mycorrhizal fungi during plant developing and planting from one site to the other among different climatic and soil conditions. On the other hand, we are also interested in the colonisation pattern of mycorrhizal ornamental plants in the Hungarian nursery gardens.

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EFFECT OF LIVESTOCK ON THE GROWTH OF ROSA RUBIGINOSA IN A MOUNTAIN RANGE: A DENDROCHRONOLOGICAL APPROACH

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Abstract. In the present study, we used dendrochronological tools to determine the effect of domestic livestock on the cumulative radial growth of *Rosa rubiginosa*, a shrub native to Europe that has been introduced to the mountains of central Argentina. We described *R. rubiginosa* growth rings, we built a ring width chronology using 18 stems from a homogeneous site, and finally compared rings width in 40 individuals distributed in areas with and without livestock but otherwise similar. Our results demonstrated the negative effect of livestock on the cumulative radial growth of invasive species *R. rubiginosa*. In this sense, the livestock would be acting as a biotic filter for the invasion of this species in the mountains of Córdoba (Argentina) and may be considered as a tool to reduce *R. rubiginosa* growth in invaded areas, where livestock will not cause other damages. We consider that the measurement of annual ring width against datable basal stems represents a simple method for the determination of radial growth rates. This approach could be particularly useful for monitoring the effects of land management practices on the invasion of woody species.

Key words: *Dendroecology, invasive species, radial growth rate, Argentina.*

Resumen. En el presente estudio se utilizaron herramientas dendrocronológicas para determinar el efecto del ganado doméstico sobre el crecimiento radial acumulado de *Rosa rubiginosa*, un arbusto nativo de Europa que ha sido introducido en las Sierras del Centro de Argentina. Se describió los anillos de crecimiento de *R. rubiginosa*, se construyó una cronología con el ancho de los anillos utilizando 18 fustes de un sitio homogeneo y finalmente se comparó el ancho de anillo en 40 individuos distribuidos en áreas con y sin ganado. Nuestros resultados demostraron el efecto negativo del ganado sobre el crecimiento radial acumulado de la especie invasora *Rosa rubiginosa*. En este sentido, el ganado estaría actuando como un filtro biótico ante la invasión de esta especie en las Sierras Centrales de Córdoba (Argentina) y puede ser considerado como una herramienta para reducir el crecimiento de *R. rubiginosa* en áreas invadidas, donde el ganado domestico no cause otros daños. Consideramos que la medición del ancho de anillo anual representa un método simple para la determinación de las tasas de crecimiento radial; en conclusión, se sugiere la utilización de la dendrocronología como una herramienta particularmente útil para monitorear los efectos de las diversas prácticas de manejo del suelo sobre la invasión de especies leñosas.

Palabras claves: *Dendroecología, especie introducida, tasa de crecimiento radial, Argentina.*

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Introduction

Biological invasions are considered a key driver of global environmental change (Vitousek et al. 1997; Sala et al. 2000; Castro-Díez et al. 2004). There is a growing number of studies reporting impacts of alien plants on recipient ecosystems, like alteration of hydrological and nutrient cycles, soil properties (Mack et al. 2000; Figueroa et al. 2004; Lake and Leishman 2004), as well as reduction of fitness and growth of resident plants (Vilà et al. 2011).

To be an invasive species, the introduced species must overcome the abiotic and biotic filters offered by the recipient ecosystem. Theses filters act differently on the different stages of the invasion process (colonization, establishment, etc.), in turn, affecting differently the different stages (reproduction, dispersion, survival, etc.) of the life cycle of the introduced species (Theoharides and Dukes 2007). Biotic filters are barriers to invasion created by the action or presence of living organisms. While biotic filters will not necessarily prevent the germination of seeds or the spread of introduced species, these filters can affect survival, growth, and reproduction (Theoharides and Dukes 2007).

In this context, livestock can act as a biotic filter delaying the growth of the introduced species (Theoharides and Dukes 2007), whether directly by browsing the aerial tissues or indirectly by effects of soil compaction and erosion. Conversely, many authors have reported that livestock can favour the introduced species' growth by reducing intense competition for light, nutrients, and water (Davis et al. 2000; Hierro et al. 2005). Given that no clear patterns have been found on the effects of livestock on the invasion rate of plant species, we consider necessary to study the effects of livestock grazing on the invasion process before deciding whether to use or not to use livestock grassing as a tool to manage plant invasions.

As a general rule, plant growth is modulated by external drivers like climate, topography, soil properties, disturbance degree, etc. (Morales Tejada 1993; Schweingruber 2007). The growth rate can be reliably estimated through repeated measurements of morphometric variables (crown diameter, total height, etc.) or by the application of dendrochronological techniques. In general, dendrochronological techniques can be used to study the annual ring growth of plant species, including trees, shrubs, dwarf shrubs, and herbs (Schweingruber 2007). A lot of work has been conducted on tree species, but little is known about other species which have been taken into account only in the last decades (Schweingruber and Poschlod 2005; Winchester et al. 2007).

Dendrochronology is a method of dating based on the analysis of ring patterns (Avilés et al. 2007), and represents a powerful tool to study the ecology of biological invasions.

The species *Rosa rubiginosa* L. known as sweet briar is native to Europe and has been introduced to several countries in the southern hemisphere, including Argentina (Damascos 1992; 2008). This opportunist shrub is found in disturbed environments with altered vegetation structure and available light increases (Damascos and Gallopin 1992; Zimmermann et al. 2011). Early studies have reported that *R. rubiginosa*, like other species of the genus *Rosa* (Schoch et al. 2004), presents well marked growth rings (Zimmermann et al. 2011; 2012).

To date, in Argentina, *R. rubiginosa* has been cited as an invasive plant in Patagonia and in the mountains of central Argentina. In the first region, this species is considered an aggressive invasive plant because it forms impenetrable mats occupying large areas and thus out-compete native species (Damascos 1992; Bran et al. 2003; Aguirre et al. 2009). In the central Argentina, *R. rubiginosa* is invasive in the sense that it replaces natural plant communities (Richardson et al. 2000), but it is not as widespread as it is in Patagonia. The limited distribution of the species in central Argentina in comparison to Patagonia is probably due to its comparatively later introduction rather than to less favourable abiotic or biotic conditions (Zimmermann et al. 2010). However, little is known about the factors that influence *R. rubiginosa* growth and distribution in the mountains of central Argentina.

The objective of this study is to determine the radial growth rate of the shrub *Rosa rubiginosa* in sites with and without livestock in the mountains of central Argentina. This work represents part of a regional effort to identify the factors that influence the process of invasion of alien woody species.

Methods

Study area

The study was performed in the Córdoba Mountains (900–2800 m a.s.l.) in central Argentina (*Fig. 1*) which extend for over 200 km in an N-S orientation. At its upper ranges the vegetation consists of a mosaic of tussock grasslands, grazing lawns, outcrop communities, *Polylepis australis* woodlands and eroded areas with exposed rock surfaces (Cingolani et al. 2004). The mean temperatures of the coldest and warmest months are 10.9 and 14.5ºC (at 1700 m a.s.l.), respectively, with no frost-free period. Mean annual precipitation is 895 mm, with most rainfall concentrated in the warmer months from October to April.

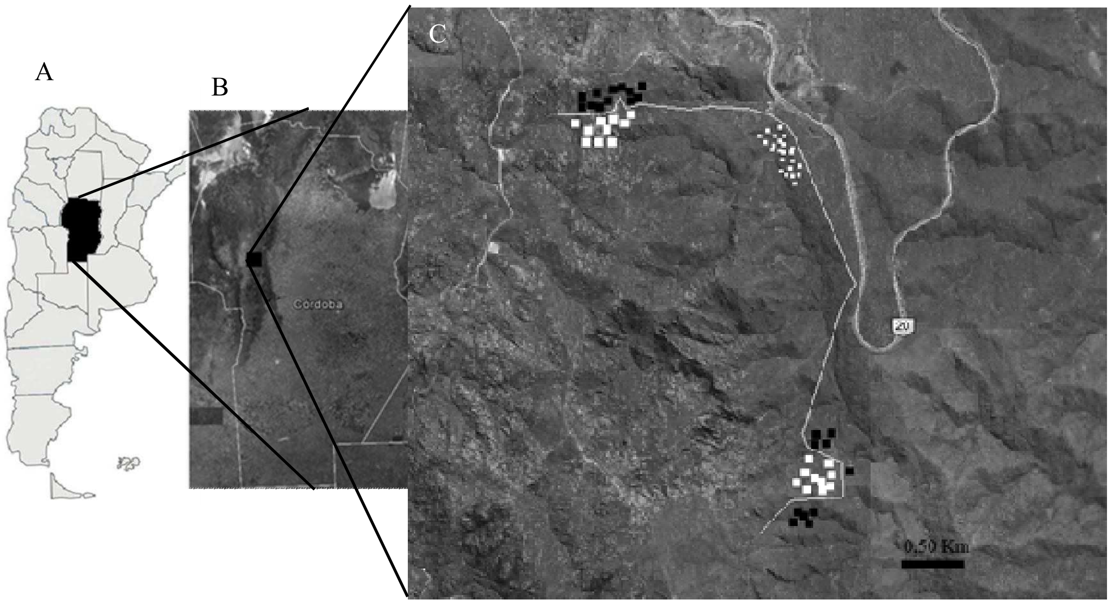

Figure 1.*(A) Location within Argentina of Province of Córdoba (in black) where mountains of central Argentina are located. (B) Córdoba province and the study area indicated by a square. (C) North-East section of "Quebrada del Condorito" National Park, where dots represent samples for the different treatments: reference chronology (small white boxes); sites without livestock (big white boxes) and with livestock (big black boxes). The continuous line indicates the limits of the National Park.*

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The main economic activity in the area is extensive livestock rearing, which together with incidence of fires ignited to promote pasture re-growth, have caused serious problems of erosion and woodland degradation (Cingolani et al. 2003; 2004; Marcora et al. 2008; Renison et al. 2010). In 1997, the creation of the National Park "Quebrada del Condorito" (hereafter "National Park") determined a substantial change in livestock management regimes, with great portions of livestock exclusion inside the National Park and grazing with moderate to intense stocking rates outside the National Park (Giorgis et al. 2010). In the sites with livestock, the predominant plants are graminoid or herbaceous creeping species like *Muhlenbergia peruviana*, *Alchemilla pinnata*, *Carex fuscula* and *Relbunium richardianum*, while sites with livestock exclusion have greater horizontal and vertical structural diversity predominating tall grasses like *Deyeuxia hieronymi*, *Poa stuckertii* and *Festuca tucumanica* (Nai-Bregaglio et al. 2002; Cingolani et al. 2003).

Sample collection

The *R. rubiginosa* samples were collected in NE sector of the National Park (1600m a.s.l., 31°37′15.0′′S and 64°40′62.1′′W), which concentrates the highest density of adult individuals in the area (Calamari and Calamari 2009). In the field, the individuals were selected haphazardly, attempting to choose the larger plants so as to obtain a longer time span. The stem with the largest circumference of each selected individuals was cut at ground level. The stems′ cross sections were allowed to air-dry for ~20 days and we then sanded them sequentially with 80 to 320 grit sanding belts. Each ring was dated to the year of formation, in accordance to Schulman convention for the southern hemisphere and using a binocular magnifier with a 40X magnification power (Stokes and Smiley 1968). Next, the transverse sections were scanned using a high resolution scanner (1200 ppp). The program Image Pro Plus 4.5 (Spring 2011) was used to determine the ring widths with a precision of 0.01 mm. The samples that presented greater degree of eccentricity or difficulty in delineating the growth rings were measured between two and four radios in each cross section.

Ring width chronology

To verify the annual formation of growth rings we built a reference chronology of *R. rubiginosa* using, 20 *R. rubiginosa* individuals from within an exclosure area (*Fig. 1C*). The samples were processed as previously described and cross-dated by visual and cross comparison of 32 series from 18 stems. Parts of the analyzed series were excluded due to lack of good cross-dating. The quality of this cross-dating and the chronology was verified by means of the COFECHA program (Holmes 1983). Similarities between the chronologies were evaluated using conventional dendrochronological statistics: correlation coefficients, mean sensibility, standard deviation and autocorrelation (Swetnam et al. 1988; Delgado 2000). To build the reference chronology, width values of the 32 series were averaged for each year ring. So, the obtained chronology represents average annual variations in radial growth of *R. rubiginosa* in the study area.

It was not necessary to standardize ring width series due to the reduced number of years of the temporal series studied and because there was no clear growth trend (Pers. com. Fidel Roig).

Effect of livestock on radial growth

In order to evaluate the effect of livestock on the growth rate of invasive *R. rubiginosa* populations we identified two sites where plants were found in adjoining fields in and out of the National Park (*Fig. 1C*). In each site, we haphazardly chose 22 samples, 11 within the National Park (without livestock) and 11 in the adjoining fields (with domestic livestock), totalling 44 samples. Livestock in adjoining fields consisted mainly of cattle, but also a few horses, at densities of around 0.8 cattle equivalent/ha. The samples were processed as previously described; they were measured between 2 and 4 radios in each transverse section. Then, radios were averaged to obtain a reliable series for each cross section. The reference chronology was used to verify the assigned calendar years in each measured series.

To compare the radial growth rate in sites with and without livestock we performed a repeated measures analysis of variance, where radial growth of year 1 through 7 was the within subject response variable, and livestock presence was the predictor variable with two categories (presence and absence, with 20 and 22 samples respectively). We did not include study site in the model after checking that there were no differences in cumulative radial growth between the two study sites. The age of seven years was selected as a cut point to include a plant in the study because few plants were older than this age and including them would lower sample size even further. We also tested at what age cumulative radial growth became significantly different with and without livestock by performing a Tukey test. The assumptions of normality and homogeneity of variances were corroborated and significant level was determined at $P \leq 0.05$.

Results

Characteristics of the Rosa rubiginosa`s wood

The transverse sections of a basal portion of the stem of *R. rubiginosa* clearly showed distinct anatomical elements (*Fig. 2*). The pith and radios were relatively large and well defined. The annual growth rings were marked, with circular to semicircular porosity (Esau 1982). The vessels were perfectly aligned and the major diameter was located in the early-wood, but they decreased in size and density as they entered the zone of the latewood. Moreover, the vessels were found to be progressively more solitary and dispersed at the end of the growing period. The analyzed samples showed a concentric radial growth during the first years, with increasing eccentricity at later ages. The limits set between two vegetative periods were unclear only, when the transverse sections with high degree of eccentricity showed stems with incomplete rings. In general, we obtained few samples with false rings (22% of the analyzed samples) and, we did not find cases with absent rings.

Chronology ring width of Rosa rubiginosa

The reference chronology covered a period of 14 years (1997-2010) and was based on the average of the annual growth rings of 18 individuals (*Fig. 3*). Ring width had an average value of 1.16 mm (EE ± 0.73) ranging between 0.06 to 4.40 mm. We confirmed similarities in ring patterns by the cross-dating of 32 series of *R. rubiginosa* stems growing within the exclosure (National Park). The statistics used to measure the quality of the chronology indicate a common signal in the interannual variations of radial growth among the individual samples that integrated the chronology. The chronology showed a high (r-bar=0.76) correlation among the 18 stems. The mean sensitivity of standardized ring width indices for standard chronology was 0.52, indicating high variability in growth rings between consecutive years. The autocorrelation index was not significant (0.32 $p > 0.01$), therefore the ring width in a particular year could not be related to the width of previous years.

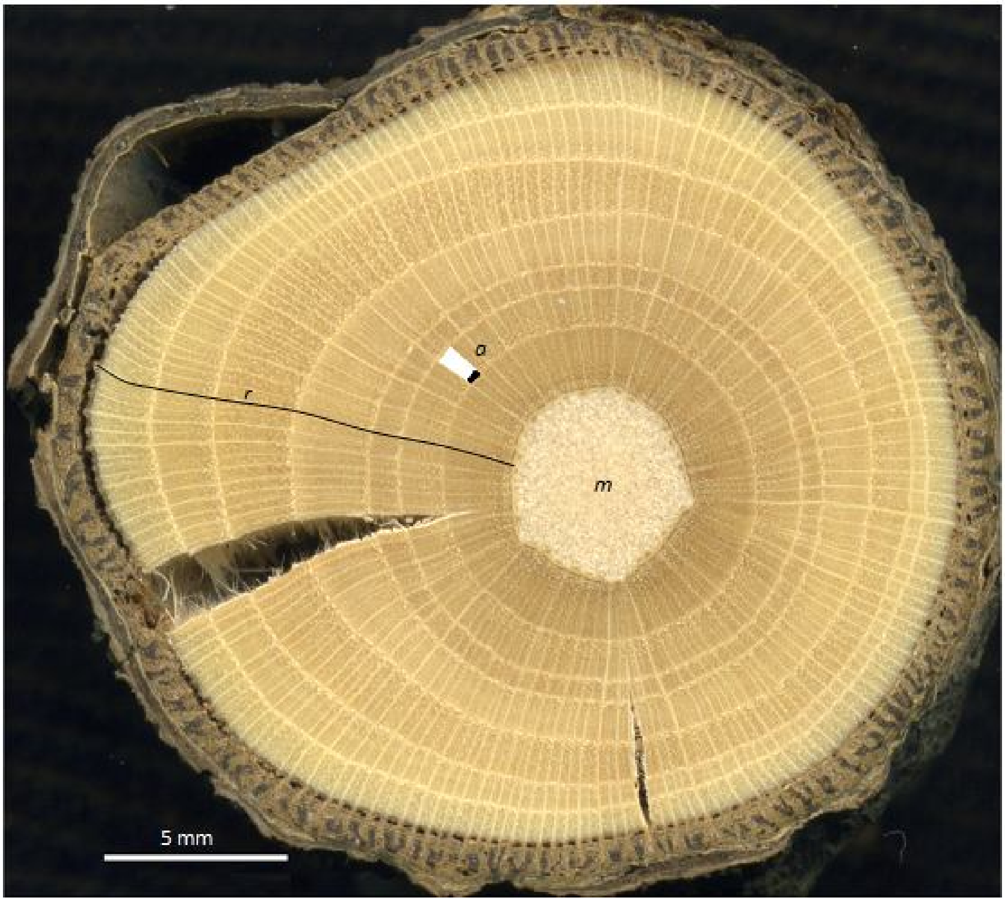

Figure 2. *Cross section of Rosa rubiginosa stem. The letters indicate: a, growth ring including early-wood (black) and latewood (white); r, radio; m, pith.*

Effect of livestock on radial growth rate

The oldest stem was 13 years old and was found in the area with livestock, while the youngest stem, 4 years, was found in the exclosure area. For plants seven years old or older (N =12 without livestock and N = 10 with livestock), ring growth was smaller in sites with livestock than without (average for the 7 years, 0.92 ± 0.06 mm/year and 1.16 ± 0.06 respectively, repeated measures ANOVA: $F_{0.05,\ 1,\ 20} = 7.94$; $p = 0.01$). The difference in ring growth between livestock conditions was similar for every age except at the 4th and 7th years when the differences were almost zero (< 0.1 mm; *Fig. 4A*). Differences with and without livestock in cumulative radial growth were not significantly different when comparing plants of ages 1 and 2 years ($p = 0.99$ and 0.26 respectively) and became significant at age 4 and thereafter up to age 7 ($p < 0.01$ in all cases; *Fig. 4B*).

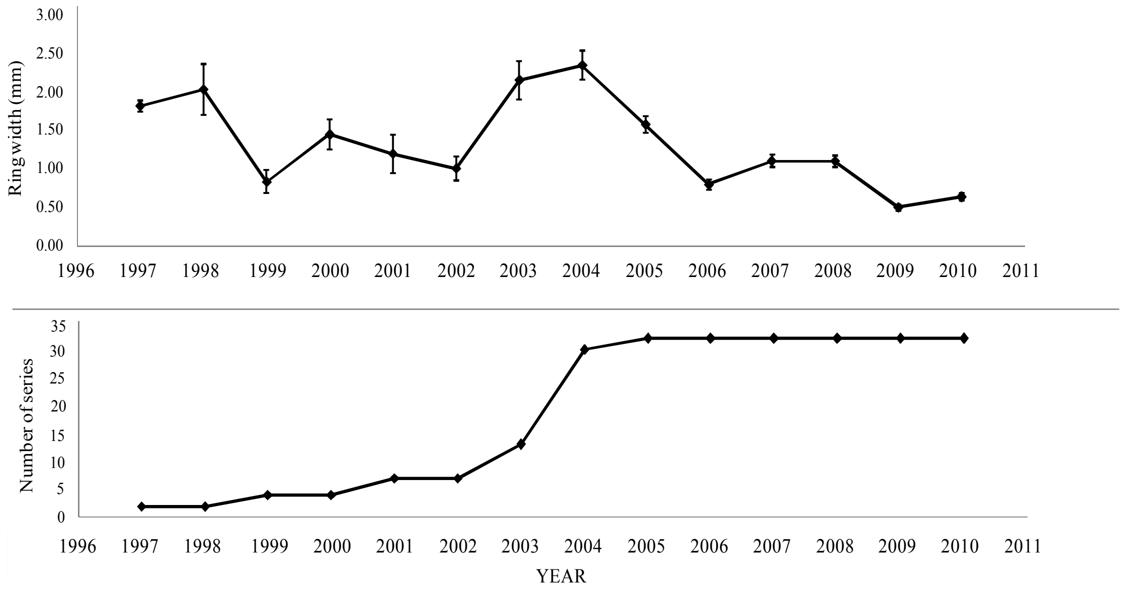

***Figure 3**. Shrub-ring chronology based on mean width of growth rings (upper graph) and corresponding number of samples (lower graph) for the Rosa rubiginosa growing within the National Park Quebrada del Condorito, Córdoba. Vertical bars represent standard error.*

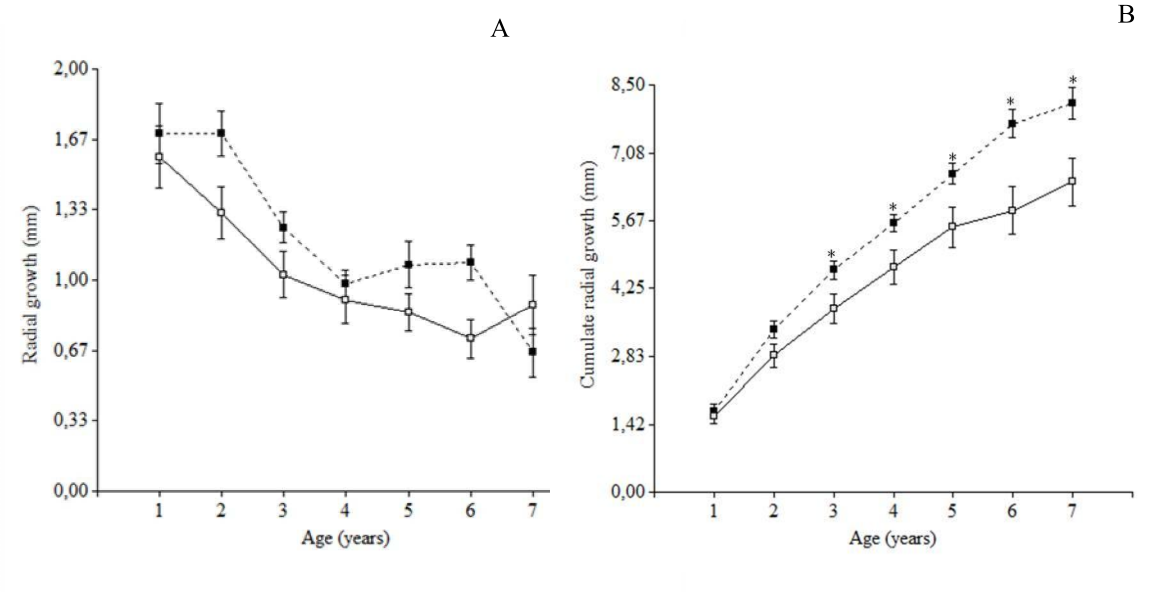

***Figure 4.** Radial growth (A) and cumulate radial growth (B) of Rosa rubiginosa in relation to age, in sites without (continuous line) and with (dotted line) livestock in the mountains of central Argentina. * Statistical differences at P≤ 0.05.*

Discussion

Characteristics of the wood and ring width chronology

The growth rings of *R. rubiginosa* were clearly defined, with circular to semicircular porosity and usually solitary and scattered vessels (*Fig. 2*). This distribution pattern of

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the vessels has been found in other species of *Rosa*, like *R. canina* (Schoch et al. 2004) and *R. glauca* (Zhang et al. 1992). Generally, the cross sections showed concentric increments in radial growth in the juvenile stage, changing gradually towards an eccentric growth with increasing age. This could be explained by light competition between stems or neighbouring plants.

The analysis of the transverse sections of *R. rubiginosa* presented an accurate chronology based on annual ring width measurements of 18 individuals selected from the ungrazed (exclosure) area (*Fig. 3*). The synchronization of growth pattern of *R. rubiginosa* was revealed by the high intercorrelation value, indicating the annual formation of growth rings. On the other hand, the mean sensitivity was high in relation to other species that grow in limiting environments of South America like *Polylepis pepei* from Andean highland (0.30, Roig et al. 2001) and *Margyricarpus pinnatus* from semiarid steppe of Patagonia (0.30, Chartier et al. 2009). The mean sensitivity represents a measure of the effect of environmental conditions on radial growth which varies annually. Seasonal and yearly fluctuations in precipitation and temperature are the most common factors correlated to growth in sensitive species (Swetnam et al. 1988). However, in our study we did not find significant correlations between width ring and climatic variables. This could be explained by the short period covered by the chronology (14 years). Similarly, the reduced chronology probably explains why *R. rubiginosa* did not present a growth trend as a function of age.

Trees and other plants with annual growth rings (including dwarf shrubs, shrubs, and herbs) can play a key role in many dendroecological studies (Schweingruber and Poschlod 2005; Bär et al. 2006). In this sense, except for long-lived tree species, plants other than trees have been largely ignored by dendrochronologist around the world (Winchester et al. 2007). However, plants other than trees have been taken into account in certain studies in the last decades, like the shrubs *Empetrum rubrum* (Roig 1988) and *M. pinnatus* (Chartier et al. 2009) from Patagonian steppe Argentina, and *Cistus ladanifer* evergreen species from European Mediterranean system (Patón et al. 1998). Thus, our results emphasize the dendroecological potential of shrubs and sub-shrubs species, especially in those areas where the presence of trees is scarce or absent, or where the shrubs are dominant.

Effect of livestock on radial growth

The results of our study show that livestock has a negative effect on radial growth of *Rosa rubiginosa*. While in the early years cumulative radial growth of *R. rubiginosa* was similar in sites with and without livestock, these differences increased with age and were significantly different at three and thereafter even with the small samples sizes we used (*Fig. 4*). Reasons for the negative effect of livestock on *R. rubiginosa* growth could be due to browsing, soil compaction and erosion, and the elimination of neighbouring competition which in harsh mountain environments may facilitate the growth of woody species acting as nurses.

A small percentage of our study plants had signs of browsing (authors personal observations), highlighting the low preference of livestock for this species at least with the low stocking rates found at our study site (as reported for *R. rubiginosa* in the Swiss Jura Mountains by Smit et al. 2007). The high density of thorns in aerial tissues of *R. rubiginosa* is probably the reason for these low browsing rates (Damascos 2008). Even though tissues lost to browsing are relatively low, given the harsh mountain environment of our study area, these losses could be contributing to the reduction on ring growth in our study plants. Livestock could also be affecting the radial growth rate indirectly through trampling and impoverishment of the soil surface conditions (e.g., infiltration, soil and nutrient loss by erosion). Due to cold dry winters the soils of mountains of Central Argentina are especially vulnerable to erosion and sensitive to the pressure of livestock hooves which compacts soils (Cingolani 2008; Renison et al. 2010). A reduction in fitness due to the effects of livestock on soil properties was shown on native shrubs and trees of *Polylepis australis* in our study area (Renison et al. 2004) and thus could also be affecting *R. rubiginosa*.

Finally, livestock had been excluded in the National Park for 15 years at the date of our study, with a consequent partial restoration of native vegetation cover and structure which could be facilitating *R. rubiginosa* growth. In stressful mountain environment like in our study area, plants can have positive effects on each other as was found for *P. australis* seedlings in our study area (Landi and Renison 2010). This facilitation by neighbour plant cover can be due to accumulation of nutrients, provision of shade, amelioration of wind or extreme temperatures (Callaway et al. 2002). Thus facilitation by existing vegetation could also be contributing to the difference found in radial growth in our study species with and without livestock sites.

In that way, the livestock would be acting as a biotic filter (Theoharides and Luke 2007), retarding to a certain extent the growth of invasive species *R. rubiginosa* in mountains of central Argentina and consequently retarding the invasion processes. As livestock is also known to favour *R. rubiginosa* by dispersing Rose hips (Vila et al. 2011), the outcome of livestock introduction in areas invaded with *R. rubiginosa* will depend on the balance between its negative effects on growth and its positive effects on dispersal, which is possibly context dependant. As an example, livestock will possibly be a useful tool for retarding the invasion processes in areas where there is no limitation by dispersal, i.e. the last remnant patches of native vegetation (tolerant to grassing) in densely invaded areas. Many studies have demonstrated that during the stage of severe invasion, eradication or control is economically unviable, producing irreversible environmental damage (Hulmes 2006), thus these sites could be used for livestock grazing if this slows down the invasion. In this context, the measurement of annual ring width against datable basal stems represents a simple method for the determination of radial growth rates which may be used by government departments, environmental managers and conservationists to help resolve the diversity of invasive alien species problems. This approach could be particularly useful for determining the effects of land management practices on recent invasion of woody species where historical data regarding this process are scarce or absent.

Conclusion

Our study demonstrated the negative effect of livestock on cumulate radial growth of invasive species *Rosa rubiginosa*. In this sense, the livestock would be acting as a biotic filter for the invasion of this species in the mountains of Córdoba (Argentina) and may be considered as a tool to reduce *R. rubiginosa* growth in invaded areas, where livestock will not cause other damages.

Moreover, our study highlights the usefulness and importance of the application of dendrochronology to understand the woody invasion processes, emphasizing the dendrochronological potential of shrub and sub-shrub species. This approach could be particularly useful for monitoring the effects of land management practices on recent invasion of woody species.

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RURAL REGIONS WITH DIFFERENT LANDSCAPE FUNCTIONS: COMPARISON ANALYSIS OF TWO PILOT REGIONS IN HUNGARY

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Abstract. Nowadays a popular research field is the use of indicators, especially landscape indicators for the analysis of landscape functions. We have elaborated 13 complex indicators in 6 groups to compare the level of different landscape services and explore the relation between landscape use and economic situation in two rural regions of Hungary. We have chosen rural regions with different natural and economic conditions. The level of regulation and supporting functions are reflected by the group of indicators of environment and biodiversity. Habitat function was analyzed by the rate of protected areas. For the assessment of the visual and cultural value we analyzed the naturalness and diversity of the landscape and cultural heritage. The provisioning services cover mostly the cultivation of the landscape; we assessed intensity of agriculture, forestry and tourism. As an additional analysis we assessed the availability and economic conditions of the pilot regions. The results have shown interesting correlation between the level of landscape functions, availability and economic situation of the analyzed regions. Based on our researches we found that in course of landscape function analysis as a base of rural development it is important to analyze not just the relevant functions but the connections between them, since they may act differently on each other in different situations. While in case of regulation, supporting and habitat function values of Gönc micro-region exceeds Csorna, the values of availability and economic situation Csorna has higher values.

Keywords: *Landscape function; Ecosystem services; Landscape management; Sustainable rural development; Micro-region of Csorna, Gönc*

Introduction

Always more attention is paid on rural development and landscape management. In our study we apply landscape function analysis using complex landscape indices to indentify the most appropriate landscape management guidelines and rural development policies. The terms of ecosystem services and landscape functions became frequently used terminologies within the scientific community. The most important difference in the approach of the terms is rooted in the field of ecology and was originally designed for the assessment of (semi-)natural ecosystems (Costanza et al. 1997). Meanwhile the approach to landscape functions came principally from landscape ecology and planning (Bastian et al., 2006; De Groot and Hein 2007).

Ehrilch and Ehrilch (1981) were the first to mentioned ecosystem services and COSTANZA et al. in 1997 assessed the economic value of ecosystems. Up till now the most detailed, extensive scientific study was elaborated by the Millennium Ecosystem Assessment program initiated by the UN in 2001 (MEA 2005). In the frames of the

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program researchers analyzed the relation of social wellbeing and ecosystem services. There is a vast literature about the theory and application of ecosystem services. There are a number of options to group assets offered by nature, very often the next being an advanced version of the previous one, thus so much similar to one other (de Groot, 1992, 2006; Costanza et al., 1997; MEA, 2005). In case of four groups of services there is a consensus of opinion: provisioning services (food, timber, etc.), regulating services (climate control, water purification, etc.), supporting (soli formation, nutrient cycling) and information services (recreation, education, etc.). These services can be described in terms of carrying capacity and spatial needs (minimum critical ecosystem size) of the natural ecosystems which provide them. Provisioning functions mean many resources of the nature for human use (food, raw material-fiber, timber, etc.). Information functions cover all the services related to recreation, reflection, cognitive development and aesthetic experience. Finally de Groot distinguishes carrier functions which include cultivation, habitation and transportation. This group of services includes all the human activities which converse the original ecosystem. Naturally there are overlaps and interactions between the different functions, for example the carrier function also offer other goods such as cultivated landscape maintain regulation services and have aesthetic qualities, etc. (de Groot, 2006).

The term of landscape functions has been developed parallel with the term of ecosystem services in other scientific field. This concept as an integrative framework establishes the connection between natural, economic, social sciences and policy sectors. So landscape services are more often defined as ‘the capacity of the landscape to provide goods and services that satisfy human needs, directly or indirectly’ (Willemen et al., 2010). Bastian (1997) grouped landscape functions according to the three pillars of sustainability, as, production (economic), anthropocentric (society) and regulatory (ecological) functions.

In the model of landscape functions Brandt and Vejre (2004) distinguished land use functions referring to material processes connected with land use. Lamarque et al. (2011) highlights the fact that a clear demarcation between landscape functions and land use functions is not possible. According to Lamarque there are four types of services, which mostly overlap with each other: ecologic services, ecosystem services, landscape functions, land use functions. Demarcation is possible along the following dimensions: service provider (species/population, ecosystems/habitats, land use/land cover) goal (biodiversity protection, multifunctional landscape), scale (global, regional, local).

The foregoing shows that the two concepts are formed from different scientific fields, using different terminology, but carrying the same meaning (Schlößer et al., 2010). For the non ecologic scientific fields is more attractive to use the term of “landscape” than “ecosystem”, furthermore term of landscape function is harmonizing with the habitat of people, the living environment (Hermann et al. 2014).

The landscape functions are provided by the biological and landscape diversity so it is inevitable to maintain the biodiversity and to turn back the unfavorable processes. It is particularly true for the rural landscapes which are the most important food production places of the humankind, which next to the production function fulfils important social/cultural and landscape/nature protection functions as well (EEC, 1992).

In European context landscape management means: "...action, from a perspective of sustainable development, to ensure the regular upkeep of a landscape, so as to guide and harmonize changes which are brought about by social, economic and environmental process" (Council of Europe 2000, Chapter I, Article 1, e). In spite of the comprehensive, international interpretation of the concept, landscape management has different meaning for researchers, decision makers, planners (Gobster, 1999).

In Hungary especially among researchers in the field of agricultural sciences is landscape management popular mostly with the expression of environment and landscape management. According to Belényesi (2008) the most important feature of the concept is to strive for activities, management forms fitting local conditions meanwhile taking into consideration the economic, social, environmental and regional functions of the landscape. Ángyán (2004) emphasized environmental and landscape management is equivalent with "multifunctional" agriculture, where next to the traditional production function environmental, social, employment and cultural tasks also come up. For landscape architects and planners according to the interpretation of Csemez (1996) it means harmonizing the objectives of landscape reclamation nature, landscape and environmental protection and sustainable regional development and fitting them into the system of landscape elements. In our interpretation in the frames of landscape management such system of different land use forms should be maintained which helps the harmonious functioning of the landscape, ensures on the long run the sustainable functioning of landscape functions furthermore strives to meet the current needs of society Filepné Kovács (2013).

In rural regions for sustainable landscape management and rural development is extremely important to analyze the level of landscape functions as in rural regions people still live from the goods of the landscape (agriculture, tourism), or people have chosen rural settlements for living because of the quality of the environment.

As pilot areas we have chosen two rural regions. According to the classification of the OECD and EU, both of them belong to predominantly rural areas (more than 50% of the area's population lives in (rural) communities, where the density of population is under 120 persons/km^2) (OECD, 2007).

Based on the typology used in the European Union 96% of the territory of Hungary is classified as rural areas accommodating 74,5% of the population. The ratio of predominantly rural areas is 58,3%, with a proportion of 31,3% in the total population, which is 3,2 times higher than the EU average (9,7%).

Rural areas mostly offer healthy living environment suitable for rest and recreation, generally have rich, although rundown landscape, architectural and cultural heritage. Public utilities, infrastructure and services are usually worse than in urban areas and the transportation and access systems are extremely bad. The general income rate is lower, rate of unemployment is higher, population is aging, high rate of migration etc. Both pilot areas have negative demographic tendencies.

We consider landscape function analysis highly important for the sustainable development of rural regions which make up the majority of Hungary and also of Europe. For our research we formed the following objectives:

- To compare the environmental and economic situation of pilot areas (regional analysis)
- To detect determinant landscape indices to reflect the level of landscape functions
- To compare the landscape functions within the pilot areas
- To explore the relation between the level of landscape functions and use of the landscape and the economic conditions of rural areas
- To highlight the importance of landscape management in case of rural development

Methods and materials

Following the concept of landscape functions, we elaborated complex landscape indicators to compare the level of different landscape services and explore the relation between landscape use and economic situation of the analyzed regions. For the assessment of landscape functions we used wide range of complex indicators (13 different indicators in 6 groups). The used landscape indicators mostly cover all types of landscape services from the most important classification systems (*Table 1*). We used the data of the Hungarian Statistic Office, and other types of databases (Spatial planning and development Information System – TEIR, database of national monuments, database of landscape values – TÉKA, nature and environmental protection databases – TIR) for the evaluation of the level of the landscape services and characterization of settlements and sub-regions.

The level of regulation and supporting functions are reflected by the group of indicators of environment and biodiversity. For the assessment of environmental integrity we adopted the method elaborated by Koschke (2012) and his fellow researchers in. This method reflects a multi-criteria approach, based on the land cover data (Corine Land Cover data base), experts assessed all landscape functions of different land use forms. The result is a schematic, map based analysis, enabling the relative simple and easy assessment of greater territorial units as well.

Biodiversity in cultivated landscape is mostly defined by the diversity of different land use forms which is reflected by the Shannon diversity index. The diversity of the land use system is measured by the Shannon diversity index which measures the type and size of different land use forms. The value of a diversity index increases when the number of the land use types increases and when evenness increases.

Due to the decades-long traditions of nature protection there are wide scale of data available so we assessed the ratio of nature protection areas of national importance, proportion of Natura 2000 areas, Ramsar sites and Biosphere reserves furthermore the proportion of National Ecologic Network (special tool of landscape protection in the spatial plans with building restrictions mostly NET areas include all other protected areas and other areas of ecologic value). The same factors appear in different indicators (Natura 2000 for example), but this does not modify the relation between them, neither the results since the whole system is relative.

In most classifications there are landscape services distinguished related to the non-material goods offered by the landscape (Anthropocentric services, Culture and amenity etc.). The assessment of the visual value of the landscape is a very difficult question. Several methods, indicators were elaborated for the assessment of visual diversity applied on different spatial level. A few indicators for visual assessment: openness and closeness (OECD, 1997), visibility (USDA, 1995), tranquility (Swanwick, 2002), land use patterns (OECD, 2001), complexity (Kaplan et al., 1989), variety (Daniel, 2001). From our point of view the quality of the landscape scenery highly depends on naturalness (Clay and Smidt, 2004) and diversity (EEA, 1998).

In our analysis the indicator of naturalness is calculated on the base of proportion of extensive land use forms. The classes of CORINE Land Cover were classified by scale of extensive use. At the end of the selection we labeled 20 land cover forms as extensive (different forest types, wetlands, bushes). Visual diversity measured by the number of distinct land use forms per unit area (Kollányi, 2004). There are several methods for determination of the index, from which (due to the spatial scale and availability of data) we have chosen the distribution of valuable edge types per unit area. From the point of landscape aesthetics forests and water edges represent the highest values. For the assessment of landscape diversity we calculated the forest (three times the weight) and water edges divided by the area of settlements. The last of the indicator group is intended to measure cultural significance. Cultural heritage indicator includes the number of monuments of the settlements.

The provisioning services cover mostly the cultivation of the landscape. In spite of the overall reduction of the weight of agriculture and forestry in GDP and employment, agricultural use is still important in rural especially backward regions from the point of economy and forming the landscape. The group of indicator of agriculture and forestry includes arable land potential (proportion of arable land indicating the fertility (AK index of land quality) as well), forestry potential (proportion of forests). The intensive fruit, vegetable gardens, vine yards increase the income-generating capacity of agriculture which is represented by the vine and fruit potential indicator (proportion of vine yards and other intensive horticulture using Corine Land Cover database).

Tourism shows another aspect of attractiveness and use of the landscape. We assessed the accommodation capacity and number of guest nights in the settlements (tourism flow indicator). Generally availability in rural development is a cardinal question. We highlighted the differences in availability between the pilot-regions and sub-regions by calculating the time of availability of motorway junction (minutes), county seat, seat of micro-region and counting railway stations which improves availability.

As we consider landscape function analysis as a tool of rural development and in order to present the differences in the economic circumstances as an additional analysis we elaborated a complex economic indicator using data of the Hungarian Statistic Office. The economic indicator highlights the income generating capacity of the settlements represented by total domestic income per taxpayers and ratio of taxpayers in the settlement.

First we aggregated the spatial data on settlement level. The values of our indicators varied between rather different scales, so we harmonized them into a common dimension. The values (32+34) of all settlements of both pilot regions were ranged one after the other. Then the values were divided on a scale from 1 to 10. So we can asses not just the situation of settlements in the pilot area but the range or relation of the settlements of both pilot regions general as well. Thanks to the employed method we could aggregate the indicators into 6 groups.

Introduction of the pilot regions (Hanság-Rábaköz and Micro-region of Gönc)

We have chosen two rural regions lying along the Western and North-Eastern boarders of Hungary (*Figure 1*). Both pilot regions contain backward settlements and suffer severe depopulation processes and are peripheries or have peripheral parts.

Micro-region of Csorna situated in the Small-Plain between the great centers of the County Győr-Moson-Sopron. The micro-region of Csorna holds most of the settlements of Hanság and Rábaköz together. For centuries especially in Rábaköz agriculture was the most important economic basis of the region. From the point of view of tourism important landscape conditions, the area is in a shadow-situation, the Rábaköz does not have outstanding attractions but it is situated between the Szigetköz/Danube and Lake Fertő recreational areas of national importance, in the vicinity of the Austrian border. The micro-region can be characterized by rural small settlement structure. The wetlands, swamps, and forests of Hanság and the banks and gallery forests of river Rába

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are of great ecological value. In the micro-region two characteristic groups of settlements can be drawn because of the different landscape conditions: 1. Hanság and Tóköz 2. Rábaköz and Rábamente.

The *micro-region of Gönc* is located in the North-Hungarian Region, in Borsod-Abaúj-Zemplén county. The region, thus the settlements belong to the most disadvantaged areas of the country. The sample area can be divided into two main parts with different landscape characteristics. The largest portion of the micro-region is located in the upper valley of the Hernád river, while about two thirds of that is on the northwestern side of the mountains of Zemplén. The subject area consists of two small towns (Gönc and Abaújszántó) and 30 villages grouped around the towns. The Slovak-Hungarian border, which runs along the northern perimeter of the research area, has got a direct influence on the settlements of the micro-region. It is important to note that the wider region's largest center, Kosice is half as far from Gönc as Miskolc, the county seat. Especially after the Schengen's border opening the proximity of the second largest city in Slovakia, Kosice with a population of 300,000 was appreciated.

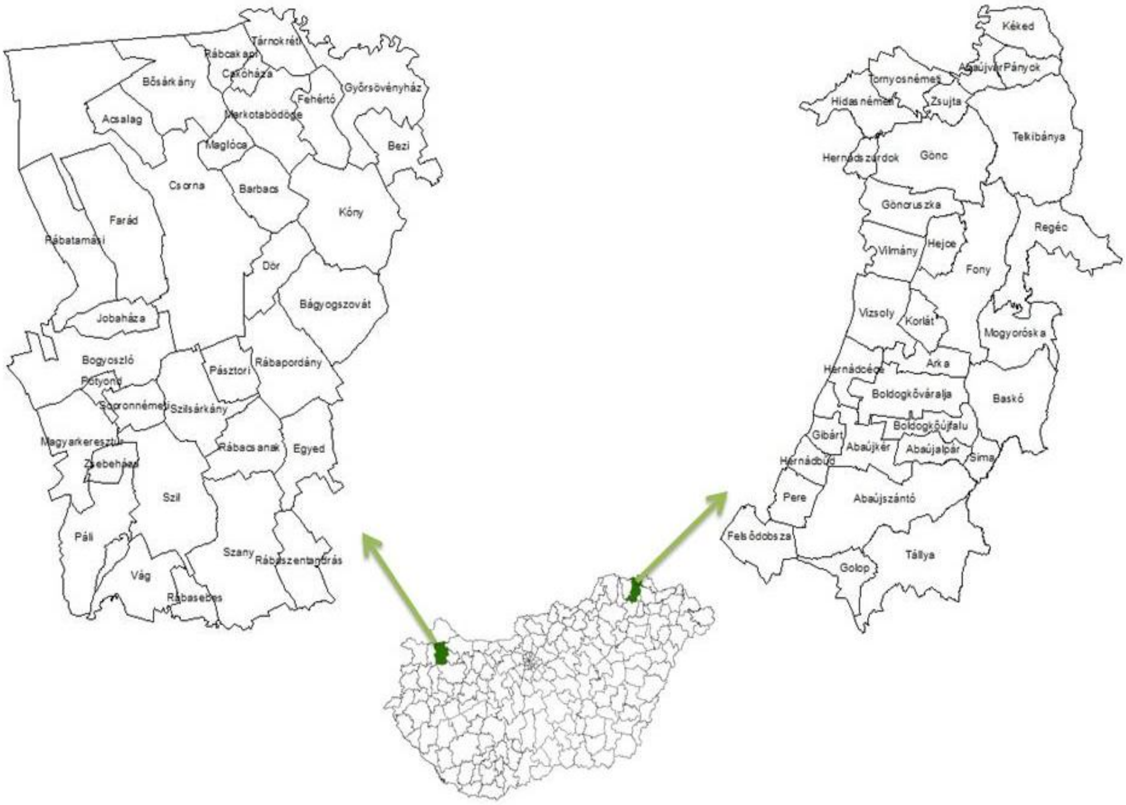

Figure 1. *Location of the pilot-regions, micro-region of Csorna, and Gönc*

The structure of the micro-region (settlement network and infrastructure network) is determined by the landscape and geographical (topography, hydrography) conditions.

Both pilot-regions lie near the borders of Hungary and are mostly characterized by high settlement density with mostly small villages. If we consider natural and economic conditions, we have chosen two characteristically different micro-regions: Gönc lies in one of the most backward region and Csorna and its surrounding in the second richest region of Hungary.

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***Table 1**. Relation between ecosystem services, landscape function concepts and the used landscape indicators of the research*

Used indicators of the research		Different classification systems for landscape functions and ecosystem services																					
Group of indicators	Indicator	Landscape functions (Bastian, 1997)			Landscape functions (Brandt & Veyre, 2004)			Landscape functions (De Groot & Hein, 2007)				Landscape functions (Williem et. al, 2008)			Ecosystem services (MA, 2003)				Ecosystem services (Costanza, 2008)				
		Regulation	Production	Anthropocentric	Ecosystem	Land use	Transcending	Regulation	Habitat	Provision	Culture and amenity	Complete delineation	Partial delineation	No delineation	Supporting	Regulation	Provision	Culture and amenity	Global non-proximal	Local proximal	Directional flow related	In situ	User movement related
Environment and Biodiversity	Biodiversity	X			X				X				X			X				X			
	Environmental integrity	X			X				X				X			X				X			
Nature protection	Protected areas of national importance	X				X				X		X				X							X
	Protected areas of international importance	X				X				X		X				X							X
	National ecologic network	X				X				X		X				X							X
Landscape aesthetic and cultural value	Cultural heritage			X			X				X	X						X	X				
	Naturalness			X			X				X	X						X					X
	Diversity			X			X				X	X						X					X
Agriculture and Forestry	Arable land potential		X			X				X			X				X					X	
	Vine and fruit productin potential		X			X				X			X				X					X	
	Forestry potential		X			X				X			X				X					X	
Tourism	Tourism flow			X			X				X		X					X				X	
Infrasturcture	Availability		X			X				X		X					X				X		

However southern Rábaköz can be characterized as an inner-periphery with decreasing population. The pilot regions consist of different landscape character types. Gönc and its surrounding is a mountainous varied landscape with the valley of Hernád river. Hanság and Rábaköz is a plain landscape and while Hanság with the remnants of the former vast marshland is rich in ecologic values, Rábaköz is mostly an intensive cultivated cultural landscape.

Results

First the chosen and employed indicators were pre-tested in both sample areas. To detect the relevance of each indicator we analyzed the differences between the highest and the lowest value and the variety of the values. *Table 2* shows, that the same indicator could not always detect the same differences or relationships in different pilot regions. We also found that some of the indicators indicate better the differences between the settlements, while others are more suitable for analysis entire micro-regions. Despite of the formers we decided to employ all of our indicators for the following steps, while as *Table 2* presents all of them have got some relevance for the evaluation.

Detailed comparison of landscape indices in the pilot regions

The research results allow detailed comparison of landscape functions between the pilot regions and a sub-regional analysis as well (*Figure 2*). First we present our results according to the landscape indicator groups and afterwards summarize them.

Table 2*. Relevance of the indices in the pilot areas*

Used indicators of the research		Relevance of the sample areas	
Group of indicators	Indicator	Micro-region of Gönc	Micro-region of Hanság-Rábaköz
Environment and Biodiversity	Biodiversity	**	**
	Environmental integrity	**	**
Nature protection	Protected areas of national importance	**	*
	Protected areas of international importance	***	***
	National ecologic network	***	***
Landscape aesthetic and cultural value	Cultural heritage	***	***
	Naturalness	***	***
	Diversity	***	***
Agriculture and Forestry	Arable land potential	***	***
	Vine and fruit productin potential	***	***
	Forestry potential	***	***
Tourism	Tourism flow	***	**
Infrasturucture	Availability	***	***

*** Relevant: difference between the highest and lowest values is triple, different values occur in at least 50% of the settlements

** Less relevant: difference between the highest and lowest values is one and half times, different values occur in at least 25% of the settlements

* Not relevant: difference between the highest and lowest values is less than half times, different values occur in less than 25% of the settlements

Environment and Biodiversity

The biodiversity indicator shows characteristic differences in both pilot regions. Settlements of *Hanság and Tóköz* dispose of high natural variety, high rate of semi natural, natural vegetation and protected areas (highest value Tárnokréti 56,4), low or medium intensity of cultivation, diverse land use, small patches of meadows, forest belts and plough fields. Meanwhile *Rábaköz* is characterized by high intensity of agricultural production which leads to a monotonous landscape with low ecologic value. This reflects in low values of biodiversity (lowest value 23). In Rábaköz the only exceptions are the settlements along river Rába where a narrow belt of gallery forests, meadows give high biodiversity (Rábasebes).

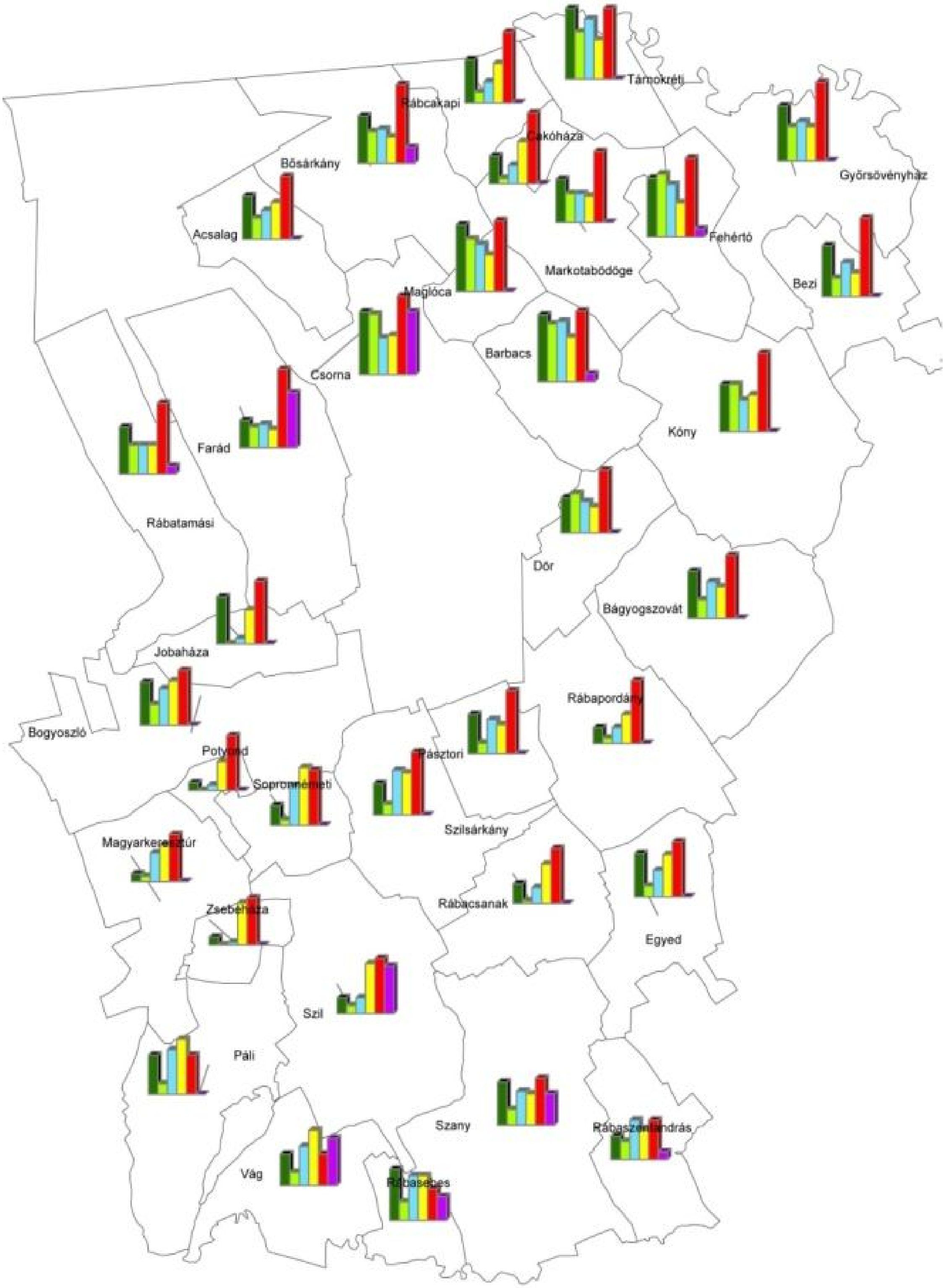
The indicator of environmental integrity reflects similar results, so similar differences between the sub-regions as the indicator of biodiversity. The highest values belong to settlements of *Hanság, Tóköz*, Tárnokréti, Csorna (51), Fehértó (49), Barbacs (47), Maglóca (46). Exceptions are Bogyoszló and Páli from Rábaköz. All settlements with low values are settlements from *Rábaköz*.

The analysis of diversity draws also in *Gönc micro-region* an interesting pattern of the settlements. The most diverse area is the northwestern part of the region and also some of the southern settlements, while the eastern half of the sample area seems to be the least diverse. This picture is rather contradictory with the natural protected areas, which are mainly located the least diverse part of the micro-regions. The reason of this is the fact, that the majority of the protected areas covered by forest and the pattern of the land uses do not show less variability (e.g. Baskó (23,76), Regéc (25,62)). In contrast, along the river Hernád plenty of small lakes (which were mainly former gravel pits), backwaters are located. In addition narrow belts of gallery forests also increase the diversity of these settlements. The highest values detected in Hernádszurdok (57,15), Zsujta (52,12) and Hidasnémeti (49,67).

In the case of environmental integrity in *Gönc micro-region* the borderline between the Mountains of Zemplén and the Valley of Hernád is outlined clearly. The highest values were calculated in the settlements, which are covered mainly with forest: Regéc (93,27), Mogyoróska (89,65), Telkibánya (89,16), Baskó (88,87) and Fony (84,96). The lowest values belong to the settlements, which have large arable areas: Felsődobsza (37,92), Vilmány (38,44), Vizsoly (39,26) and Göncruszka (40,14).

Nature protection

In *micro-region of Csorna* the share of protected areas in the region of *Hanság and Tóköz* is really high, here we can find areas of the National Park Fertő-Hanság (Fehértó, Barbacs, Csorna, Maglóca, Kóny, Dör, Bősárkány, Győrsövényház, Markotabödöge, Acsalag) and also the protected areas of international importance (Natura 2000 areas, Ramsar site) are here. Up till the 18th century Hanság was a vast marshland but following the drainage works most of the lakes of Hanság and Tóköz disappeared; only the Lake Fehér and the Lake Barbacsi remained. As a result of turf-cutting smaller lakes emerged (Lake Király), all these areas are as strictly protected areas the part of the National Park Fertő-Hanság. A very interesting and successful wetland reconstruction project in the Hanság started in 2001 to restore the open wetlands and the marshes. Its first step was realized in the area Nyirkai-Hany (420 ha) and as a following project in Osli-Hany (1322 ha) in 2013.


Tárnokréti
Rábcakapi
Cakóháza
Győrsövényház
Bősárkány
Acsalag
Fehértó
Markotabödöge
Bezi
Maglóca
Barbacs
Csorna
Kóny
Farád
Rábatamási
Dör
Bágyogszovát
Jobaháza
Rábapordány
Bogyoszló
Potyond
Pásztori
Sopronnémeti
Szilsárkány
Magyarkeresztúr
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Vág
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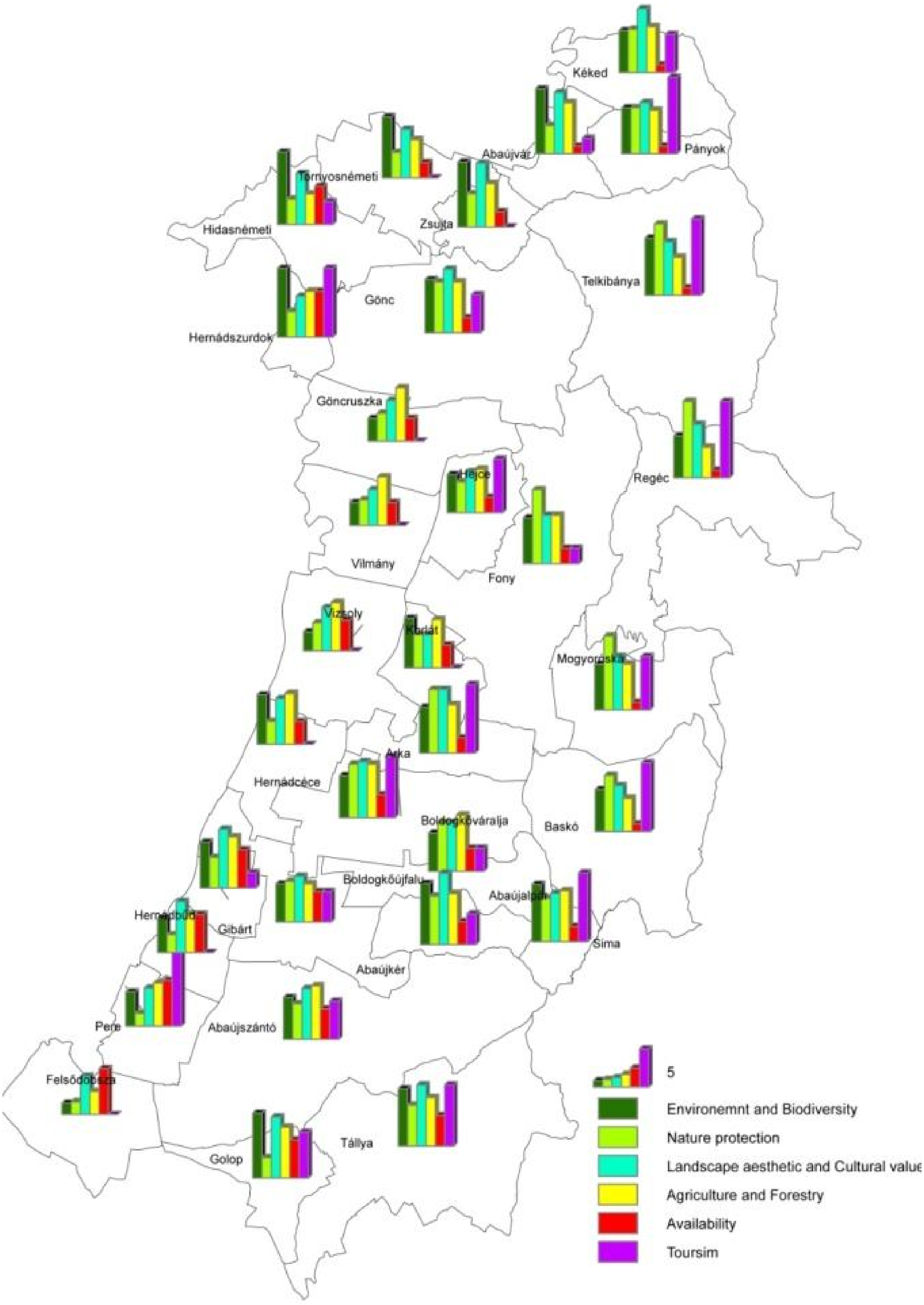

Figure 2. Level of landscape functions on the settlements of micro-region Csorna and Gönc

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The flooded Nyirkai-Hany was quickly detected by the breeding and migratory birds. The area became a wetland of international importance (Ramsar site) in 2006 (http://www.ferto-hansag.hu/fhnp-en).

In almost all settlements we can find areas of National Ecologic Network. But meanwhile in settlements of Hanság, Tóköz the National Park and Natura 2000 areas dominate in Rábaköz the areas of National Ecologic Network are in majority. In Rábaköz the areas of National Ecologic Network are mostly meadows. It is important for the farmers because while in the area of Natura 2000 different support schemes are available for farming, in the area of National Ecologic Network there are no such support available which does not help the maintenance of the ecologic values in this intensive agricultural land.

In *micro-region of Gönc* the Landscape Protection Area of Zemplén has got a big portion of area of some eastern settlements of the micro-region (e.g. Fony, Mogyoróska, Regéc, Arka, Telkibánya). The Landscape Protection Area is one of the most undisturbed and undetected region in Hungary. For several years there have been efforts to declare the area as a national park. The Landscape Protection Area was established in 1984 on 26,496 acres. Certain parts of Nagy–Sertés Mountain, which belongs to Telkibánya, are also forest reserves (F. Nagy, 1996). About 85% of the Landscape Protection Area's territory is covered by forest, which determines its character.

The share of protected areas of international importance (Natura 2000 areas and Ramsar sites) is high, especially in the western settlements (along the Hernád-river) and the eastern settlements (related to the Landscape Protection Area of Zemplén).

In all of the settlements we can find areas of National Ecological Network. Most of these areas overlap the protected areas of national and/or international importance. The differences can be detected along the small streams, which arise from the Mountains of Zemplén and held toward the Hernád-river. One of our important results is the lack of the Ecological Network in the Valley of Hernád, especially in those settlements, which have big arable fields (e.g. Göncruszka, Vizsoly, Vilmány, Hernádcéce). In the sample area it causes a big problem, because the lack of the ecological corridors between the core areas (in the mountains and along the river) means, that the ecological system can not work as a real network.

Landscape aesthetic and cultural value

In the *micro-region of Csorna* the only outstanding cultural value is the baroque Premonstratensian Abbey Church of Csorna but the small villages are rich in folk architecture. So the indicator of cultural heritage shows the highest values in Szilsárkány, Barbacs with old folk houses. The indicator of naturalness show similar values to the indicators of biodiversity and environmental integrity. The values of naturalness are high in settlements of *Hanság* and Tóköz and mostly low in *Rábaköz.* As the indicator of diversity represents the length of forest and water borders the values are high in settlements along river Rába (Rábasebes, Vág, Rábaszentandrás) and Rábca and the major canals and lakes of Hanság (Győrsövényház, Tárnokréti). Forests are mostly situated in Hanság. Because of the intensive drainage works in Hungary the highest density of canal system can be found in Hanság.

We can distinguish four major landscape character types in the micro-region:

1. The remnants of the former marshland mostly characterized by wetlands, pastures, forests, mosaic landscape.

2. Drained marshland mostly characterized by low and medium intensity cultivations (Konkoly-Gyúró, 2010).
3. Plain landscape with dominant plough-land
    a. Monotonous plain landscape with large plough fields.
    b. Plain landscape with mosaic like cultivation.
4. Riverside landscape characterized by high rate of gallery forests with small pastures.

In *micro-region of Gönc* the most important cultural heritages are located in Gönc (e.g. Bible Museum and Reformed church, Hussite House), in Telkibánya (e.g. Reformed fortified church and wooden headboards cemetery), in Boldogkőváralja (e.g. Castle of Boldogkő), in Vizsoly (e.g. Reformed Church) and in Tállya (e.g. vineyards and wine cellars on the World Heritage Site). Besides these, our results show high value of the cultural heritage in Hejce, Zsujat and Felsődobsza. The reason for this is the richness of folk architecture (e.g. folk houses). The indicator of naturalness shows similar results than the indicator of environmental integrity in *micro-region of Gönc*. The settlements, located in the Mountains of Zemplén reached high values (e.g. Baskó, Regéc, Mogyoróska,Telkibánya), while the settlements with big cultivated areas have got low values (Felsődobsza, Vizsoly, Vilmány, Pere). Since the indicator of diversity is based on the length of the water and forest borders, the value of it is the highest in the settlements along the Hernád river, especially in the north half of the sample area, where plenty of small lakes and backwaters are located (Zsujta, Abaújvár, Hidasnémeti, Hernádszurdok). The values of the indicator are also high in the eastern settlements, where the forests of the Mountains of Zemplén meet the arable areas (orchards, vineyards). The diversity values are low between these two sides of the micro-region.

We can distinguish four major landscape character types in the sample area:

1. Mountains of Zemplén mainly characterized by broadleaf forest
2. Transition areas on the foothills of Zemplén characterized by old orchards, vineyards and meadows
3. Valley of the Hernád river characterized by big arable areas with narrow stream valleys and along them small groups of trees and bushes
4. Riverside landscape with the Hernád river and along that with gallery forests, backwaters and small lakes

Agriculture and Forestry

In the micro-region of Csorna the significance of agriculture in the economy, employment and land use is above the national average. The *Rábaköz* is characterized by intensive agriculture. The ratio of plough fields are extremely high in the micro-region (national average 48%, local 66% with great local differences), in spite of the traditional predominance of grazing the ratio of the grass fields in the region is average today. Hanság, Tóköz is characterized by low, medium intensity of agriculture.

Fruit production is not characteristic either, but there are a few large plantations for example in Sopronnémeti (fruit plantation), Tárnokréti (fruit, vegetable from ecologic production), Szilsárkány. The proportion of area of fruit and vegetable gardens is much less than the national average in spite of the considerable growth during the socialism and during the 1990’s when the vegetable growing especially the cucumber which was called the “gold of Rábaköz” was significant.

Forestry is not important in the region. Most of the forests are situated in the marshland of Hanság (high values of indicator in Csorna) or along the river Rába (Vág, Páli).

Based on the indicator of agricultural potential we can also divide *Gönc micro region* into two significantly different parts. In the Valley of Hernád the ratio of arable land is very high. These settlements are characterized by big, intensive agricultural fields (Göncruszka, Vilmány, Vizsoly, Felsődobsza, Hernádcéce, Tornyosnémeti). This region historically was called as the "pantry of Kosice", so the agriculture has a great tradition. While the settlements in the Mountains of Zemplén have very small agricultural areas due to the huge forests (Regéc, Fony, Telkibánya, Mogyoróska).

Two main hotspots of vine and fruit potential indicators were detected in *Gönc micro-region*. First, the southern settlements of the micro-region belong to the "Tokaj Wine Region Historic Cultural Landscape" World Heritage Site, that is why Tállya, Abaújszántó and Golop have a really high value of this indicator. Especially in Tállya and Abaújszántó have big historical vineyards and plenty of wine cellars.

The other interesting area is Gönc and the settlements in its surroundings, which are traditionally fruit product areas ("pálinka of Gönc"). Nowadays plenty of old abandoned orchards are located next to the forests of Zemplén. Despite of this, the fruit production is still a significant sector of the region. Plenty of new, modern orchards were deployed in Gönc, Göncruszka, Hejce, Boldogkőváralja and Boldogkőújfalu.

In Gönc micro-region the result of forestry indicator is more or less the inverse of the indicator of arable land potential results. The settlements situated in the Mountain of Zemplén reached high value of forestry potential. In their economy the forestry has an important role, especially in the case of Fony, Regéc, Mogyoróska, Baskó, Telkibánya, Pányok and Kéked.

Tourism

Analyzing the accommodation capacity and number of guest nights the results are disappointing in the *micro-region of Csorna*: the indicator show high tourism potential just in a few settlements: in Hanság and Tóköz just in Csorna, Farád, Bősárkány, and in Rábaköz with the exception of the settlements along the river Rába (Vág, Szany, Szil, Rábasebes) we can not talk about tourism at all. If we look at the recreational activities related to natural endowments we can see almost just ceasing possibilities: the thermal bath of Csorna in spite of the great development plans of the self-government is not functioning (because of ownership conflicts), the former beaches of Rába are abandoned, the lookout tower in Fehértó (Hanság) is run down.

Analyzing the tourism in the surrounding of *Gönc* our results show duality among the settlements. There are some villages, in which we can not talk about real tourism, in these settlements officially there are no guest accommodation and guest nights. These villages located mainly in the Valley of Hernád (e.g. Zsujta, Göncruszka, Korlát, Vilmány, Hernádbűd).

On the other end of our results is Telkibánya, which reached the significantly highest value of this indicator. We can detect other settlements in the Mountains of Zemplén which have also quite high values (Regéc, Pányok, Sima, Arka, Baskó, Tállya, Hejce). It is interesting, that the two towns of the micro-region have only on intermediate position of our results. Based on this, one can argue, that the main tourism types in the sample area is the rural and the nature-based tourism. We found some settlements with relatively high values along the Hernád river. In this area the water tourism is the main tourism type. In the villages some guest accommodations and tourism services help to stimulate the tourism (Pere, Hernádszurdok).

Availability

The level of availability has great differences in *micro-region of Csorna*. The settlements along the main traffic corridors and in the vicinity easily accessible even by public transportation as well. The values of availability are insufficient in Southern Rábaköz.

In the micro-region of Gönc similar differences were detected as in micro-region of Csorna. The settlements on the southern and western parts are relatively easily accessible thanks to the main roads of the region. The same villages have got better connections by public transportation as the settlements in the Mountains of Zemplén. The availability of this part of the sample is very low (e.g. Kéked, Pányok, Mogyoróska). Some of the villages are "dead-end" –villages.

Economic situation of the pilot regions

Figure 3 highlights the unfavorable economic situation of most of the settlements of micro-region of Gönc and the Southern settlements of Rábaköz. In *micro-region Csorna* the city of Csorna, Bősárkány, Győrsövényház, Kóny, Bősárkány, Szilsákány have the highest values of the economic indicator. From the point of economic development the northern part of micro-region can be also divided in two parts. Settlements of Tóköz neighbouring Csorna have unfavourable values. In the eastern part of Tóköz in the vicinity of the county seat the settlements are popular and developing. In *micro-region Gönc* the settlements in the Mountains of Zemplén have got relatively high values of the indicator. The economy of these villages is mainly based on the tourism, wine production and forestry. In general, the smaller villages in the Valley of Hernád have got the biggest economic and —related to this— social problems.

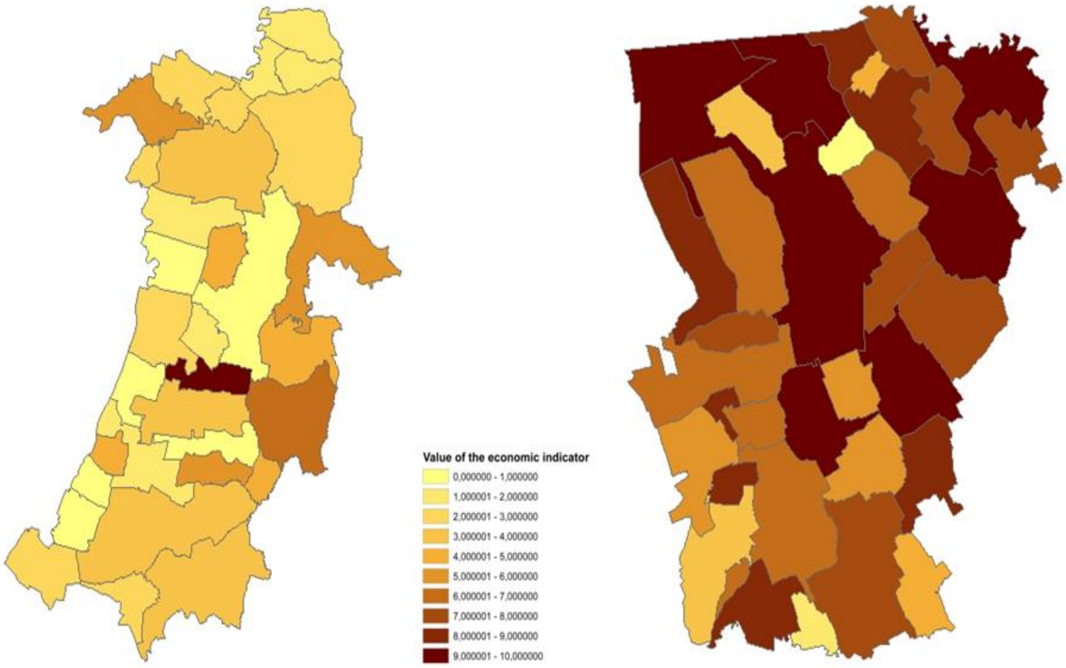

Figure 3. *Economic indicator in the settlements of micro-region Csorna and Gönc*

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Differences in level of landscape functions, relevant landscape functions between the pilot regions (general indices)

In order to highlight the main differences and similarities between the pilot-regions we calculated the averages of the settlement values. The diagram shows characteristic differences in the level of landscape functions (*Figure 4)*. The value of most landscape indicators of micro-region Gönc exceeds those of Csorna. The average value of environmental integrity, naturalness, nature protection, diversity in Gönc is mostly double or triple of Csorna. Considering the indicators which influence and represents the economic situation of the regions Csorna has the highest values. Remarkable differences exist in the level of availability and economic situation. Values of tourism and agriculture are higher in Gönc regions, unfortunately these sectors have a lower influence on economic development and employment. In the field of agricultural production in Gönc vine and fruit production have the highest values, arable land potential is higher in Csorna.

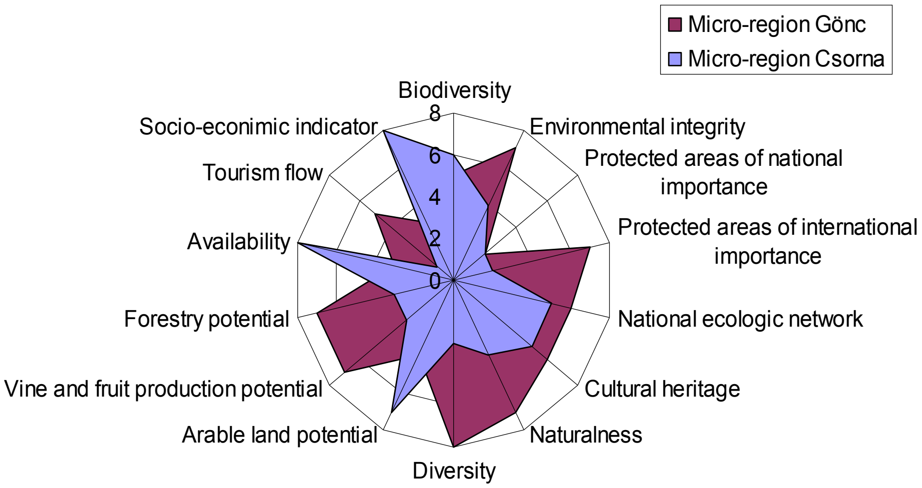

Figure 4*. Comparison of values of landscape indicators and level of landscape functions and economic situation between pilot regions*

Discussion

We have found interesting relations between landscape functions, availability and economic situation, but because of distinct interactions between the functions in different situations the correlations vary. Rábaköz is a plain landscape cultivated since centuries where the most common way to increase the amount of agricultural products was obtaining land by meadow-plough land transition. This processes resulted in low rate of extensive areas which causes the low values of environment and biodiversity. Regulation, supporting landscape functions and the aesthetic, cultural value of the landscape are insufficient in Rábaköz.

The other major landscape unit which differs characteristically in micro-region of Csorna is Hanság and Tóköz, which still bears the values of the former vast marshland and the majority of that is under nature protection. Looking at the landscape aesthetics the protected areas are of high value but in the cultivated areas especially plough fields the landscape is monotonous. Significant part of protected areas are strictly protected, there is only one study trail in the region and only two exhibitions of smaller scale.

There are no guest houses, apartments of appropriate quality, so tourism; eco-tourism is marginal in Hanság.

In the micro-region of Csorna there is a very strong relation between availability and development potential. Those settlements have good economic conditions which are easily accessible. The settlements in South-Rábaköz where the values of availability are low suffer severe depopulation. Because of the poor conditions of availability the most important economic sector is agriculture whose income-generating capacity and role in employment is insufficient because of the low rate of high value-added agricultural sectors. Agriculture, however, as in the case of most of the settlements of low commuting and other economic activities does not provide competitive salaries to the public. The low rates of intensive horticulture furthermore the low level of processing of agricultural products and a limited scale of complementary activities and income reduces the profitability of agriculture. The monocultural agricultural production causes the insufficient level of several landscape functions. This and the low level of availability have a strong correlation with the poor economic situation, and depopulation trend of Southern-Rábaköz. Landscape management should focus in Rábaköz on diversification of agricultural production and development of the ecologic value of the landscape.

We can also observe correlation between landscape functions, availability and economic situation in Hanság and Tóköz which are rich in natural values. In spite of the high rate of areas of high natural value, the landscape values do not appear as attractions; there are limited opportunities for ecotourism because of the lack of study trails, exhibitions, and lookout towers. All of this reduces the additional income from tourism for local people. In spite of the lack of jobs due to the good availability of the county center the settlements of Eastern-Tóköz remained attractive for the society. Settlements of unfavorable availability in Tóköz in the vicinity of Csorna (Western-Tóköz) became deprived communities in spite of the richness of natural values.

Micro-region of Gönc can be divided into different parts according to the landscape function analysis. As a result of our research we identified different landscape function combinations and mechanism. This region was the pantry of Kassa (today the second biggest city of Slovakia) before 1920. Agriculture dominated in the fertile areas, typically in valley of Hernád. The cultivation structure which evolved in times of socialism and maintained up till now caused the marginalization of environmental and natural values. Consequently we measured relative low values in valley of Hernád in case of the groups of environmental and natural indices. Parallel with the marginalization of supporting and regulation functions the provision function is dominant as a consequence of high rate of agricultural use. Similarly to micro-region of Csorna the values of availability are low and this is in strong correlation with the economic development. Mountain of Zemplén characteristically differs from landscape unit of valley of Hernád where we explored different correlations. The cultural and amenity, supporting, and regulation functions are much stronger in these settlements. Forestry, and nature protection are dominant and as a consequence tourism. However, it is interesting that this area has the worst values of accessibility. In this case (in contrast to the micro-region of Csorna) this strengthens the position of tourism and thus of the economy (tourists arriving here are mainly attracted by the relatively untouched nature).

In the transition zone we find a mosaic like system of landscape functions characteristic for Zemplén or Valley of Hernád. The economic situation is not homogeneous either. Those settlements where tourism has a significant role or agricultural sectors with relatively high additional value (fruit and vine production) are dominant have better economic indicators.

We can clearly state on the basis of the foregoing that diversified landscape management is needed in both pilot regions. In the Valley of Hernád and Rábaköz the restructuring of agriculture is proposed, where the agricultural sectors of higher additional value shall be more significant (e.g. horticulture, organic farming). The improvement of ecologic value is also important so the planting of elements of mosaic like and the traditional landscape such as vegetation along canals, forest belts, trees and greater areas of pastures should be enhanced. Parallel to it the improvement of availability and cross-border co-operation is needed. In the Mountains Zemplén the main directions of landscape management shall follow the preservation of natural values, nature protection and tourist development (rural, eco and active tourism). The situation of settlements of Hanság and Western-Tóköz are the same but there tourism shall be developed ‘from scratch’.

Based on the regional comparison we can clearly state that the economic situation has a strong correlation with availability. It is also obvious that in many cases high environmental-natural potential does not result in economic development. In our opinion this is due to poorly targeted regional and rural development policies. For the better use of landscape potential we call for more professional foundation of these policy documents by detailed landscape management guidelines based on landscape function analysis.

Relation between intensive agriculture and economic situation of the settlements is controversial. The labor intensive agriculture, the higher value-added sectors such as vine production, horticulture or organic farming result in higher rate of employment and better economic situation. The intensive cereal production is not appropriate anymore to provide economic base for the people of villages because of its low labor needs. Furthermore intensive cereal production leads to a decrease of the ecologic and aesthetic values.

Naturally there is a strong correlation between natural and cultural values and tourism in general, however the settlements of Tóköz can not benefit from their natural values due to the strict nature protection regulations and the lack of tourism infrastructure.

To analyze the landscape functions in the pilot-regions we used complex indicators. Unfortunately in many cases there are no appropriate indicators to highlight the multiple functions of the landscape. Indicators often describe and characterize just a narrow fraction of certain landscape functions. For example indicator of monuments (cultural value) describes just the number of cultural values and do not give information about the significance of the monument (local, regional, national, international significance).

Furthermore we aggregated the spatial data for settlements level which also may cause loss of data. For more detailed analysis or possibilities for correction it is also possible to involve wider range of indicators for describing environmental, aesthetic, economic functions of the landscape (agriculture in employment, wooden production etc.).

In conclusion, rural regions possess of different system of landscape functions (looking at structure and functioning). At the beginning of detailed analysis the identification of appropriate indicators is needed, which helps to explore the characters of landscape functions. Next to the identification of landscape functions it is also essential to explore their spatial pattern, combinations and the relations between them.

In our study we have shown example for an indicator-system adaptable in Hungary. Our studies we demonstrated that it is reasonable to carry out landscape function analysis because of the complexity of system of landscape functions in all rural regions.

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THE COMBINED IMPACT OF URBAN HEAT ISLAND, THERMAL BRIDGE EFFECT OF BUILDINGS AND FUTURE CLIMATE CHANGE ON THE POTENTIAL OVERWINTERING OF *PHLEBOTOMUS* SPECIES IN A CENTRAL EUROPEAN METROPOLIS

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Abstract. Leishmaniasis is one of the most important emerging vector-borne diseases in Western Eurasia. Although winter minimum temperatures limit the present geographical distribution of the vector *Phlebotomus* species, the heat island effect of the cities and the anthropogenic heat emission together may provide the appropriate environment for the overwintering of sand flies. We studied the climate tempering effect of thermal bridges and the heat island effect in Budapest, Hungary. Thermal imaging was used to measure the heat surplus of heat bridges. The winter heat island effect of the city was evaluated by numerical analysis of the measurements of the Aqua sensor of satellite Terra. We found that the surface temperature of thermal bridges can be at least 3-7 °C higher than the surrounding environment. The heat emission of thermal bridges and the urban heat island effect together can cause at least 10 °C higher minimum ambient temperature in winter nights than the minimum temperature of the peri-urban areas. This milder micro-climate of the built environment can enable the potential overwintering of some important European *Phlebotomus* species. The anthropogenic heat emission of big cities may explain the observed isolated northward populations of *Phlebotomus ariasi* in Paris and *Phlebotomus neglectus* in the agglomeration of Budapest.

Keywords: *climate change, epidemiology, Phlebotomus, sandfly, vector, leishmania, overwintering, prediction, model*

Introduction

In the warm temperate and tropical areas of the World, leishmaniasis is one of the most important emerging diseases with prevalence of at least 12 million cases per year,

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and the incidence of all clinical forms reaches 2 million new cases per year in the world (Naderer et al. 2006; WHO, 2000). The causative agents of leishmaniasis are different *Leishmania* protozoans (Killick-Kendrick, 1990). Two species of them have major importance in Europe: *Leishmania infantum* and *Leishmania tropica* (Ready, 2010). The main reservoir hosts of the parasites are domestic dogs (Marty et al., 2007; Shaw et al., 2003). The European Phlebotomus species and the Leishmania parasites they can transmit can be seen in *Table 1. Phlebotomus mascittii* Grassi (1908) has never been proven to be a vector of leishmaniasis (Naucke and Pesson, 2000).

Table 1. *European Phlebotomus species, their subgenus, auctor, and the transmitted Leishmania species (Minter, 1989; Killick-Kendrick, 1990; WHO, 1984; Léger et al., 2000). The last column shows whether the species was studied in this research and if so what is the group number which the species is included in.*

Species	**Subgenera**	**Auctor**	**Transmitted Leishmania species**	**Group**
Ph. ariasi	*Larroussius*	Tonn.	*L. infantum*	1.
Ph. neglectus	*Larroussius*	Tonn.	*L. infantum*	3.
Ph. mascittii	*Transphlebotomus*	Grassi	–	3.
Ph. papatasi	*Phlebotomus*	Scop.	*L. donovani, L. killicki, L. tropica, L. arabica, L. major*	3.
Ph. perfiliewi	*Larroussius*	Parrot	*L. infantum*	3.
Ph. perniciosus	*Larroussius*	Newst.	*L. infantum*	2.
Ph. sergenti	*Paraphlebotomus*	Parrot	*L. tropica, L. major*	not studied
Ph. similis	*Paraphlebotomus*	Perfiliev	*L. tropica*	not studied
Ph. tobbi	*Larroussius*	Adler, Theodor et Lourie	*L. infantum*	not studied

In wide urban areas of European cities the existence of a high canine seroprevalence of leishmaniasis indicates the urban presence of the parasite's vectors (Tselentis et al., 1994; Dantas-Torres, 2006). As an example, in Apulia (Italy) where *Leishmania infantum* is endemic, sandfly species prefer the urban environment (Tarallo et al., 2010). It is known that e.g. *Phlebotomus perniciosus* – one of the most important vector of *Leishmania* parasites in Europe – is able to colonize rural, peri-urban and urban areas (Bettini et al., 1991; Biocca et al., 1977; Maroli and Bettini, 1977). The life-cycle of *L. infantum* is distinctly peridomestic. In Portugal, it was also found that leishmaniasis cases are associated with dogs and urban areas (Cortes et al. 2007).

Many authors suggest the future expansion of vector *Phlebotomus* species in Europe, and according to these models Hungary will also provide adequate environment (Trájer et al., 2013). It is, however, notable that in case of Heteroptera species migration is the function of those differences which are actually existing between the temperature-

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dependent potential and the locally and historically developed number of individuals (Hufnagel and Gaál, 2005).

Climate change in Hungary (eg. according to the ENSEMBLES simulations; Pongrácz et al., 2011) is expected to change the distribution ranges of many species and even the boundaries of biomes (Bede-Fazekas, 2012; Garamvölgyi and Hufnagel, 2013). Shift of the distribution of species to north due to the global warming have been already observed (Gimesi et al., 2012).

Our aim was to explain the extrazonal occurrence of two resident *Phlebotomus* species in the agglomeration of Budapest by creating a model based on urban heat pollution (thermal bridges and heat island effect) and model the possible future extension of the suitable urban areas in case of different sandfly species. Our hypothesis was that urban heat pollution can allow for the overwintering of the sandflies.

Review of literature

Sandflies in natural and urban areas

In this section we attempted to collect all the relevant data on the lifestyle, nutrition and behavior of sandflies, considering their preferred environment and their connection to the built human environment. By reviewing the literature, we found references for the distribution of leishmaniasis from Brazil, South America, and four European countries, which were Italy, French, Portugal and Hungary (Budapest). Moreover, leishmaniasis was also reported by an expert committee of the WHO in 1984.

According to Bettini and Mellis (1988) the pre-imaginal stages of the sand fly species are associated with a comparatively stable, humid, cool environment, protected from rain, direct solar radiation and wind and rich in clay and organic nitrogen. Imagoes seek refuge in both anthropogenic and natural environments: e.g. inside buildings, barns, wall fissures, tree holes, animal nests (Maroli et al., 1994; Ascione et al., 1996; Maroli and Khoury, 1998). According to Moncaz et al. (2012) *Phlebotomus sergenti* rests and breeds inside caves and in tree nests of several rodent species. Hanson (1961) and Vanni (1940) found sand fly pupae [*Phlebotomus perfiliewi, Phlebotomus major, Phlebotomus papatasi*] in natural areas on the surface of fallen dead leaves and forest soil. Due to the fermentation heat of the compost, they can overwinter during cold conditions in the nature.

Human factors, travelling, and livestock are also important determinants of the abundance (Ready, 2010). In the Mediterranean parts of Europe, *Phlebotomus* species can also have their larval development in man-made environments – old buildings, rifts on the walls; (Killick-Kendrick, 1987a and 1987b; Naucke 2002). Supporting walls constructed with large boulders were also identified as breeding habitat for *Phlebotomus sergenti* although they are less important than caves. A great amount of larvae of *Phlebotomus perfiliewi* flies and a few larvae of *Phlebotomus perniciosus* were collected by Bettini et al (1986a) from a breeding site inside an abandoned cement building in Sardinia. Dantas-Torres et al. (2010) found a colony of *Phlebotomus papatasi* on the second floor of an old building in a highly urbanized area of Southern Italy (the old town of Bari). They found the insects near a bed and in a bathroom and potential blood sources (from e.g., pigeons and dogs) were abundant in the neighborhood. In Germany, *Phlebotomus mascittii* can usually be found close to human dwellings (Naucke and Pesson, 2000). Kamhawi et al. (1991) made the largest *Phlebotomus* collections from natural habitats, uninhabited and inhabited houses. They concluded that sand fly populations are able to recover, and often exceed their original natural densities in man-made artificial habitats.

Phlebotomus *species and leishmaniasis in Hungary*

The first observations of *Phlebotomus perfiliewi* in Hungary were reported from houses in Hódmezővásárhely (southwestern Hungary) in the summers of 1931-32, since the local residents noticed unusual skin symptoms related to insect biting (Szentkirályi and Lőrincz 1932). Leiashmanisis can become a real emerging disease in Hungary: in 2007 and 2008 autochthonous canine leishmaniasis cases were observed in Southwestern Hungary and Budapest (Tánczos et al., 2012). *Phlebotomus perfiliewi* and *Phlebotomus papatasi* are endemic in Baranya county (Southwestern Hungary), *Phlebotomus mascittii* and *Phlebotomus neglectus* are endemic in Csongrád county (Southeastern Hungary) and in Törökbálint (a suburb of the capital), (Szentkirályi and Lőrincz 1932). In contrast to the statement of Kuhn (1999), *Leishmania infantum* complex is not prevalent only in areas with average temperatures ranging between 5–10 °C during the coldest month (January), while in the Southwestern part of Hungary the mean temperature in January is approximately 0 °C and the canine leishmanias is prevalent in this area.

The most northward observed geographical distribution of **Phlebotomus** *species*

The known, most northward distribution of *Phlebotomus neglectus* observed in Europe in 2012 was Törökbálint, one of the suburbs of Budapest, at latitude 47°28 N, (VBORNET, 2012), while that of *Phlebotomus ariasi* was in Paris (VBORNET, 2012) at latitude 48°51 N. In both cases, the areas are isolated from the main, continuous distribution ranges.

The studied **Phlebotomus** *species and the abiotic factors of overwintering and activity*

We studied the potential overwintering of six *Phlebotomus* species (*Table 1.*). According to Killick-Kendrick (1987a, 1987b), the *Phlebotomus* species in the temperate areas of Europe can overwinter at the ontogenetic stage of 4th instar larvae. Larvae need enough heat, humidity and organic matter to survive and develop (Naucke, 2002; Lindgren et al., 2004). The different *Phlebotomus* species have different minimum temperature tolerances: -4 °C of *Phlebotomus perfiliewi*, *Phlebotomus neglectus*, *Phlebotomus papatasi*, *Phlebotomus mascittii*; 0 °C of *Phlebotomus perniciosus;* and 5 °C of *Phlebotomus ariasi* (Killick-Kendrick et al., 1984; Killick-Kendrick, 1999; Singh, 1999; Naucke and Schmitt, 2004). According to Lindgren et al. (2004) the preferred humidity for adults of *Phlebotomus neglectus* and *Phlebotomus perfiliewi* is between 60-80%, and for *Phlebotomus papatasi* and *Phlebotomus sergenti* is below 45%. While the number of days with suitable temperature for reproduction and larval growth, and moisture index have a strong correlation with the ontogeny and growth of the *Phlebotomus* larvae (Killick-Kendrick, 1987a; Oshagi et al., 2009), low temperatures and precipitation are expected to limit *Phlebotomus* distributions.

Heat island effect of Budapest

Heat island effect is an important additional climate-moderating effect, which has similar importance attenuating the winter minimum temperatures to the effect of heat bridges (Santamouris, 2001). The main causes of heat island effect are: increased retention of solar heat energy by open concrete and asphalt surfaces, infrared radiation, which is absorbed and reflected by photochemical smog, and the lack of shading and transpiring vegetation and evaporating wet soil surfaces (Bradley, 2007).

Urban heat island intensity depends on the size of the city (the spatial scale and the population size), the characteristics of the surface (e.g. the albedo of the surfaces, the amount of green spaces) and the natural and modified topography, the anthropogenic heat release (thermal pollution), and the prevailing wind direction (Kim et al., 2004). There is a positive correlation between the maximum heat island intensity and the order of magnitude of the population size of the settlements. The steepness of correlations shows differences in North America, Japan and Western Europe (Oke, 1973; Heinrich et al., 2006). The mean ambient temperature is increasing from the border of the populated area to the geometrical center of the city (Santamouris, 2001).

In the case of Budapest, according to the measurements of the MODIS (Moderate Resolution Imaging Spectroradiometer) multispectral sensor of satellite Aqua and the meteorological stations, the maximum difference between the downtown of the city and the Plain of Pest, which is surrounding Pest (the east part of Budapest), can be (-6)–(-7) °C in winter nights (Pongrácz et al., 2006; Bartholy et al., 2009; Lelovics et al., 2012)

The tempering effect of thermal bridges

Female sandflies lay eggs in batches, tree holes, animal shelters, and inartificial crevices, fissures, as well as cracks of weathering construction materials which are dark and rich in organic matter (Shortt, 1930; Szentkirályi and Lőrincz 1932; Yuval, 1988). Since fissures of walls, joint gaps, foot separations interrupt the continuity of the walls and the isolations form thermal bridges. The survival of the eggs and the larvae is also dependent on the appropriate humidity and temperature of their microenvironment. If protection from excessive sun radiation and availability of organic materials are assured, thermal bridges can provide such microenvironment on buildings.

In heated buildings, temperature increases toward the interior of the building. A thermal bridge is created when materials that are poor thermal insulators come into contact, allowing heat to flow through the path of minimum thermal resistance (R-value or a material's effectiveness of resisting the conduction of heat) created, although nearby layers of material separated by airspace allow little heat transfer (Binggelli, 2010). Thermal bridges can be either integral parts of a construction or they can be generated by weathering processes. Weathering can also liberate minerals essential for microbial life, which will cause the enhancement of organic materials (Loughnan, 1969). Formation of fissures, cracks or loss of surface material occurs on the outer surface, consequently, thermal conductivity of the deteriorated parts increases. Moreover, cracks and fissures can provide “artificial micro-caves” with sufficient survival and growth conditions for the larvae, as it is described below. Furthermore, microbial activity itself accelerates the chemical weathering of the material by producing organic acids (Banfield, 1999; Drever, 1997). The presence of organic materials is essential for the colonization of the spot by the *Phlebotomus* larvae (Killick-Kendrick, 1987.a; Bettini, 1988). Despite the low solar radiation in the outer surface of buildings, one can observe the colonization of mosses or even little ferns in the entrance, green algae and blue bacteria.

The permanently wet surface plays the role of an absorber for the polluted air and dust (Török, 2002) which can carry organic and mineral matters which are suitable for mold species. *Aspergillus versicolor* Vuillemin requires at least 79% moisture to grow. Besides condensation on the surfaces due to the thermal bridges, permanently wet surfaces can also contribute to the wetting of the wall by capillary absorption from the soil. Broken rain water conducting elements, such as gutters or hanging gutters, or intense seasonal precipitation wetting the walls, such as during a monsoon-period have similar effects.

Altogether, the main role of thermal bridges in the survival of larvae is that they can conduct heat and moisture to the outmost parts of the building partitions, thus creating a sufficient microenvironment for larval growth. These places are much easily accessible for the insects than interior areas.

Materials and methods

The approach of the study

According to the above detailed literature, natural refuges, such as leaf piles and artificial ones, as well, such as cracks of the buildings may be adequate habitats for sandflies and can serve as winter shelters. We aimed to study the additional heat surplus of thermal bridges (microclimatic factor) and the urban heat island effect (mesoclimatic factor), and also synthesize these effects. Thermal bridges of cracks and corners are air-polluted outdoor micro-environments where sandflies can overwinter.

Thermal imaging

We used a Testo thermometer. The camera has 845 measure points as close as ¼ inch. It displays temperature with minimum and maximum values within the same thermal image.

Not only the ambient air temperature, but also the temperature of the wall surfaces was measured, both within and outside of the thermal bridges. Since the temperatures of the warm thermal bridge and the surrounding cold wall surfaces were recorded within one thermal image, we characterized the temperature intensity of the wall surfaces, which was characterized by the measured minimum and maximum temperatures of the image.

Temperature values obtained with the Testo thermal imager may differ from the actual temperature values, thus they were calibrated with the temperature values measured on the spot with the help of a GANN Elektronik-Feuchtemesser [Nr.: 6483 Hydromette RTU 600 (sensor) IR 40 (head)] electronic thermometer.

We measured environmental temperatures in two periods, one was a colder period (six measurements on six days between the 23rd–30th of January), the other was relatively warmer (six measurements on six days from 25th of February to 13rd of March). These two time periods represented average winter conditions in Budapest with average outdoor temperatures. Surface temperatures were measured in the mornings, around 7:30 AM and 8:00 AM, since the morning temperatures are the lowest during the day, in general. This time the spots were shaded. During the period of measurements the sun rose between 7:21 AM (23rd of January – first day) and 6:54 AM, respectively (25th February – last day).

Site of the study

Budapest is the capital and the largest city of Hungary and also the largest city in East-Central Europe with its 1.74 million inhabitants (and with the 525 km^2 area). Together with the population of the Commuter Area, the population of the urban and their suburban settlements reaches 3.3 million. (Hungarian Central Statistica, 2011). The North-South extension of Budapest is 25 and 29 kilometers in East-West direction.

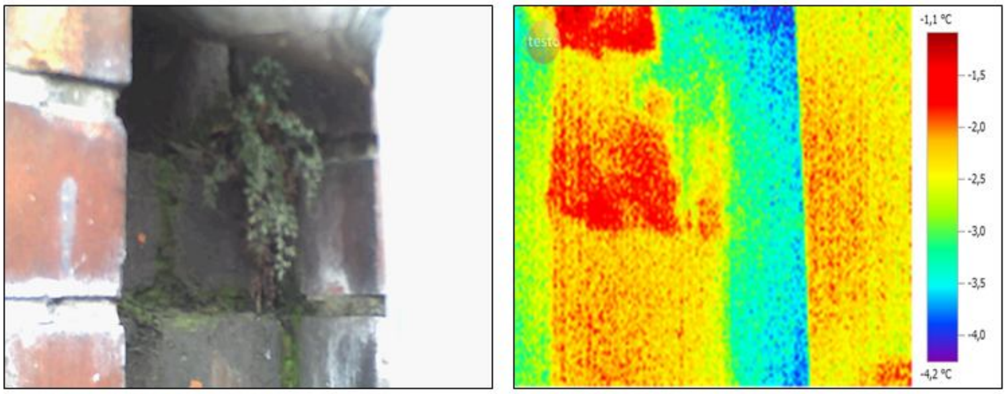
In the 1960's, the fellows of the National Public Health and Medical Services observed the presence of *Phlebotomus perfiliewii* in the Paulin Monastery in the Gellért Hill Cave [47.486628N, 19.048276E or 47° 29′ 5,62″ N, 19° 03′ 7,66″ E; the elevation from the mean see level is 235 m;] (Szentkirályi and Lőrincz, 1932) in Budapest, which is very close to the recent site of the study – the distance is about 700m. More recently, in 2009-2006 the presence of *Phlebotomus neglectus* and *Phlebotomus mascittii* were observed in Törökbálint [47.435648N, 18.915658E or 47° 26' 8.33" N, 18° 54' 56.37" E]. The distance from the study area is about 12 km (Tánczos, 2012).

Since the 1960's, when the internal surface of the cave has been renovated by cement-based binder, we found no alive sandflies in that location. In our research, several parts of two old buildings were examined [47° 28′ 53″ N, k. h. 19° 03′ 19″ E]. Both buildings are situated along the riverside of the Danube and in the proximity of Gellért Hill.

In our research, two building parts were examined of two university buildings, on two different spots. Both buildings are situated in the campus of the Budapest University of Technology and Economics, which lies on the riverside of the Danube. Two thermal bridges with a moderate-low heat intensity were selected, which can be found in many places in Budapest. It should be noted, that there are even warmer thermal bridges, but our aim was to study general spots of general buildings. One of the two spots is located on a vertical and full-height nook on the Southern facade (marked with S1), the other one in a negative building edge facing South-East (marked with S2). Spot S1 is situated on a brick wall facade inside a nook with varying depth between 13 and 3 cm, and protected by a plastic gutter pipe (d=90 mm) against the sun rays. Moreover, due to the capillary adsorption of water from the soil and wetting due to the broken gutter-pipe, spot S1 is almost always wet. This is further proved by the presence of moss colonies on the surface. Negative edge S1 has an ooidic, porous limestone cover, and it is partly shaded by a plastic gutter pipe with a diameter of 15 cm. The appearance and the location of the two spots can be seen in *Fig. 1*.

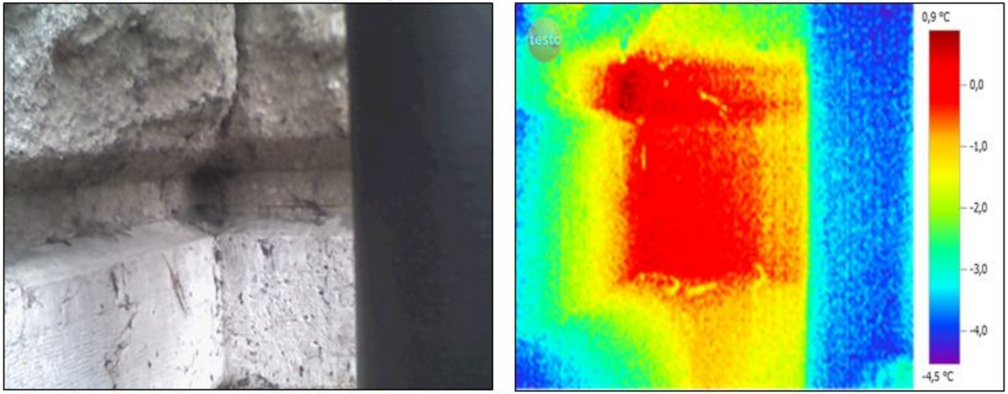
On the Spot S2 a wall rue, *Asplenium ruta-muraria* L. individual fern and *Bryum argenteum* Hedw. and green algae math were found. *Asplenium ruta-muraria* is a typical lithophyta, which originally lives on dry to moist, shaded limestone outcroppings, calcareous rocks (Vogel et al., 1999). The presence of this fern and the non-vascular plants indicate moisture and shading. *Bryum argenteum* is a common moth which can tolerate dry and sunny environments and prefers alkali soils.

Not only the surface materials, but the involved structures of the two spots are also different. Both of them are outer walls, separating heated internal spaces and environmental conditions outdoor. Spot S1 is located in a wall, whose structure is the following from the inside to the outside: 2 cm of color plaster on lime plaster, 44 cm large sized brick and 12 cm small-sized covering brick. The structure of the wall involving spot S2 is the following from the inside to the outside: 2 cm lime plaster, 48 cm large-sized brick and 25-20 cm porous and freshwater limestone covering. The location of the spots, as well as details and layers of the wall can be seen in *Fig. 2a.*, and *Fig. 2b*.



Spot S1 – brick cover and nook of the gutter

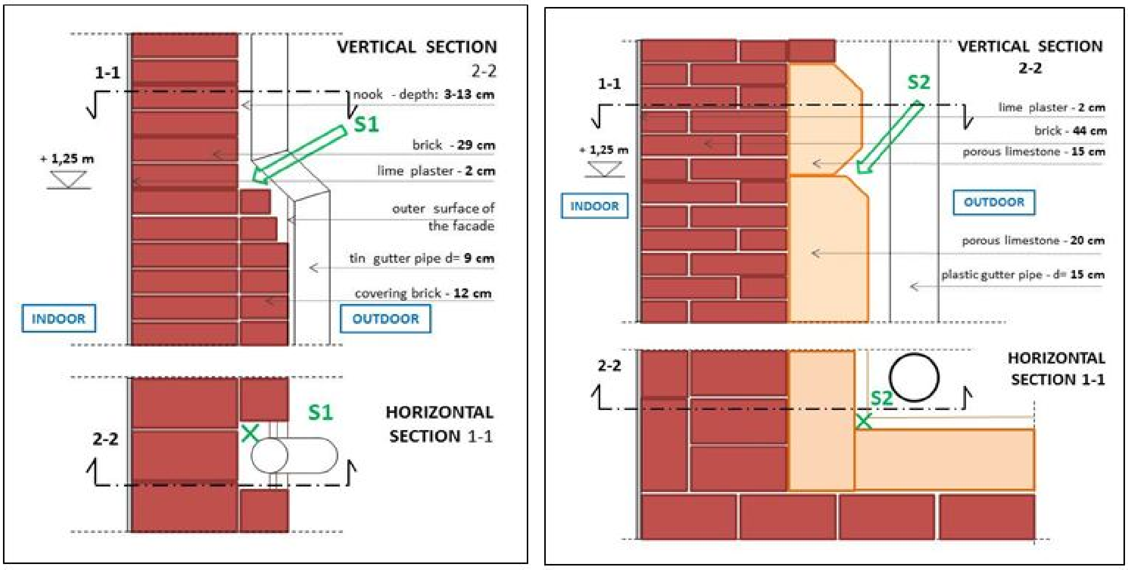
Thermal image of Spot S1



Spot S2 – Joint of porous and freshwater limestone

Thermal image of Spot S2

Figure 1. *Appearance and thermal images of the two spots. Colors represent the temperature differences between the coldest (purple) and the warmest points (red)*



a., Spot S1 **b.,** Spot S2

Figure 2. *Details and layers of the wall structures involving spots S1 and S2 (marked with green arrow and green ×)*

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These two spots are partly protected from weather conditions. The gutter pipes serve as a shader from solar radiation, as a wind-stopper, as well as a source of water in the case of S1 spot. Due to their orientation and location, both spots are protected from the relevant direction of the wind, which is North-West to South-East, since the Gellért Hill develops a wind-tunnel. From the Northern side both spots are protected by the buildings itself, and due to the height of the buildings, the effect of airflow is minimal. From the Southern direction S1 is partly protected by trees and bushes growing nearby and by the body of the gutter. S2 is protected from this direction by the Southern wing of the U-shaped building, and also by the gutter-pipe. As for protection against excessive rain-wash, both the pipes and the drips of the buildings are effective.

Data sources

Climatic data of Budapest

The ground temperature data of Budapest and its surroundings were obtained from MODIS (Moderate Resolution Imaging Spectroradiometer) sensor of the Aqua satellite of NASA (Gutro et al. 2002). The satellite has quasi-polar orbit and makes images between 02-03 UTC and between 12-13 UTC (Wan 1999). Since the colder temperatures had importance in this research, the images were obtained at night. A grid with 4900 points was selected between latitude 47.2208N-47.7958N and longitude 18.6073E-19.6740E. Between 2003-01-01 and 2008-12-31 643 images were taken which had less than 20% data absence. The 12 images which were selected among them can be seen in *Fig. 3* with the urban mean temperature and the difference of urban and rural temperatures (calculated by Lelovics et al. 2011). The selected images were taken in 2007-11-29, 2007-01-27, 2005-01-24, 2003-01-16, 2008-11-23, 2003-12-07, 2007-02-04, 2004-12-07, 2006-12-11, 2005-11-03, 2005-04-05, and 2003-10-14 with urban mean temperatures -6.02899, -5.91848, -3.99395, -3.88729, -1.96469, -1.9346, 0.16965, 0.31202, 2.0499, 2.11026, 3.93641, and 4.19791 °C, respectively.

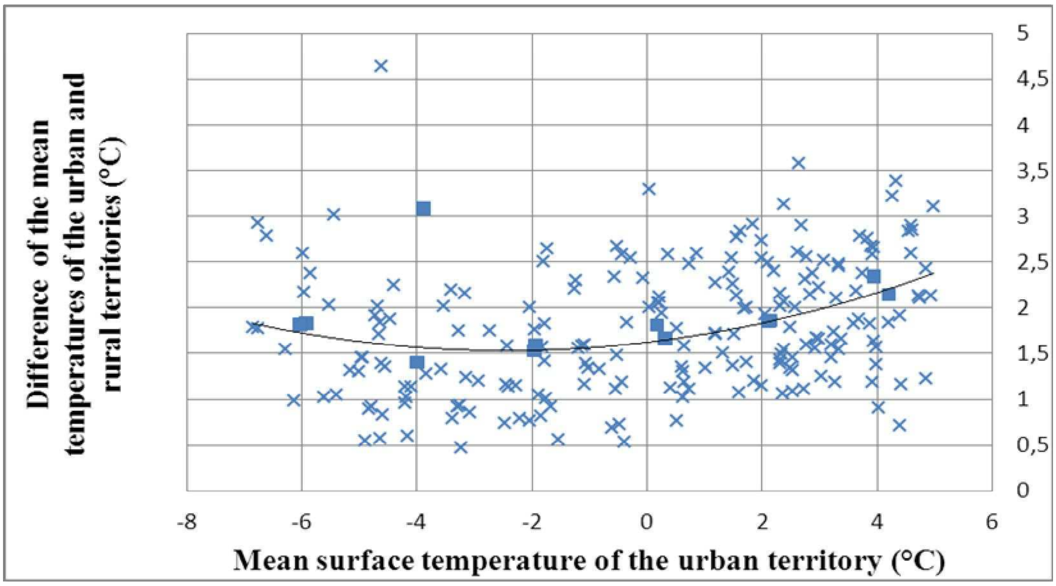

Figure 3. *The difference of the mean temperatures of the urban and rural territories with a second order regression line. Only the days with less than 20% data absence and with urban mean temperature between -7 – 5 °C are displayed. The selected 12 days are marked with square symbol.*

Software and statistics

For processing the thermal images, Testo’s own software, IRSoft Testo program was used. For linear regression analysis Microsoft Excel 2010 was used. R statistic analyzer and Microsoft Excel 2010 were used as data preprocessors. GIS (Geographic Information System) modeling was achieved by ESRI ArcGIS 10 software, and the output layouts were edited by Adobe Photoshop.

The physical characteristics of the covering materials of the studied surfaces

The covering material of the first thermal bridge was brick, and that of the second one was limestone. The composition of the covering material determines e.g. the pH of the surface, the insulation properties, the rate of water absorption and the albedo. In the present study, thermal conductivity and heat loss of the thermal bridges were considered. Firstly, thermal conductivity value and the thickness of the different layers determine the thermal gradient of the layers (*Table 2).* With the help of these data, the heat loss in a building element (the u-value) was calculated in W/m^{2}K. The importance of such a calculation is that temperature inside a deep crack located in an outer layer with very low thermal conductivity value can be much higher than the external temperature. Both thermal properties of the materials, as well as the facilitated calculation method used for the heat-balance were obtained from the educational material of the Building Physics courses of the Faculty of Architecture and Faculty of Civil Engineering of the Budapest University of Technology and Economics. Properties and calculated gradients of the layers are listed in *Table 2*.

Table 2. *Detailed dimensional, quantitative and thermal properties of the layers in the wall structures of the spots S1 and S2*

Layers	**thick-ness**	**Appa-rent density**	**Thermal conduct. (λ)**	**Heat transfer coeff.**	**Thermal resistance (R)**	**Proportion of thermal resistance**	**Fall of temper. inside the layer**	**Fall of tempe-rature**
	m	**kg/m³**	**W/mK**	**W/m²K**	**d/λ**	**Ri/R**	**at dTi-e = 23oC**	**per cm**
				S1				
h_e				24,000	0,042	0,060	1,38	
Covering brick	0,12	1800	0,930	7,750	0,129	0,186	4,29	0,357
Brick wall	0,29	1730	0,780	2,690	0,372	0,537	12,35	0,426
Lime plaster	0,02	1650	0,810	40,500	0,025	0,036	0,82	0,410
h_i				8,000	0,125	0,181	4,15	
Summa					0,692	1,000	23,00	
Thermal insulance (u)					**1,44**			
				S2				
h_e				24,000	0,042	0,040	0,92	
Porous limestone	0,15	1300	0,520	3,467	0,288	0,276	6,36	0,424
Brick wall	0,44	1730	0,780	1,773	0,564	0,540	12,43	0,282
Lime plaster	0,02	1650	0,810	40,500	0,025	0,024	0,54	0,272
h_i				8,000	0,125	0,120	2,75	
Summa					1,044	1,000	23,00	
Thermal insulance (u)					**0,96**			

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Modeling

It was supposed that the impact of urban heat island effect, the shelter of the thermal bridges and future climate change allow sand fly species to overwinter in heat-polluted environments. Three different models were built in order to investigate whether Budapest and its surroundings provide suitable environment in terms of climate for the six sand fly species. The studied species were assorted by their cold tolerance in three groups (*Table 1.*). There are three components used in the models: a., heat island effect (*Eq. 1*), b., the effect of thermal bridges, and c., the effect of the predicted climate change. In Method 1. only component a., was considered. In Method 2. components a., and b., (*Eq. 2*), and in Method 3. all the three components were considered (*Eq. 3*).

$$T_1 = T_0 - \Delta T_{UHI} + \Delta T_{air} \quad \text{(Eq. 1.)}$$

$$T_2 = f_{TB}(T_0 - \Delta T_{UHI}) + \Delta T_{air} \quad \text{(Eq. 2.)}$$

$$T_3 = f_{TB}(T_0 - \Delta T_{UHI}) - \Delta T_{FP} + \Delta T_{air}, \text{ where} \quad \text{(Eq. 3.)}$$

ΔT_{air}: difference of air and ground temperature
ΔT_{UHI}: temperature difference caused by urban heat island
$f_{TB}(T)$: sheltering effect of the thermal bridges
ΔT_{FP}: temperature difference of the reference period and the future prediction

Conversion from ground temperature to air temperature (at 2 m height) was applied in all of the three methods based on averaged differences in six observation points in the studied domain (Lelovics, 2012). The observations were made in winter at night. The calculated difference (1.84 °C) has the opposite sign to the three aforementioned temperature altering effects (the air temperature is greater than the ground temperature).

The effect of urban heat island was calculated by a script written in R for all the 4900 studied points. Our preliminary calculation showed that both second order polynomial regression and linear regression with major axis method are highly unstable for calculating the base temperature dependence of the volume of the urban heat island. Therefore simple averaging was done based on the 12 selected images. The maximum heat surplus of the urban heat island was +4.10 °C.

The sheltering effect of the thermal bridges was calculated based on the measurements made on the spot S2. Linear regression was made, since the volume of the temperature difference showed correlation with the base temperature. The warming effect of the predicted climate change was approximated by averaging the warming in the 48 points of the domain. The value occurred to be -14.36 °C in the reference period; -10.28 °C in the prediction period, therefore 4.08 °C was subsequently used as the difference.

The result of the three methods in the case of the three groups was displayed by ESRI ArcGIS 10 software. The grid was interpolated by inverse distance weighted (IDW) method with power 2 and 12 neighboring points. The results were clustered between isotherms of the even integer numbers in the Celsius scale. Digital NUTS3 polygon borders were displayed to help orientation (GISCO 2013).

Results

Differences in thermal behavior of the two spots

Great difference was calculated between the thermal insulation capacities of the two wall structures containing the two spots S1 and S2. The wall containing S2 turned out to be 33% better insulator (u-value of 0.96 versus 1.44) than S1, which suggests that heat loss is more intensive in the case of spot S1. However, temperatures of the limestone surface were found to be higher in most of the cases: in 66.67% of the measured minimum temperatures and in 75% of the measured maximum temperatures. Moreover, strong correlations were found (S1: $y = 1.011x + 3.765$; $R^2=0.9785$ and S2: $y = 0.824x + 4.715$; $R^2=0.946$) between the minimum and the maximum temperatures of the thermal bridges – see *Fig. 4.* Different gradient and intersection of trend lines fitted on the measured values showed that the two thermal bridges behave differently due to the changes of the ambient temperature.

Moreover, regarding both the minimum and maximum temperature values, relative differences are higher in the colder period (rel. difference of minimum values: 1.48 ± 1.26 °C and 3.02 ±1.07 °C for the maximum values) than in the milder period with higher temperature values (rel. difference of minimum values: 0.65 ± 0.89 °C and 1.23±1.60 °C for the maximum values). According to the fitted linear trend this means a 3.88–3.59 °C permanent difference in the 10–(-15) °C interval for S1 and a 2.96–7.35 °C difference in the 10–(-15) °C interval for the S2 – see the two trend lines moving away from each other in *Fig. 4.*

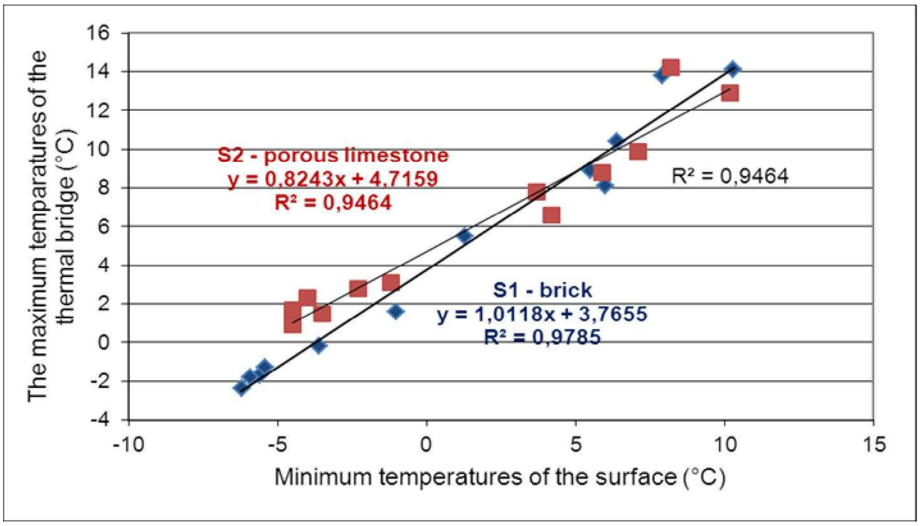

Figure 4. *Correlation between the minimum and maximum surface temperatures measured on the two spots*

Using the linear trends we calculated the expected maximum temperature of the thermal bridges according to the minimum temperatures of the surfaces. The intersection point of the two trend lines was calculated to be P (5.0688 °C; 8.894 °C), which shows that, for the minimum temperatures lower than $x=5.0688$ °C, the differences between the maximum and minimum temperatures measured on the spots will be higher for the limestone cover.

Secondly, the maximum surface temperatures were compared to the limits of the temperature tolerance of the different species, in order to evaluate the ambient minimum temperature limiting the survival of the *Phlebotomus* species – see *Fig. 5*. By calculating the first coordinates of the intersection points of the trend lines and the horizontal limits, the critical minimum temperatures were determined. It is noticeable that due to the calculated heat surplus of the thermal bridges, different sandfly species are able to tolerate environmental temperatures ranging from +1.60 (brick surface – *Phlebotomus ariasi*) to -12.61 °C (limestone surface – *Phlebotomus mascittii, Phlebotomus neglectus, Phlebotomus papatasi* and *Phlebotomus perfiliewi*).

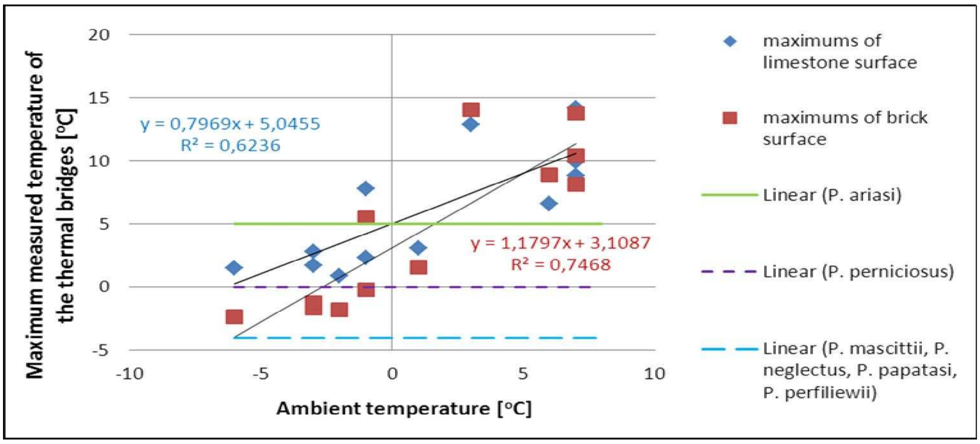

Figure 5. *Correlation between the maximum surface temperatures measured on the two spots and the ambient temperature (S2 – blue dots; S1 – red dots) with the minimum temperature tolerance of the sandfly species groups (marked with vertical coloured lines).*

Impact of urban heat emission on the potential distribution of sand flies

The result of the linear regression based on the measures at spot S2 is shown in *Eq. 4*.

$$T_{min} = 0.1481 * T_{max} - 5.3280\ °C \tag{Eq. 4.}$$

The descriptive statistics of the three methods in the case of the three studied groups was summarized in *Table 3*.

Table 3. *Descriptive statistics of the three group (1: Phlebotomus ariasi, 2: Phlebotomus perniciosus, 3: Phlebotomus (Tr.) mascittii, Phlebotomus neglectus, Phlebotomus papatasi, Phlebotomus perfiliewi) and the three methods (specified in the text).*

(°C)	Minimum	Maximum	Mean (all)	Mean (rural only)	Mean (urban only)
Method 1. (Group 1.)	2,7250	9,4451	6,5981	6,8295	4,9017
Method 1. (Group 2.)	-2,2749	4,4451	1,5981	1,8295	-0,0982
Method 1. (Group 3.)	-6,2749	0,4451	-2,4018	-2,1704	-4,0982
Method 2. (Group 1.)	-2,4718	5,2433	1,9747	2,2404	0,0271
Method 2. (Group 2.)	-8,2122	-0,4970	-3,7656	-3,4999	-5,7132
Method 2. (Group 3.)	-12,8046	-5,0893	-8,3579	-8,0922	-10,3055
Method 3. (Group 1.)	-6,5545	1,1606	-2,1079	-1,8422	-4,0555
Method 3. (Group 2.)	-12,295	-4,5797	-7,8483	-7,5826	-9,7959
Method 3. (Group 3.)	-16,8873	-9,1720	-12,4407	-12,1750	-14,3882

The result of the GIS modeling can be seen in *Fig. 6*, *Fig. 7* and *Fig. 8*.

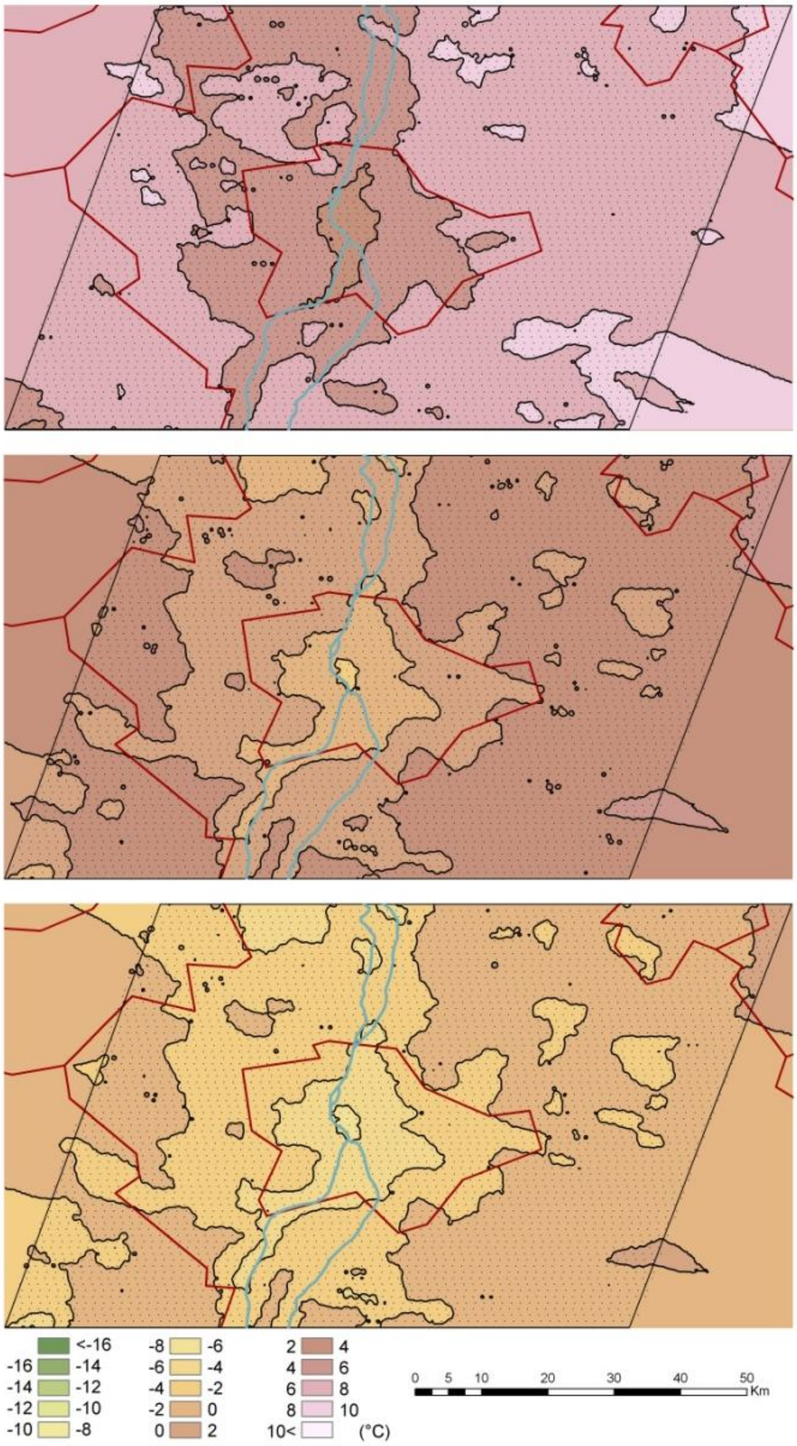

Figure 6. *The average air temperature of the region which can be tolerated by Phlebotomus ariasi in a certain location with urban heat island (Method 1., upper image), with urban heat island and the sheltering effect of the thermal bridges (Method 2., middle image), and with urban heat island and the sheltering effect of the thermal bridges in the period of 2025-2050 (Method 3., lower image)*

The displayed temperature values mean the average air temperature of the region which can be tolerated by the species in a certain location in the case of the three methods.

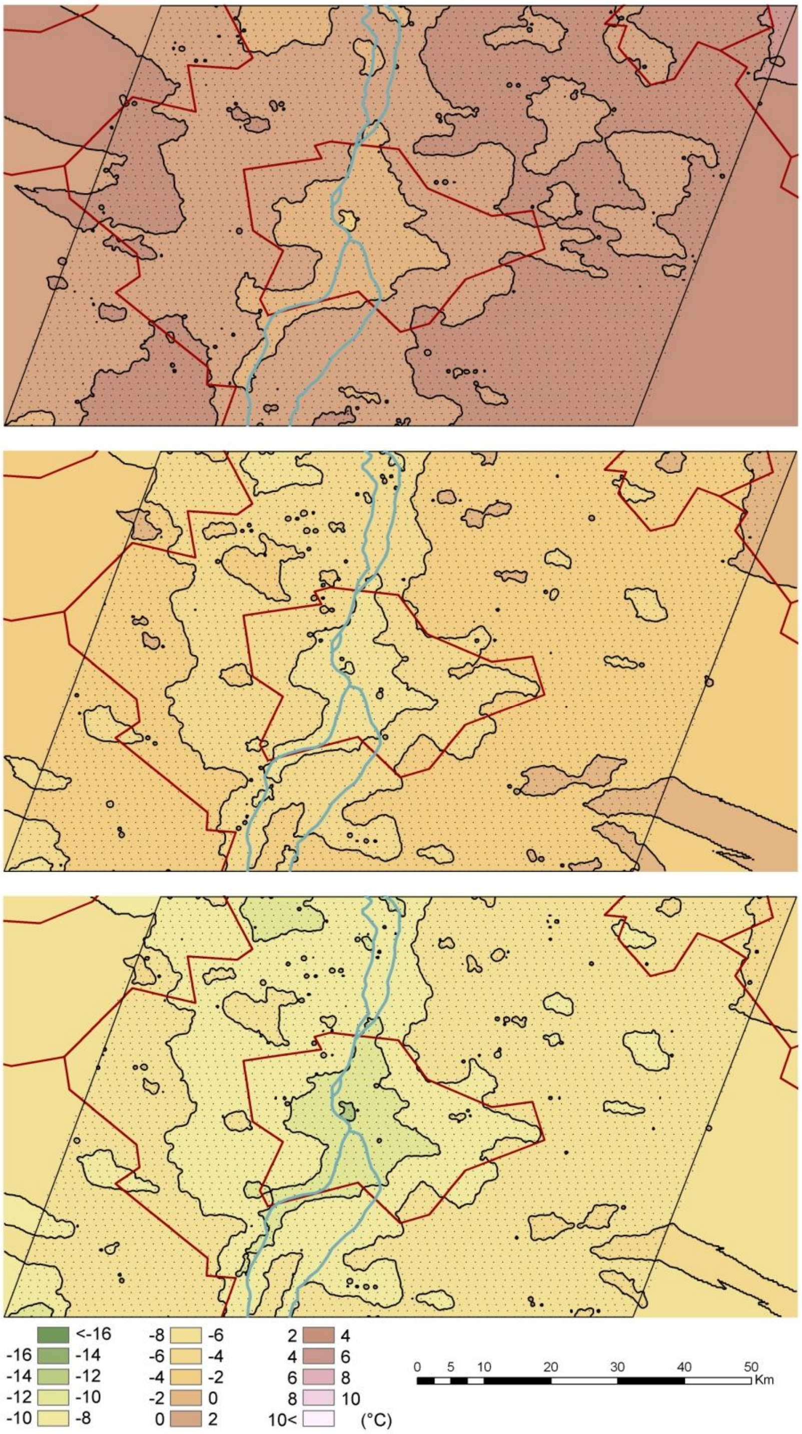

Figure 7. *The average air temperature of the region which can be tolerated by Phlebotomus perniciosus in a certain location with urban heat island (Method 1., upper image), with urban heat island and the sheltering effect of the thermal bridges (Method 2., middle image), and with urban heat island and the sheltering effect of the thermal bridges in the period of 2025-2050 (Method 3., lower image)*

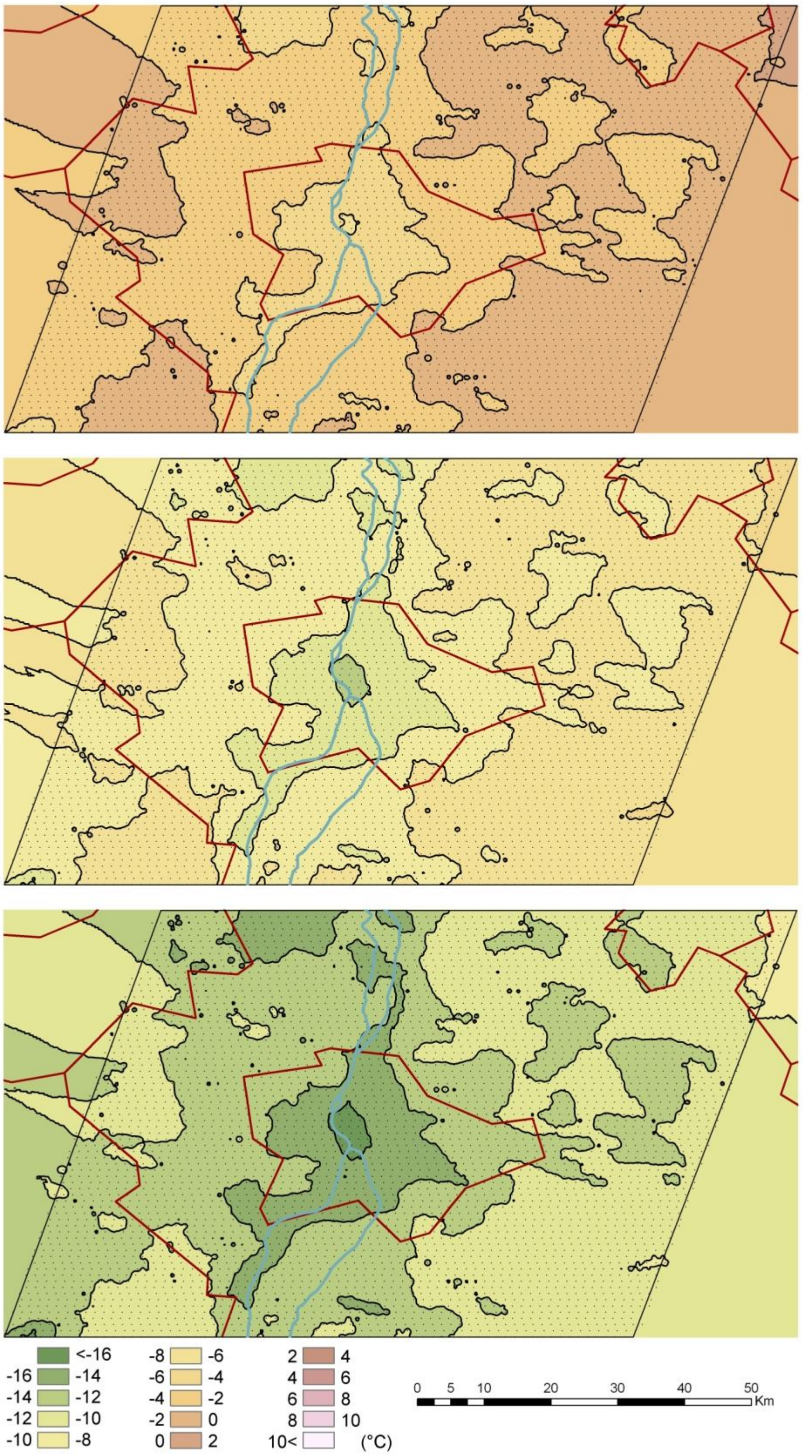

Figure 8. *The average air temperature of the region which can be tolerated by Phlebotomus mascittii, Phlebotomus neglectus, Phlebotomus papatasi and Phlebotomus perfiliewi in a certain location with urban heat island (Method 1., upper image), with urban heat island and the sheltering effect of the thermal bridges (Method 2., middle image), and with urban heat island and the sheltering effect of the thermal bridges in the period of 2025-2050 (Method 3., lower image)*

Discussion and future perspectives

The recent occurrence of sandfly species (*Phlebotomus neglectus*, *Phlebotomus mascittii)* in the agglomeration of Budapest could not have been achieved without the tempering effect of the heat-polluted shelters, since the recent winter outdoor ambient temperatures of Budapest could not allow the outdoor overwintering of *Phlebotomus* species since the annual average minimum temperature of the surrounding areas of Budapest is between approximately -17.8 to -15.0 °C. According to our model results, only Method 3 predicts possible overwintering, and only for the *Phlebotomus mascittii*, *Phlebotomus neglectus* in the non-heated, non-protected environments. Since the average annual minimum temperature is about -15 to -12.2 °C, according to the Method 1 (using only the present effect of the urban heat island) at the observation point of these species in Törökbálint (in the agglomeration of Budapest), *Phlebotomus mascittii*, *Phlebotomus neglectus* can overwinter only in milder shelters which have a +10 to +13 °C surplus compared to the external environment. Using Method 2 (which operates with both the urban heat effect and thermal bridges), *Phlebotomus mascittii*, *Phlebotomus neglectus* can overwinter in warmer shelters where the temperature is warmer by 0.8 to 5 °C than the external environment. This means that the observed two sandflies can overwinter only in protected or indoor environments in the studied area. The winter hardiness of the species of the other two groups is less pronounced.

According to the *in situ* measurements, the maximum surface temperatures were 3–7 °C higher than the ambient temperature due to the effect of the thermal bridges at the studied points. Moreover, temperatures of the limestone surface were found to be higher in most of the cases: in 66.67% of the measured minimum temperatures and in 75% of the measured maximum temperatures. The first reason for the higher surface temperatures of the limestone surface compared to the brick covering in colder weather is that the latter one is a wetted surface. Since the specific heat of the water is very high (4.2 kJ/kg ^{o}C), a wetted material cannot follow the changes of the environmental temperature as fast as a dry material. Moreover, the higher surface temperature of the limestone surface can also be contributed to the lower thermal conductivity value of the limestone (3,467 W/m^{2}K) compared to the brick (7,750 W/m^{2}K), and the overall better thermal insulance value of the limestone-covered wall. Lower thermal conductivity means higher specific heat, thus higher heat capacity. The higher heat capacity of the limestone covered wall due to the higher u-value means that limestone adapts to the changes of the environmental temperature much slower than brick. Moreover, with decreasing the environmental temperature, the limestone cover has a larger amount of heat support from the backing wall due to its higher heat capacity, which slows down the temperature decrease of the covering material. The correlation was found to be stronger between the minimum and maximum temperatures of the surfaces, than between the maximum surface temperatures and the ambient temperatures. This result further indicates that the two wall structures show a high inertia towards changes of the ambient temperature.

The above mentioned results showed that factors, for instance water-content and type of the material highly affect the intensity of thermal bridges. The flora and the microbial math of the studied wet brick thermal bridge (S1) showed that due to the leaving gutter, organic matter accumulation is observable in the cracks and in the join gaps, which is essential for the potential colonization of sand fly larvae. Below 5 ºC, the surface temperature of the wetter brick surface (S1) is lower than the surface temperature of the limestone (S2), maybe due to the evaporation of the water. In this comparison, the limestone surface seems to be milder, but since *Phlebotomus* larvae prefer wet mats and high air vapor-content, our opinion is that wetted bricks provide (as well as wetted non-porous, dense stone surfaces, concrete, etc.) a more adequate basis for modeling and predictions. However, thermal bridges in a wall structure with high heat capacity, thus high thermal insulation property, covered with well-insulating materials (for example porous stone materials: tuffs, sandstone and limestone) can provide high and permanent heat surplus even in the case of sudden falls of temperature.

The heat island effect of Budapest is notable and can exceed or can be as important as the minimum temperature moderating the effect of thermal bridges. We found that any scale of the thermal pollution – as the local microclimate-moderating thermal bridges or the mesoclimate moderating heat island effect – can highly change the original outdoor climate patterns. Our findings are in accordance with those results of Révész (2008) that the climate change in Hungary may cause serious public health and veterinary problems.

As *Phlebotomus* species have a wider distribution area than leishmaniasis itself, the expected future migration of sand flies due to climate change does not imply the same spread of the disease into the Northern regions of Europe. This contradicts with the observation in Hungary that geographical occurrence of canine leishmaniasis and the distribution of the potential vector *Phlebotomus* species of *Leishmania infantum* is nearly the same, except for the area of the Hungarian capital (Tánczos, 2012).

Our results suggest that the heat island effect of the capital and its agglomeration can explain the observed isolated, most Northern presence of some *Phlebotomus* species, while anthropogenic heat pollution (e.g. in the case of thermal bridges and cracks, fissures in the walls) can create the conditions for winter survival for sand flies. It is important to note that the importance of these isolated *Phlebotomus* populations can play very important role as the transmitters of *Leishmania* parasites to human beings, since more than 2.2 million people live in Paris and about 1.7 million in Budapest and with the commuters of the agglomerations the affected population can be 2-3 times higher.

It is important to emphasize that the future population, the structure of the city, the isolation techniques and the matter of energy production will probably not be the same some decades later.

Conclusions and adaptation strategies

Regarding the fact that the agglomeration of Budapest in the Carpathian Basin is the most Northern and isolated occurrence of the above mentioned species, it is plausible that sandflies can overwinter in heat polluted refugees. These refugees can be garages, unheated outbuildings, major cracks in the wall and protected surfaces of thermal bridges, as well. The highly populated Northern cities in the temperate areas are candidates to become the most Northern habitants of *Phlebotomus* species, and maybe that of leishmaniasis due to urban heat pollution. It is plausible that anthropogenic heat emission and the high domestic dog population can be the main causes of these distant, but existing distributions from the continuous endemic areas. In the further planning of the cities, several strategies should be taken into consideration, for instance reduction, or at least mitigation of the overwintering suitability for sandfly species by decreasing human heat emission. The adequate and modest use of the renewable energy sources may also provide a mitigation chance (Raghuvanshi and Chandra, 2008).

This purpose may be achieved by opening new wind tunnels, increasing the area of green spaces (green roofs, parks instead of parking places) and increasing the efficiency of thermal insulation of buildings.

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EDGE TRANSITION IMPACTS ON SWAMP PLANT COMMUNITIES IN THE NILGIRI MOUNTAINS, SOUTHERN INDIA

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Abstract. Swamps represent a relatively understudied ecosystem in many regions, which contrasts markedly with the research attention which other wetlands and Mangrove ecosystems have received. In the upper Nilgiris of southern India, montane swamps are restricted to geographic areas with flat surfaces and bounded by different edge transition vegetation types including grasslands and shola forests. Our study examined whether species richness, endemism, edge and the composition of swamp interior communities have a significant relationship with swamp area. Using species-area curves we continued sampling for species in each swamp until species richness reached the asympote within that swamp. Our results suggest that species richness (log n), log endemism, and edge and swamp interior species composition do not increase significantly with increasing area due to edge effect. Moreover, swamp area and vegetation parameters showed no significant relationships. However our results did indicate that swamps species richness and endemism were affected by abiotic and biotic edge effects, particularly physical topographic environment and the structure of adjoining matrix vegetation. Therefore swamp protection and restoration, in addition to the preservation and management of buffer regions may be important conservation criteria to preserve these fragile ecosystems.

Keywords: *endemism, ecotone, grasslands, Nilgiris, species richness, swamp area, sholas*

Introduction

Habitat loss represents one of the major drivers of regional species loss, and even total species extinction across much of the globe. Therefore developing methods to

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manage and mitigate the impact of habitat destruction on species diversity are major components of conservation strategy (He and Hubbell, 2011). Research is urgently required to develop the best methods to mitigate species loss, through targeted conservation and managing remaining intact habitat regions.

One of the oldest tenets within ecology is that of the species-area relationship (Arrhenius 1921; Gleason 1922; Rosenzweig 1997), whereby the number of species any habitat patch can retain is directly related to the size of that patch. Species-area relationships are also useful predictors to estimate the species richness a patch should be capable of retaining, and thereby a means of calculating the number lost through either habitat loss or other human actions (Rybicki and Hanski, 2013). A further debate is how the shape of the habitat, and the relative proportion of habitat edge (often characterised by denser vegetation and a higher incidence of invasion by alien species) to core habitat effects the ability of that patch to sustain species and how much it impacts (relative to overall area) on the retention of species within habitat fragments (Gonzalez et al., 2010).

Edge effects are also major drivers of change in many fragmented landscapes, but are often highly variable (in terms of structure, and effects) across space and time (Laurance et al. 2007; Murcia 1995; Ries et al. 2004; Laurance et al. 2007). However edge effects can have serious impacts on species diversity and composition, dynamics and ecosystem functioning (Saunders et al. 1991; Chen et al. 1992; Laurance et al. 2007), yet have only been properly considered in a small number of ecosystems.

The total area of a habitat patch/fragment has been found to relate directly to species richness in studies of many ecosystems across the globe (Rosenweig, 1997; Whittaker et al., 2001; William, 1943; Wilcox, 1980; Schoener and Schoener, 1981; Rydin and Borgegard, 1988; Holt et al., 1999; Moody, 2000; Gonzalez et al., 2010). This area to richness relationship exists across extended scales, with mid-domain effects also contributing at larger extents of intact habitat; such as the Amazon basin (Laurance et al. 2007). Interestingly, the number of endemic species is also significantly related with area (Moody, 2000), though this relationship is much more complicated as many endemic species occur in small areas, which are either isolated or have high turnover due to high topographic heterogeniety-and therefore endemism is a much more complex measure than simple species richness. Thus an understanding of floristic patterns and, the species-area relationship of any habitat type (especially rarer habitat types) potentially provides useful insights into the planning of conservation strategies for plant communities within such habitats.

Swamps (wetlands) represent biologically diverse assembelages of species, yet are amongst the most vulnerable ecosystems (Nirmal Kumar and CiniOommen, 2011; Puyravaud et al., 2012; Pitman et al., 2014). In addition to pressures on swamps through drainage and conversion to agriculture, swamps are made more vulnerable by their sparse distribution, as they represent isolated islands, patchy in nature and are dominated by endemic and endangered herbaceous species. This high endemism has been recorded and analysed in a number of regions including in the upper Nilgiris of southern India (Mohandass, 2008; Puyravaud et al., 2012; Mohandass, 2013). Within the Nilgiris region human usage, agricultural conversion and grazing pressures threaten many of the swamps within a formerly highly diverse region and thus these pressures may drive local, or even total extinction of species within the Nilgiris region (Puyravaud et al., 2012). As a consequence of these pressures swamps are becoming increasingly rare and fragile ecosystems throughout the Nilgiri Mountains.

However, knowledge of swamp plant communities succession and dynamism and community function has received little attention, and therefore determining the impacts of human mediated pressures on biodiversity are difficult to quantify, making prioritisation for conservation difficult or impossible. Because of the lack of baseline information for many regions the use of the relationship between swamp diversity and endemism relative to the area could provide a means of gauging the impacts on habitat loss or alteration on species diversity.

Tropical montane evergreen forest in the upper Nilgiri Mountains (locally termed *sholas),* are normally stunted and occur in discrete patches, and are confined to sheltered valleys, hollows and depressions, and frequently surrounded by grasslands and swamps. Our former studies investigated that the relationship between species richness and area among 18 shola patches and found a positive relationship between species richness and patch area (Mohandass and Davidar 2010). Many of the swamps within the study area have been affected by various anthropogenic factors including exotic tree plantation, burning, grazing and conversion into agricultural fields. Long-term grazing of swamps within our study area is linked with a higher susceptibility to drought stress in resident plant species (Dong et al., 2011). We build on this knowledge in the present study by exploring natural succession in vegetation surrounding swamp plant communities. This study sets to examine the relationship between swamp species richness and endemism with swamp area, to understand process of succession surrounding the swamp, and ecological response in terms of diversity and endemism to swamp area.

Swamps of the Nilgiri region may be affected in a similar way to those in previous studies which reported that the structure of the adjoining matrix vegetation had a major effect on swamp communities and diversity (Mesquita et al. 1999; Cronin 2003; Pohlman et al. 2007). Thus, this study focused on testing the relationship between total species richness, endemism, edge and swamp interior species richness an assessing the interactions between the swamp edge region with core swamp plant communities.

Therefore, the study examines how swamp species richness and endemism varies at edge transition zones around swamp margins, and estimates relative capacity of swamps to maintain diversity using species-area relationships to understand swamp community dynamics. We explore if there is a relationship between species richness and endemism in both edge and interior regions compared to swamp area, to explore if the swamp buffer region has a significant effect on the capacity of the swamp to maintain species diversity.

Materials and Methods

Study area

The study was conducted in the tropical montane swamps of the Korakundah and Upper Bhavani Reserve Forest of the upper Nilgiri Mountains. Reserve Forests represent a category of forest which has lower levels of protection than National Parks (Mohandass and Davidar, 2010). The study sites lie between 11° 13’ latitude N and 76° 35’ longitudes E and the elevation ranges between 2100–2400 m above mean sea level (*Fig. 1*). The mean annual rainfall recorded at Upper Bhavani Electricity department and Korakundah tea estate, during the periods for ten years (1993–2006) was 2637mm and 1887mm respectively. The distance between these two sites was approximately 8-

13 kms. The climate and geological information for the region has been published in previous studies (Mohandass, 2008; Puyravaud et al., 2012).

Swamps in the study area are generally flat-tables in depressions between slopes and grasslands and are normally small and isolated from one another, although large extensive swamps do occasionally occur. Swamps are a reflection of local topography coupled with drainage and local climate, and therefore can only occur in specific, limited regions. Slope gradient and topographic structure determines swamp location, through influencing soil nutrients and enhancing water holding capacity, and therefore swamp plant communities are non-randomly distributed. As a consequence, swamp ecotones may be more sensitive to disturbance and colonization especially of grassland species, as any changes which alter the retention of moisture or cause the drainage properties to change (Kent et al., 1997), are likely to facilitate the transition of the swamp to other types of habitat.

Flowering of most swamp species occurs during the monsoon period (June to November). Swamp communities are largely dominated by grasses and forbs, however some woody species also occur and generally flower from January to May (Mohandass pers. obs.). Most of the swamps in the study region were dominated by exotic tree plantations, of Australian black wattle and various Eucalyptus species (introduced in the 1950s) and pine trees which were planted from the 1960s onwards. Many swamps have already been converted into grasslands, grazing land and agricultural areas and belong to private owners or local government bodies. Local government agencies generally consider swamps as wastelands and there is generally a lack of awareness of both the floristic diversity of swamps and their ecological value.

Methods of data collection

A floristic survey was carried out from September 2001 to February 2002, and August 2002 to October 2002 in thirteen swamps throughout the Korakundah and Upper Bhavani regions *(Fig. 1*) (surrounding vegetation types including exotic tree species such as *Acacia dealbata* Link. *Eucalyptus globules* Labill, and *Pinus patula* Schiede ex Schltdl. & Cham., within plantations, in addition to native sholas and grasslands. All the studied swamps were marked by GPS points and topographical maps were made (*Fig.1; Table 1*).

Various anthropogenic activities including grazing, exotic tree plantation and fire have occurred in all swamp regions included within the study (Puyravaud et al., 2012). For all thirteen swamps, sampling effort success and effectiveness was quantified through construction of cumulative species-area curve for each individual swamp starting from the first sample unit (0.5 m^2) to the largest swamp present within the study region (1024 m^2) (*Fig. 2*). For five swamps included within this analysis, abundance and ground cover of plant species had previously been recorded and published (Mohandass, 2008; Puyravaud et al., 2012). In all thirteen swamps sampling effort was terminated once the species-area curve reached its asymptote. All plants were identified to species using various regional published floras (Bor, 1960; Fyson, 1932; Gamble, 1967; Matthew, 1999). The botanical revised names were followed according to APG III plant classification (Bremer et al. 2009). Species identification was confirmed by consulting herbaria collections at the Botanical Survey of India, Coimbatore, and Survey of Medicinal Plants and Collection Unit, Ootacamund. Rare, Endangered and Threatened (RET) and Endemic species were categorized according to the Flora of endemic plants (Nair and Henry, 1983; Ahemdullah and Nayar, 1986). A voucher specimen of each species was deposited at the Department of Ecology and Environmental Sciences, Pondicherry University, India.

***Table 1**. showed site code, swamp names, geographical location, , altitude (m), swamp size (ha), sampling unit of each site, number of species, number of edge species, number of swamp interior species, number of endemics, and surrounding vegetation types status of the 13 surveyed swamps in the upper Nilgiris Mountains, India.*

Site code	Geographical coordinates	Altitude (m)	Shapes	Size (ha)	Sampled area (m^2)	Species richness	Number of edge species	Number of swamp interior species	Number of endemic species	Surrounding vegetation types
GD	11° 14. 367′ N 76° 35. 657′ E	2236	Rectangle /triangle	0.8	64	50	18	32	10	Grassland
KT	11° 12. 387′ N 76° 34.158′ E	2224	Rectangle /triangle	4,2	128	51	19	32	7	Grassland & shola forest
KG	11° 12. 879′ N 76° 33. 352′ E	2270	oval/round	4	256	47	17	29	9	Grassland & Plantation
KK	11° 12. 992′ N 76° 33. 638′ E	2251	oval/round	2	512	53	16	36	9	Grassland & Plantation
KV	11° 14. 978′ N 76° 35. 200′ E	2279	oval/round	6	256	52	18	31	8	Grassland & shola forest
NT	11° 12. 069′ N 76° 33. 628′ E	2254	Rectangle /triangle	0.7	256	50	14	33	8	Grassland & Plantation
NU	11° 13. 082′ N 76° 32. 989′ E	2289	oval/round	1.2	128	53	15	36	10	Grassland
OT	11° 13. 889′ N 76° 36. 173′ E	2218	Rectangle /triangle	2.8	512	63	12	51	12	Grassland & Shola forest
PT1	11° 12. 580′ N 76° 34. 523′ E	2209	oval /round	1.5	128	57	10	43	8	Grassland & Shola forest
PT2	11° 12. 542′ N 76° 34. 527′ E	2206	Rectangle /triangle	1	256	56	19	34	9	Grassland & Shola forest
PC	11° 14.632′ N 76° 35. 488′ E	2258	Rectangle /triangle	0.9	128	51	17	31	10	Grassland
QT	11° 12. 898′ N 76° 34. 482′ E	2215	Oval /round	3.3	128	66	19	45	10	Grassland & Plantation
TE	11° 13. 186′ N 76° 32. 581′ E	2259	Rectangle /triangle	1.8	256	46	16	29	7	Grassland & Plantation

All swamps species were pooled for estimation of species richness. The area of each swamp was determined using data provided by the Tamilnadu Forest Department. RET/species were categorized as per the Flora of endemic plants (Ahmedullah and Nayar, 1986; Nair and Henry, 1983). Edge zone species were considered as the species found within 5-10 metres (m) of swamp edges. Species found at a distance of ≥ 10 m from the edges were considered as swamp interior species (*Fig. 2*). Life form was recorded through morphological and vegetative characters with field observation made during 2001 to 2002. Plant group was categorized according to plant classification of angiosperms viz., dicots and monocots. Life form was classified as: annual forbs, perennial forbs, perennial grass, annual grass and perennial woody stems (Rogers and Hartnett, 2001).

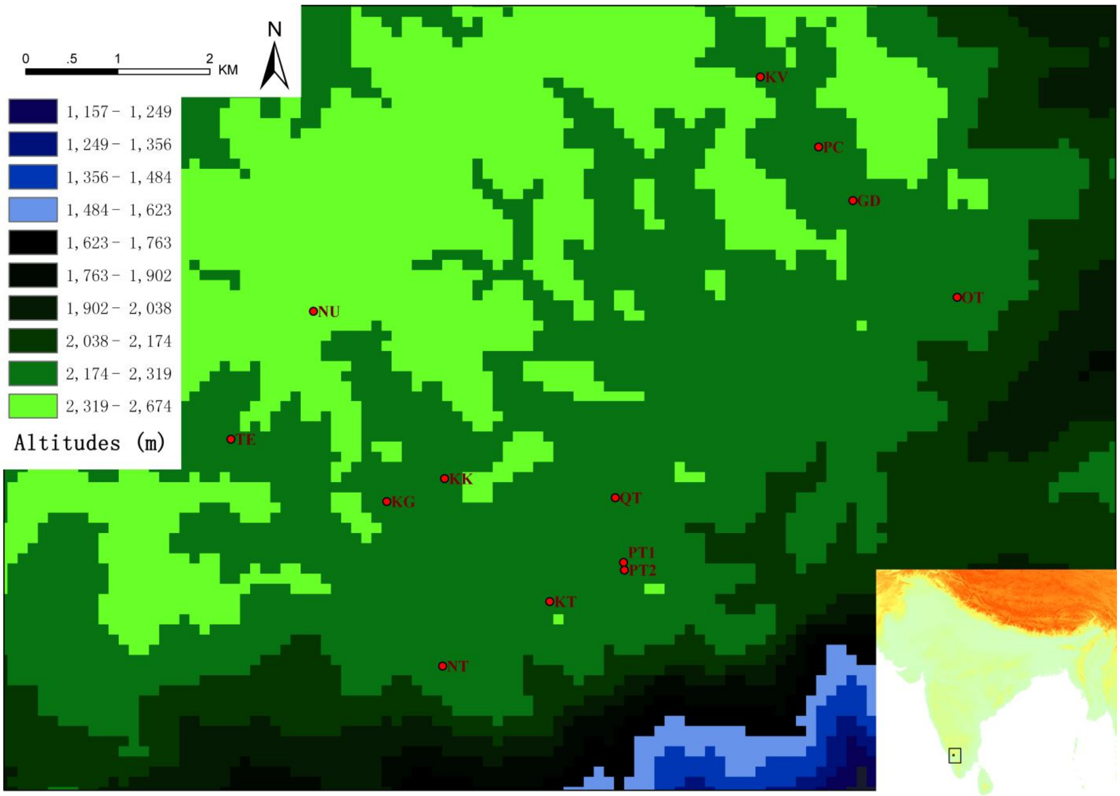

Figure. 1. *Location of the surveyed thirteen swamps with site code of the upper montane rainforest of the Nilgiris, Western Ghats, India.*

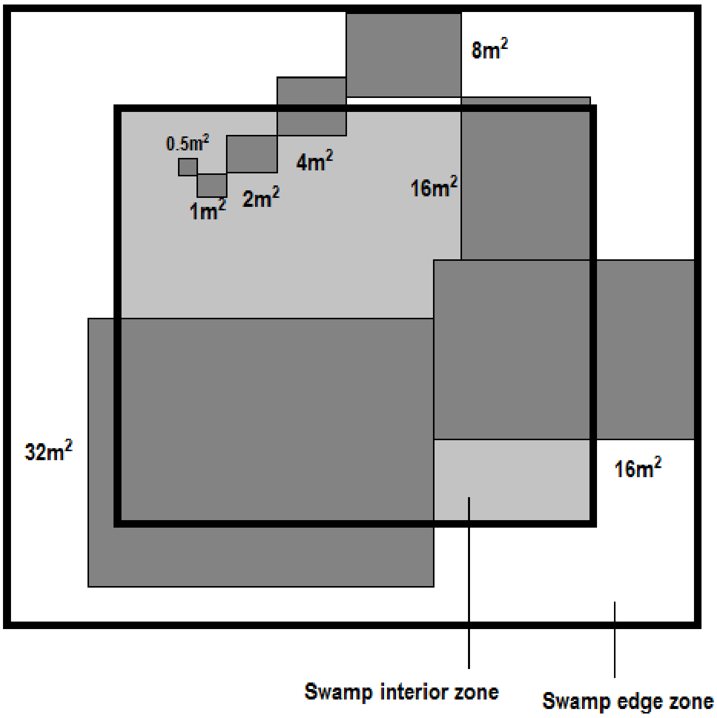

Figure 2. *Sampling quadrat pattern in each swamp that have made sampling effort to cover edge zone and swamp interior zones of the Nilgiri mountains.*

Floristic analyses

A Chi-square test was used to determine the variation of species richness among different vegetation variables. The species richness, endemism and area size was log transformed for analysis. The relationship between swamp area and vegetation parameters were analyzed using linear regression and ANOVA. Pearson correlation coefficient was used for analysis between swamp size (ha) and vegetation parameters. Differences between life forms were tested using one way ANOVA. Significant association was tested between life and plant group by likelihood ratio chi-square (G-test) of two-way contingency tables. All statistical tests were conducted using SPSS Inc (2000) and Past version 2.12 (Hammer et al., 2001).

Species area curves

In all the sampled swamps, the cumulative number of species was terminated when it reached a plateau or asymptote. In twelve of the swamps, the cumulative number of species was terminated at the area unit of 512 m2 however a large swamp reached a plateau at 1024 m2 (*Fig. 3*).

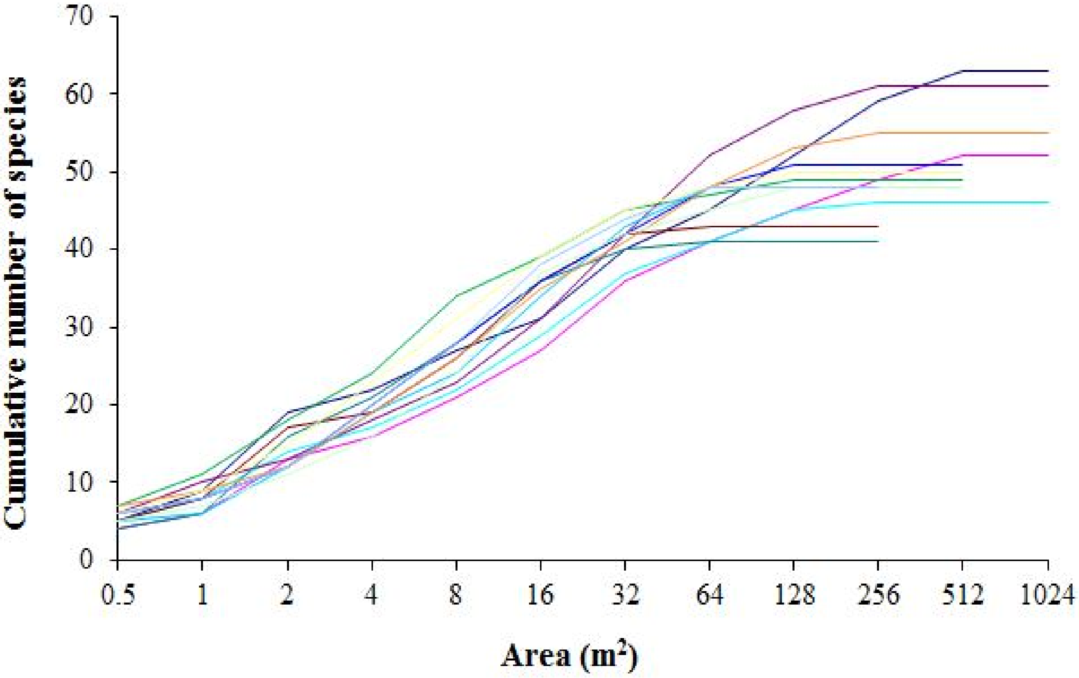

Figure 3*. It exhibits sampling effort made showing cumulative number of species increases and reaches a plateau at 512 m2 in twelve swamps and a large swamp reached a plateau at 1024 m2 in thirteen swamps of the upper Nilgiri Mountains, India.*

Results

A total of 84 species belonging to 67 genera and 33 families were recorded in the thirteen swamps (Appendix 1). The average species richness of each swamp was 53.4 (*Std. Deviation SD* = 5.8, range 46–66) (*Table 1*). Overall annual forbs represented 39%

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of species found, followed by perennial grass (31%), perennial forbs (23%), perennial woody stems (5%) and annual grasses (2%) (*Fig. 4*). The frequency of life-form was not significantly different among different swamps (F = 0.07, df = 44, ns). However, there is a significant association between life form (such as grasses, forbs, and woody plants) and plant group (i.e dicot and monocot) (G-test = 60.81, df = 4, P = 0.0001) (Appendix 1).

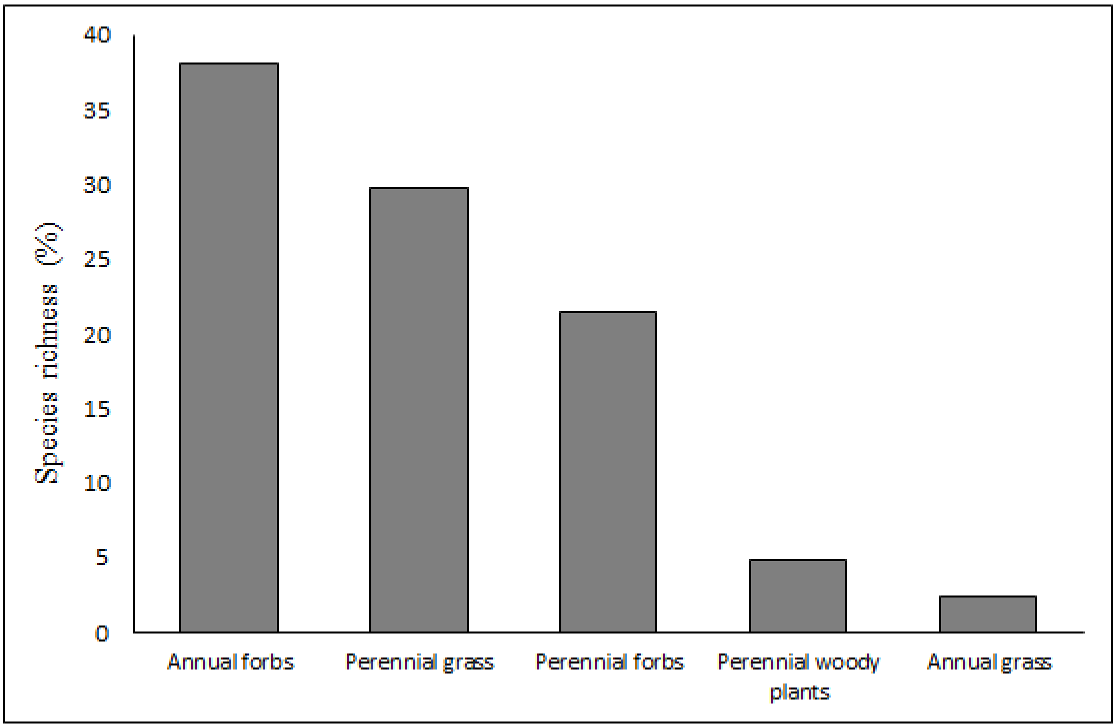

***Figure 4**. Percentage frequency of swamp life-form species among thirteen swamps in the upper Nilgiri Mountains*

Of these, 17% of species recorded were RET/endemic to Nilgiri/Palni hills and Western Ghats, with a mean value of 8.84 *(SD = 1.1)* and shared species ranging from 7–10 (approximately 15% of total species encountered). Additionally, the average edge species richness was 16.2 *(SD = 2.8)* and the average swamp interior species was 35.5 *(SD = 6.7)* for each swamp studied.

The frequency of endemism was not significantly varied from total species richness (χ^2 = 1.57, df = 12, ns), thus showed endemism was relatively higher among all swamps. Besides, the frequency of edge species richness was not significantly varied from total species richness (χ^2 = 7.12, df = 12, P = 0.85), thus indicates edge species richness was relatively high.

The log species (log*n* species richness) did not significantly increase with swamp area (R^2 = 0.052, n =13; P = 0.45; *Fig.5a* ANOVA $F_{12, 13}$= 0.60, ns). When analyzed separately edge and swamp interior species were found not to increase significantly with swamp area (R^2 = 0.00, ns, and R^2 = 0.04, ns), respectively. Similarly log endemism did not increase with swamp area (R^2 = 0.072, n = 13; P = 0.38, *Fig. 5b*; ANOVA $F_{13, 12}$ =

2.73, ns). This shows that regionally, swamps plant communities are impacts on edge effect caused by the structure of the adjoining matrix vegetation such as grasslands and sholas with the influence of topographic structure.

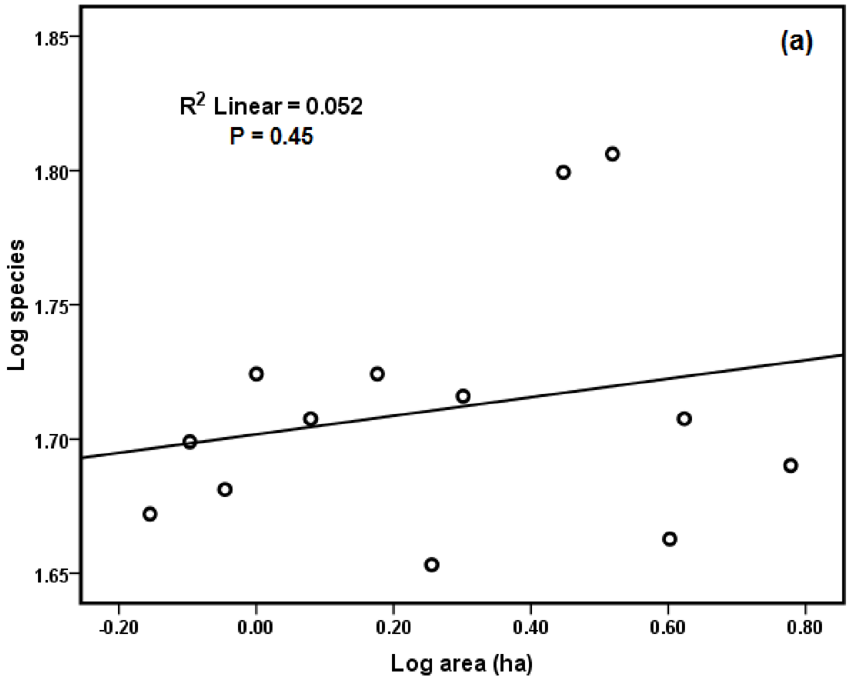

Figure 5a. *The log species and (log) and swamp log area (ha) (R2 = 0.05 N =13, n.s) with each point representing an individual swamp*

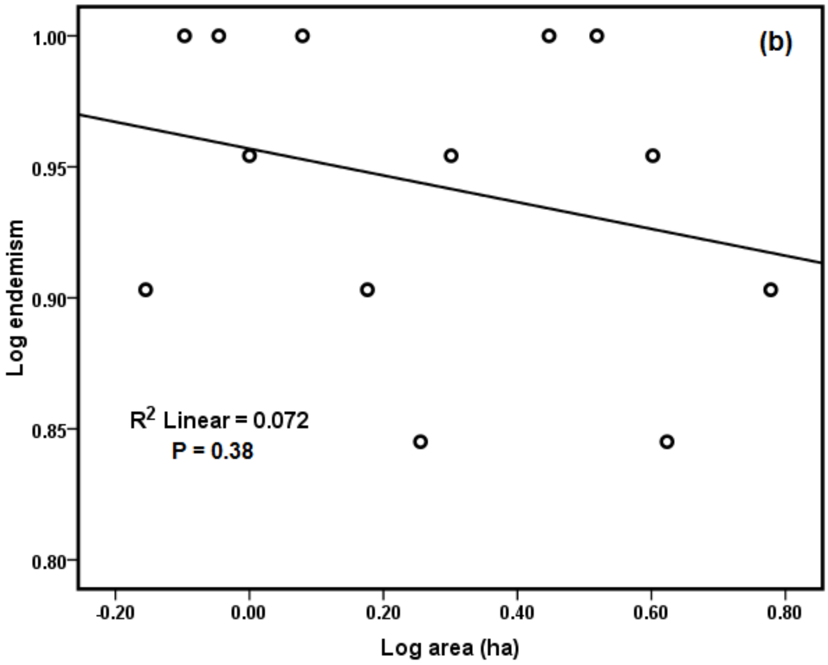

Figure 5b*. The log endemism to (log) and swamp log area (ha) (R2 = 0.072, N= 13, n.s.) of the thirteen surveyed swamps (shown as dot points) from thirteen swamps of the Upper Nilgiris, India.*

Swampy areas and vegetation community were not significantly correlated with swamp area among the thirteen swamps (*Table 2*). In particularly, there is no significant positive correlation between swamp-area (log) and total species richness (r = 0.12; n = 13; P = 0.70) or area size relative to endemism (r = −0.29; n = 13; P = 0.33) respectively. There was a marginal significant negative correlation found between swamp edge and interior species (r = −0.51; n = 13; P = 0.07). However, no significant relationship was found between edge or interior species richness with swamp area (edge: r = 0.30; n = 13; P = 0.32; interior: r = − 0.02; n = 13, P = 0.94).

Table 2*. Pearson (s) Correlation Coefficient was tested between vegetation parameter and swamp size (ha) among thirteen swamps in the Nilgiri Mountains.*

VEGETATION PARAMETER	SWAMP AREA	EDGE	ENDEMISM	SWAMP INTERIOR
EDGE	$0.30^{n.s}$			
P-VALUE	0.32			
ENDEMISM	$-0.29^{n.s}$	$0.03^{n.s}$		
	0.33	0.91		
SWAMP INTERIOR	$-0.02^{n.s}$	$-0.51^{n.s}$	$0.40^{n.s}$	
	0.94	0.07	0.18	
SPECIES RICHNESS	$0.12^{n.s}$	$-0.12^{n.s}$	$0.48^{n.s}$	0.91**
	0.70	0.71	0.09	0.00

***significant at < 0.0001; n.s = not significant*

Discussion

The effect of swamp area on species community and diversity

Our findings suggest that swamp plant communities are not significantly influenced by area but are primarily controlled by the two habitat transition zones on their outer margins, predominantly shola and grasslands *(Fig 6)*. This finding is contrary to our earlier study of shola forest species richness which significantly increased with increasing shola area (Mohandass and Davidar, 2010). However, among the shola forest, area significantly influences plant assemblages and vegetation features and in terms of local species richness, basal area and proportion of large trees, which all show positively relationships with area (Mohandass and Davidar, 2010). Swamp area and swamp size are determined and maintained by natural geological topography (physical environment) (Debski et al., 2000) including by various degrees of mountainous slopes which are integrated into aquatic habitat and edges (ecotonal boundaries). Furthermore, the relatively homogenous topographic environments within shola forests suggests that variation in eco-climatic factors has relatively little effect on species composition, due to the relative consistency within the regional conditions (Davidar et al., 2007; Mohandass and Davidar, 2009). However, species composition in swamps is influenced by annual rainfall, which couples in effects with steep mountainous slopes to create highly temporally variable conditions in terms of water influx. Former studies suggest that species richness in peat swamps is influenced by soil nutrients and rainfall (Sylvester Tan, 2004), our study indicates the same may be true within swamp environments. Swamp buffer areas also reflect physical environmental factors including topography, and drainage (soil properties), in addition to regional climatic factors (Fortin et al., 1997).

Figure 6 *(A) Swamps surrounded by pine plantation at the edges (B) Swamps bounded by grassland communities along with lower slope edges (C) One side of the swamp bounded by grassland and other side (arrow mark) bounded by sholas (native vegetation) (D)Swamps are bounded and surrounded by different vegetation types grassland, sholas, and plantation.*

The relative diversity of the buffer zone

The transition zone between swamps and surrounding vegetation types (i.e forests or grasslands) may have a higher biological diversity than either forest type which they mark the transition between, and therefore in themselves should be considered of high conservation importance (Leopold, 1933; Odum, 1983; Petts, 1990; Risser, 1995). In our study the composition of this transitory region includes both grassland and swamp species (Mohandass, 2008), however previous studies indicate that these transition regions may not facilitate the persistence of many species if frequently subject to intense disturbance (van der Maarel, 1990).

Species distributions and community composition in swamps

Communities and species abundance was found to change in the core (interior region) of the swamp, in addition to edge and buffer regions. In Swamp interior communities the majority of species are specialists for swamp environments. In the Nilgiris region the average species richness of 35.2 from the core regions of the swamp is significantly higher than in edge regions, with an average richness of 16.2 species. Given that species on swamp margins represent a subset of species found in interior regions of swamps, preventing further encroachment into swamps by grassland species may be a priority issue to maintain species which were only found in core regions of swamps.

A small proportion of species is shared across all swamps (15%), but communities present vary between the interior and edge regions of the swamp. There are two dominant species common to multiple swamps across the Nilgiri region (*Andropogon polyptychos* and *Eriochrysis rangacharii*). However these species are found in different zones of the swamps, *A. polyptychos* is specialized in both edge and swamp interior, whereas *E. rangacharii* is specialized only swamp interior. Within the swamps, both *Eriochrysis rangacharii* and *Coelachne perpusilla* are endemic to swamp areas and endangered, due to their specialist habitat requirements, and the decreasing availability of swamp areas.

Threats to swamp environments

Within this study we also noticed a high presence of what we would consider grassland species within the swamp itself, and not only limited to the transition, or surrounding grassland. We suggest that grassland species present in such high levels within the swamp may reflect regular disturbance or may relate to natural succession, or the drying out of swamps and slow transition to grassland. Swamp disturbances may facilitate colonization by grassland species and increase the potential for these swamp environments to be replaced by grasslands into the future. Thus swamps habitat might be highly sensitive to disturbance, in addition to climate change, especially in climate parameters which reflect changes in seasonality of precipitation.

There is also strong evidence that exotic species may invade highly disturbed ecotones and cause these regions to gradually transition to other habitat types (Fox et al., 1997; Lloyd et al., 2000; Watkins et al., 2003). Study area locations where exotic tree species were introduced by the forest department (more than five decades ago) have become increasingly dry, and no longer support the original swamp community, many have transitioned or are transitioning to grassland. Plantations further impact the swamp community through increasing the local water deficit during the dry season thereby assisting new colonizers during drier periods of the year. The diversity of tree species in tropical rainforests in the Western Ghats decreases significantly with increasing length of dry season (Davidar et al., 2005). Therefore the length of dry season may influence the plant community and the recruitment of grassland species into areas which are currently occupied by swamps.

Within the Upper Nilgiris, sholas have increased in species richness and overall area through various deterministic processes (Mohandass and Davidar, 2010). Shola species have progressively spread into grasslands through edge expansion, natural colonization by seed dispersal, indicating that shola species have ability and capacity to germinate in the grasslands. But in swamps, edge effects refers to the effect of a prevailing boundary between contrasting spatial environments makes swamps vulnerable to invasion by surrounding ecotones, whilst being topographically limited and therefore unable to expand (Mesquita et al. 1999; Cronin 2003; Pohlman et al. 2007). Moreover, exotic tree plantations at the boundary threatening to swamp plant communities, because high water demands of plant communities on swamp boundaries cause soils to progressively dry and therefore encroachment of other species increases the potential for further invasions and progressive conversion of swamp areas into other habitat types. The vulnerability of individual swamps therefore depends on the topography and climate of the region, in addition to the degree of disturbance and modification to the area, as disturbance can act to exacerbate soil drying (and thereby transition to other habitat types) especially if the climate is also changing seasonal water availability.

Summary

Swamps of upper Nilgiris region represent fragile ecosystems and are the sole habitat for a number of endemic threatened species. The conservation of these fragile ecosystems presents a challenging task for conservationists, as enhanced restoration and rehabilitation of swamp habitat within the upper Nilgiris must occur in conjunction with sustainable management and development. In addition further experimental research is needed to understand the swamp community evolutionary processes especially through a detailed assessment on effect of ecotone and soil dynamics.

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***Appendix 1**. Complete species list surveyed from thirteen swamps includes, species names, family, life-form, habit, plant group, and total number of frequency recorded in which swamps found in the Upper Nilgiri mountains, India.*

Species names	**Family**	**Life form**	**Plant groups**	**Habit**	**Total number of frequency**
Ageratina adenophora (Spreng.) R.M.King & H.Rob.	Compositae	Forb	Dicots	Annual forbs	5
Anaphalis brevifolia DC.	Compositae	Forb	Dicots	Annual forbs	13
Andropogon lividus Thwaites	Poaceae	Grass	Monocots	Perrenial grass	10
Andropogon polyptychos Steud	Poaceae	Grass	Monocots	Perrenial grass	12
Anemone rivularis Buch.-Ham. ex DC.	Ranunculaceae	Forb	Dicots	Annual forbs	2
Athyrium hohenackerianum T. Moore	Athyriaceae	Forb	Pteridophytes	Perennial forbs	3
Bolboschoenus maritimus (L.) Palla	Cyperaceae	Forb	Dicots	Perennial forbs	4
Bupleurum distichophyllum Wight & Arn.	Apiaceae	Forb	Dicots	Annual forbs	3
Carex capillacea Boott	Cyperaceae	Forb	Monocots	Perrenial grass	5
Carex lindleyana Nees	Cyperaceae	Forb	Monocots	Perrenial grass	7
Carex nubigena D.Don ex Tilloch & Taylor	Cyperaceae	Forb	Monocots	Perrenial grass	4
Carex phacota Spreng.	Cyperaceae	Forb	Monocots	Perrenial grass	6
Centella asiatica (L.) Urb.	Apiaceae	Forb	Dicots	Perennial forbs	7
Chrysopogon nodulibarbis (Hochst. ex Steud.) Henrard	Poaceae	Grass	Monocots	Perrenial grass	9
Cirsium abukumense Kadota	Compositae	Forb	Dicots	Annual forbs	*8*
Coelachne perpusilla (Nees ex Steud.) Thwaites	Poaceae	Grass	Monocots	Perrenial grass	*11*
Commelina clavata C.B.Clarke	Commelinaceae	Forb	Monocots	Annual forbs	7
Conyza bonariensis (L.)	Compositae	Forb	Dicots	Annual forbs	2
Cyanotis obtusa (Trimen)	Commelinaceae	Forb	Monocots	Perrenial	7

Trimen				grass	
Cyrtococcum deccanense Bor	Poaceae	Grass	Monocots	Annual grass	13
Dichrocephala chrysanthemifolia (Blume) DC.	Compositae	Forb	Dicots	Annual forbs	9
Digitaria stricta Roth	Poaceae	Grass	Monocots	Annual grass	13
Drosera burmanni Vahl	Lentibularaceae	Forb	Dicots	Annual forbs	12
Drosera peltata Thunb.	Lentibularaceae	Forb	Dicots	Annual forbs	3
Eleocharis congesta D.Don	Cyperaceae	Forb	Dicots	Perennial forbs	4
Erigeron karvinskianus DC	Compositae	Forb	Dicots	Perennial forbs	10
Eriocaulon brownianum Mart.	Eriocaulaceae	Forb	Monocots	Perrenial grass	13
Eriocaulon odoratum Dalzell	Eriocaulaceae	Forb	Monocots	Perrenial grass	11
Eriocaulon robustum Steud.	Eriocaulaceae	Forb	Monocots	Perrenial grass	11
Eriochrysis rangacharii C.E.C.Fisch.	Poaceae	Grass	Monocots	Perrenial grass	13
Eulalia phaeothrix (Hack.) Kuntze	Poaceae	Grass	Monocots	Perrenial grass	9
Fimbristylis quinquangularis (Vahl) Kunth	Cyperaceae	Forb	Monocots	Annual forbs	1
Fragaria nilgerrensis Schltdl. ex J.Gay	Rosaceae	Forb	Dicots	Annual forbs	11
Fragaria vesca L.	Rosaceae	Forb	Dicots	Annual forbs	9
Gaultheria fragrantissima Wall.	Ericaceae	Tree	Dicots	Perennial woody plants	4
Gentiana pedicellata (D.Don) Wall.	Gentianaceae	Forb	Dicots	Perennial forbs	13
Gentiana pedicellata subsp. *zeylanica* (Griseb.) Halda	Gentianaceae	Forb	Dicots	Perennial forbs	12
Gentiana quadrifaria Blume	Geraniaceae	Forb	Dicots	Annual forbs	11
Hydrocotyle sibthorpioides Lam.	Araliaceae	Forb	Dicots	Annual forbs	12
Hypochoeris argentina Cabrera	Compositae	Forb	Dicots	Annual forbs	13
Impatiens chinensis L.	*Balsaminaceae*	Forb	Dicots	Annual forbs	5
Impatiens rufescens Benth.	*Balsaminaceae*	Forb	Dicots	Annual forbs	13
Isachne kunthiana (Wight & Arn. ex Steud.) Miq.	Poaceae	Grass	Monocots	Perrenial grass	13
Ischaemum commutatum Hack.	Poaceae	Grass	Monocots	Perrenial grass	10
Juncus effusus L.	Juncaceae	Forb	Monocots	Perrenial grass	12

Juncus inflexus L.	Juncaceae	Forb	Monocots	Perrenial grass	12
Juncus prismatocarpus R.Br.	Juncaceae	Forb	Monocots	Perrenial grass	13
Kyllinga melanosperma Nees	Cyperaceae	Forb	Monocots	Perrenial grass	7
Laurembergia coccinea Kanitz	Haloragaceae	Forb	Dicots	Perennial forbs	1
Leucas marrubioides Desf.	Lamiaceae	Forb	Dicots	Perennial forbs	12
Lipocarpha chinensis (Osbeck) J.Kern	Cyperaceae	Forb	Monocots	Perrenial grass	9
Neanotis indica (DC.) W.H.Lewis	Rubiaceae	Forb	Dicots	Perennial forbs	13
Ophelia corymbosa Griseb.	Gentianaceae	Forb	Dicots	Annual forbs	4
Osbeckia brachystemon Naudin	Melastomataceae	Forb	Dicots	Annual forbs	13
Osbeckia leschenaultiana DC.	Melastomataceae	Shrub	Dicots	Perennial woody plants	4
Oxalis corniculata L.	Oxalidaceae	Forb	Dicots	Annual forbs	12
Oxalis spiralis Ruiz & Pav. ex G.Don	Oxalidaceae	Forb	Dicots	Annual forbs	3
Parnassia mysorensis F. Heyne ex Wight & Arn.	Celastraceae	Forb	Dicots	Annual forbs	10
Pedicularis perrottetii Benth.	Orobanchaceae	Forb	Dicots	Annual forbs	1
Persicaria nepalensis (Meisn.) Miyabe	Polyagonaceae	Forb	Dicots	Perennial forbs	10
Pimpinella leschenaultii DC.	Apiaceae	Forb	Dicots	Perennial forbs	4
Pinalia polystachya (A.Rich.) Kuntze	Orchidaceae	Forb	Monocots	Annual forbs	1
Pinus patula Schiede ex Schltdl. & Cham.	Pinaceae	Tree	Gymnosperms	Perennial woody plants	2
Plantago asiatica subsp. *erosa* (Wall.) Z.Yu Li	Plantaginaceae	Forb	Dicots	Perennial forbs	11
Pleiocraterium verticillare (Wall. ex Wight & Arn.) Bremek.	Rubiaceae	Forb	Dicots	Perennial forbs	8
Polytrias indica (Houtt.) Veldkamp	Poaceae	Grass	Monocots	Perrenial grass	10
Potentilla leschenaultiana Ser.	Rosaceae	Forb	Dicots	Perennial forbs	12
Pteridium aquilinum (L.) Kuhn	Denstaedtiaceae	Forb	Pteridophytes	Annual forbs	7
Pycreus flavidus (Retz.) T.Koyama	Cyperaceae	Forb	Monocots	Perrenial grass	12
Ranunculus diffusus DC.	Ranunculaceae	Forb	Dicots	Perennial forbs	3
Ranunculus reniformis Wall. ex Wight & Arn.	Ranunculaceae	Forb	Dicots	Perennial forbs	13
Rhododendron arboreum Sm.	Ericaceae	Tree	Dicots	Perennial woody plants	6

Rhynchospora rugosa (Vahl) Gale	Cyperaceae	Forb	Monocots	Perrenial grass	12
Rotala fysonii Blatt. & Hallb.	Lythraceae	Forb	Dicots	Annual forbs	11
Rubus racemosus Genev.	Rosaceae	Shrub	Dicots	Perennial woody plants	4
Satyrium nepalense D.Don	Orchidaceae	Forb	Monocots	Annual forbs	8
Senecio wightii (DC. ex Wight) Benth. ex C.B.Clarke	Compositae	Forb	Dicots	Annual forbs	11
Swertia minor T.Cooke	Gentianaceae	Forb	Monocots	Annual forbs	1
Themeda tremula (Nees ex Steud.) Hack.	Poaceae	Grass	Monocots	Perrenial grass	5
Utricularia graminifolia Vahl	Lentibulariaceae	Forb	Dicots	Annual forbs	11
Utricularia scandens Benj.	Lentibulariaceae	Forb	Dicots	Annual forbs	3
Viola pilosa Blume	Violaceae	Forb	Dicots	Perennial forbs	11
Wahlenbergia marginata (Thunb.) A.DC.	Campanulaceae	Forb	Dicots	Perennial forbs	5
Xyris capensis Thunb.	Xyridaceae	Forb	Monocots	Annual forbs	13

Appendix 2. *List of frequency of each species distributed in each swamp based on presence and absence qualitative assessment among thirteen swamps in the Korakundah and Upper Bhavani Reserve Forest, Nilgiri Mountains, southern India.*

Plant species	Family	Life-form	GD	KT	KG	KK	KV	NT	NU	OT	PT1	PT2	PC	QT	TE	Grand Total
Ageratina adenophora (Spreng.) R.M.King & H.Rob.	Compositae	Forb	0	0	0	0	1	0	0	0	1	0	1	1	1	5
Anaphalis brevifolia DC.	Compositae	Forb	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Andropogon lividus Thwaites	Poaceae	Grass	0	1	0	1	1	0	1	1	1	1	1	1	1	10
Andropogon polyptychos Steud	Poaceae	Grass	1	1	1	1	1	1	1	1	1	1	1	1	0	12
Anemone rivularis Buch.-Ham. ex DC.	Ranunculaceae	Forb	1	0	0	0	0	0	0	0	0	0	0	1	0	2
Athyrium hohenackerianum T. Moore	Athyriaceae	Forb	0	0	0	0	1	0	0	1	0	0	0	1	0	3
Bolboschoenus maritimus (L.) Palla	Cyperaceae	Forb	1	0	1	0	1	0	0	0	0	0	1	0	0	4
Bupleurum distichophyllum Wight & Arn.	Apiaceae	Forb	0	0	0	1	0	0	1	0	1	0	0	0	0	3
Carex capillacea Boott	Cyperaceae	Forb	0	0	0	0	1	0	0	0	1	1	1	1	0	5
Carex lindleyana Nees	Cyperaceae	Forb	1	1	1	0	0	0	0	1	0	1	1	1	0	7
Carex nubigena D.Don ex Tilloch & Taylor	Cyperaceae	Forb	1	0	0	1	0	0	0	1	0	0	0	1	0	4
Carex phacota Spreng.	Cyperaceae	Forb	0	1	1	1	0	0	0	1	0	1	0	1	0	6
Centella asiatica (L.) Urb.	Apiaceae	Forb	1	1	1	0	1	0	0	1	0	1	0	1	0	7
Chrysopogon nodulibarbis (Hochst. ex Steud.) Henrard	Poaceae	Grass	0	0	0	1	1	1	1	1	0	1	1	1	1	9
Cirsium abukumense Kadota	Compositae	Forb	1	0	0	0	1	0	1	1	0	1	1	1	1	8
Coelachne perpusilla (Nees ex Steud.) Thwaites	Poaceae	Grass	1	1	0	1	1	1	1	1	1	1	0	1	1	11
Commelina clavata C.B.Clarke	Commelinaceae	Forb	1	0	1	1	0	0	1	1	0	0	1	1	0	7
Conyza bonariensis (L.)	Compositae	Forb	1	0	0	0	0	0	0	1	0	0	0	0	0	2
Cyanotis obtusa (Trimen) Trimen	Commelinaceae	Forb	1	1	0	0	0	0	1	1	0	1	1	1	0	7
Cyrtococcum deccanense Bor	Poaceae	Grass	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Dichrocephala chrysanthemifolia (Blume) DC.	Compositae	Forb	0	1	0	0	1	1	1	1	1	1	1	1	0	9
Digitaria stricta Roth	Poaceae	Grass	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Drosera burmanni Vahl	Lentibularaceae	Forb	0	1	1	1	1	1	1	1	1	1	1	1	1	12
Drosera peltata Thunb.	Lentibularaceae	Forb	0	0	0	1	0	0	0	0	1	0	0	0	1	3
Eleocharis congesta D.Don	Cyperaceae	Forb	0	1	0	1	0	0	0	0	0	1	1	0	0	4
Erigeron karvinskianus DC	Compositae	Forb	1	0	1	1	1	0	1	1	1	1	1	1	0	10
Eriocaulon brownianum Mart.	Eriocaulaceae	Forb	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Eriocaulon odoratum Dalzell	Eriocaulaceae	Forb	1	1	1	1	1	0	1	1	1	0	1	1	1	11
Eriocaulon robustum Steud.	Eriocaulaceae	Forb	1	0	1	0	1	1	1	1	1	1	1	1	1	11
Eriochrysis rangacharii C.E.C.Fisch.	Poaceae	Grass	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Eulalia phaeothrix (Hack.) Kuntze	Poaceae	Grass	0	0	1	0	1	1	1	1	0	1	1	1	1	9
Fimbristylis quinquangularis (Vahl) Kunth	Cyperaceae	Forb	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Fragaria nilgerrensis Schltdl. ex J.Gay	Rosaceae	Forb	1	1	0	1	0	1	1	1	1	1	1	1	1	11
Fragaria vesca L.	Rosaceae	Forb	0	1	0	0	1	1	1	1	1	1	0	1	1	9
Gaultheria fragrantissima Wall.	Ericaceae	Tree	0	0	1	1	1	0	0	0	0	0	0	0	1	4
Gentiana pedicellata (D.Don) Wall.	Gentianaceae	Forb	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Gentiana pedicellata subsp. *zeylanica* (Griseb.) Halda	Gentianaceae	Forb	1	1	1	0	1	1	1	1	1	1	1	1	1	12
Gentiana quadrifaria Blume	Geraniaceae	Forb	0	1	1	1	0	1	1	1	1	1	1	1	1	11
Hydrocotyle sibthorpioides Lam.	Araliaceae	Forb	1	1	1	0	1	1	1	1	1	1	1	1	1	12
Hypochoeris argentina Cabrera	Compositae	Forb	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Impatiens chinensis L.	*Balsaminaceae*	Forb	0	0	1	0	1	0	1	0	0	1	0	1	0	5
Impatiens rufescens Benth.	*Balsaminaceae*	Forb	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Isachne kunthiana (Wight & Arn. ex Steud.) Miq.	Poaceae	Grass	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Ischaemum commutatum Hack.	Poaceae	Grass	1	1	1	1	0	0	1	1	1	1	1	1	0	10
Juncus effusus L.	Juncaceae	Forb	1	1	1	1	0	1	1	1	1	1	1	1	1	12
Juncus inflexus L.	Juncaceae	Forb	1	1	1	1	0	1	1	1	1	1	1	1	1	12
Juncus prismatocarpus R.Br.	Juncaceae	Forb	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Kyllinga melanosperma Nees	Cyperaceae	Forb	1	0	1	0	0	0	1	1	1	1	0	1	0	7
Laurembergia coccinea Kanitz	Haloragaceae	Forb	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Leucas marrubioides Desf.	Lamiaceae	Forb	1	1	1	1	1	1	0	1	1	1	1	1	1	12
Lipocarpha chinensis (Osbeck) J.Kern	Cyperaceae	Forb	0	1	1	1	1	1	0	1	1	1	0	1	0	9
Neanotis indica (DC.) W.H.Lewis	Rubiaceae	Forb	1	1	1	1	1	1	1	1	1	1	1	1	1	13

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Ophelia corymbosa Griseb.	Gentianaceae	Forb	0	1	0	1	0	1	0	0	1	0	0	0	0	4
Osbeckia brachystemon Naudin	Melastomataceae	Forb	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Osbeckia leschenaultiana DC.	Melastomataceae	Shrub	0	1	0	0	0	0	0	0	1	1	1	0	0	4
Oxalis corniculata L.	Oxalidaceae	Forb	1	1	1	1	1	1	1	1	1	1	1	1	0	12
Oxalis spiralis Ruiz & Pav. ex G.Don	Oxalidaceae	Forb	0	1	0	0	0	0	0	1	0	0	0	1	0	3
Parnassia mysorensis F. Heyne ex Wight & Arn.	Celastraceae	Forb	0	1	0	1	0	1	1	1	1	1	1	1	1	10
*Pedicularis perrotteti*i Benth.	Orobanchaceae	Forb	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Persicaria nepalensis (Meisn.) Miyabe	Polyagonaceae	Forb	0	1	1	1	1	1	1	1	1	0	1	1	0	10
Pimpinella leschenaultii DC.	Apiaceae	Forb	1	1	0	0	0	1	0	0	1	0	0	0	0	4
Pinalia polystachya (A.Rich.) Kuntze	Orchidaceae	Forb	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Pinus patula Schiede ex Schltdl. & Cham.	Pinaceae	Tree	0	0	0	1	0	1	0	0	0	0	0	0	0	2
Plantago asiatica subsp. *erosa* (Wall.) Z.Yu Li	Plantaginaceae	Forb	1	1	1	1	0	1	1	1	1	1	0	1	1	11
Pleiocraterium verticillare (Wall. ex Wight & Arn.) Bremek.	Rubiaceae	Forb	1	0	0	1	1	1	1	1	0	0	0	1	1	8
Polytrias indica (Houtt.) Veldkamp	Poaceae	Grass	1	1	0	1	0	1	1	1	1	1	0	1	1	10
Potentilla leschenaultiana Ser.	Rosaceae	Forb	1	1	0	1	1	1	1	1	1	1	1	1	1	12
Pteridium aquilinum (L.) Kuhn	Denstaedtiaceae	Forb	0	1	1	0	0	1	1	1	1	1	0	0	0	7
Pycreus flavidus (Retz.) T.Koyama	Cyperaceae	Forb	1	1	1	1	1	1	1	1	1	1	1	1	0	12
Ranunculus diffusus DC.	Ranunculaceae	Forb	0	0	0	1	1	0	0	1	0	0	0	0	0	3
Ranunculus reniformis Wall. ex Wight & Arn.	Ranunculaceae	Forb	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Rhododendron arboreum Sm.	Ericaceae	Tree	0	0	1	1	1	1	0	0	0	0	0	1	1	6
Rhynchospora rugosa (Vahl) Gale	Cyperaceae	Forb	0	1	1	1	1	1	1	1	1	1	1	1	1	12
Rotala fysonii Blatt. & Hallb.	Lythraceae	Forb	0	0	1	1	1	1	1	1	1	1	1	1	1	11
Rubus racemosus Genev.	Rosaceae	Shrub	0	1	0	0	0	1	1	0	1	0	0	0	0	4
Satyrium nepalense D.Don	Orchidaceae	Forb	1	0	0	1	1	0	1	0	1	0	1	1	1	8
Senecio wightii (DC. ex Wight) Benth. ex C.B.Clarke	Compositae	Forb	1	0	1	0	1	1	1	1	1	1	1	1	1	11
Swertia minor T.Cooke	Gentianaceae	Forb	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Themeda tremula (Nees ex Steud.) Hack.	Poaceae	Grass	0	0	0	0	1	1	0	0	1	0	1	1	0	5
Utricularia graminifolia Vahl	Lentibulariaceae	Forb	1	1	1	1	1	1	0	1	1	1	0	1	1	11
Utricularia scandens Benj.	Lentibulariaceae	Forb	1	0	0	1	1	0	0	0	0	0	0	1	0	4
Viola pilosa Blume	Violaceae	Forb	1	1	1	1	1	1	1	1	1	1	0	1	1	12
Wahlenbergia marginata (Thunb.) A.DC.	Campanulaceae	Forb	0	0	0	0	0	0	0	1	1	0	1	1	1	5
Xyris capensis Thunb.	Xyridaceae	Forb	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Grand Total			50	51	47	53	52	50	53	63	57	56	51	66	46	

EFFECT OF AUSTRIAN PINE ON NATURALNESS AND SUCCESSION OF VEGETATION IN RECLAIMED BAUXITE QUARRIES

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Abstract: Phytosociological and nature conservation assessment of the herb layer of 6-, 15- and 20-year-old post-mining Austrian pine stands was conducted in reclaimed bauxite quarries in Hungary. Great differences among the vegetations were found. Disturbance-tolerant species were dominant, subdominant and subordinated in the youngest, middle-aged and oldest pine stand, respectively. In parallel, proportion of the species characteristic for natural habitats increased gradually, leading to growing diversity and naturalness. The increasing pine cover reduced the species number and the coverage of herb layer. Post-mining flora differed significantly from both the potential mature oak forest vegetation of the areas and from the associations developed through regenerative succession on clear-cut areas of oak forests. Vegetation of the reclaimed quarries had lower naturalness: relative abundances of disturbance-tolerant and ruderal species were higher, but ratio of natural broad-leaved forest's species was smaller than in the potential vegetation. Similar differences were shown by comparison of the flora of bauxite quarries with the same-aged stages of regenerative succession of oak forests. In pine stands the repression of weeds parallel to the spreading of natural competitors was slower, and natural geophytes and protected species remained absent. Deficiency of propagule sources in reclaimed areas could contribute to the retentive effect of Austrian pine on vegetation succession.

Keywords: *Pinus nigra, bauxite quarries, phytosociological succession, naturalness value, reclamation*

Introduction

At present, an area of 67 200 hectares is covered by the monodominant stands of the alien Austrian pine (*Pinus nigra* Arn.) in Hungary (source: NFCSO, Hungarian Forest Management Inventory, 2013). Although these plantations occupy only 3.7% of the total forested lands of the country, *P. nigra* is responsible for some serious ecological and nature conservation problems (Tamás, 2003). Several studies called the attention to the various aftermaths of the creation of pine stands, such as to the effects on the soil and ground layer including serious impoverishment of the species-rich native grassland vegetation (Bódis, 1993; Csontos et al., 1997, 2012, Szalai et al., 2012), the promoted spreading of other alien plants (Török et al., 2003; Cseresnyés and Csontos, 2012a), as

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well as the highly increased fire risk as a consequence of the considerable accumulation of resinous needle litter (Cseresnyés et al., 2011).

Austrian pine has been widely used for biological reclamation of various degraded areas in several European countries since the 1950s (Fettweis et al., 2005; Zagas et al., 2010). In Hungary, successful afforestations were executed on open-pit dolomite, bauxite, lignite and brown coal mines, waste-rock piles, rubbish dumps, slag heaps and red sludge reservoirs (Szerémy, 1981; Baranyi, 1986; Horváth, 2002; Károly et al., 2006). The good applicability of Austrian pine is presumably related to its wide scale habitat tolerance. The cover substrate deposited through the technical reclamation is generally characterized by physical and chemical conditions improper for plant cultivation, such as low nutrient supply, extreme acidity or basicity, as well as high spatial heterogeneity in texture and subsequently in water-holding and water-conducting capacity (Jochimsen, 2001). Materials of dead rocks, but particularly of slag and red sludge usually contain high level of mobile and easily mobilizable toxic heavy metals and compounds, *i.e.* sulphides (Liu et al., 2011). Austrian pine is quite tolerant to dry and nutrient-poor soils as well as to high concentration of toxic metals (*i.e.* Al, As, Cd, Cu, Sb, Sn, W) and pyrite (Pratas et al., 2005). The intensive early root expansion enables the species to uptake water and nutrients from the soil efficiently, even if unfavourable environmental conditions prevail (Richardson, 1998; Baumann et al., 2006). Soil melioration effect of the pine proved to be unsatisfactory in many cases, but the dense root system, the quick canopy closure and the intensive litter formation markedly reduce soil erosion (Filcheva et al., 2000; Miletić et al., 2011).

After land reclamation, post-restoration monitoring systems are commonly used both for examining characteristic changes in environmental factors and to study the rate and stage of area regeneration. Such type of research offers good opportunities for improving the land restoration practice. The realized monitoring activities are ordinarily include the investigation of refuse soil as well as surface and subterranean water, but the comprehensive survey of the biota in the reclaimed area is neglected in most cases. Phytosociological studies of the vegetation developed during or after biological reclamation may be of great importance in terms of future planned area utilization, particularly in case when the restoration of the botanically valuable potential vegetation is aimed. Therefore, present study was designed to investigate the vegetation of abandoned bauxite quarries reclaimed with Austrian pine and to assess the naturalness of the flora in order to evaluate the effect of the alien tree on the regenerative secondary succession.

Materials and methods

Three reclaimed bauxite quarries were chosen for study in Transdanubia, Hungary. Locations of the study sites and percentage cover of *P. nigra* are given in *Table 1.*

(1) 6-year-old site (6.9 ha in area). Pine stand is situated 2.6 km south of village Szőc, on a north-facing slope covered by the materials of former waste-rock piles. The young pine plantation is surrounded mostly by sessile oak – Turkey oak forests, grown in the neighbouring undisturbed areas. Following the open-pit bauxite mining (1977–86), the area was technically reclaimed until 2005 (Kovács, 1998). Sawdust and inorganic fertilizers were used in order to create a more suitable material

Table 1. *Data of Austrian pine stands established for reclamation of bauxite quarries in Hungary, and the code of quadrats used for phytosociological studies.*

Study site age; code of forest management unit	Code of quadrat	GPS coordinates	Cover of *P. nigra* (%)
6 years old; Sáska 6I, Szőc	A1	N 46°59'59.9"; E 17°31'33.8"; 282 m	15
	A2	N 46°59'59.2"; E 17°31'35.0"; 284 m	10
	A3	N 46°59'58.6"; E 17°31'35.3"; 289 m	10
	A4	N 46°59'57.9"; E 17°31'34.1"; 294 m	15
	A5	N 46°59'57.2"; E 17°31'33.5"; 299 m	15
15 years old; Bicske 7A, Nagyegyháza	B1	N 47°32'38.5"; E 18°32'48.2"; 276 m	40
	B2	N 47°32'39.3"; E 18°32'49.4"; 278 m	70
	B3	N 47°32'48.6"; E 18°32'56.1"; 297 m	25
	B4	N 47°32'47.5"; E 18°32'57.7"; 291 m	90
	B5	N 47°32'43.5"; E 18°32'52.1"; 290 m	70
20 years old; Sáska 63G	C1	N 46°59'49.6"; E 17°30'55.8"; 261 m	20
	C2	N 46°59'49.1"; E 17°30'54.4"; 260 m	60
	C3	N 46°59'48.7"; E 17°30'56.0"; 261 m	55
	C4	N 46°59'49.8"; E 17°30'56.5"; 262 m	20
	C5	N 46°59'49.3"; E 17°30'58.3"; 263 m	75

for the afforestation, thereafter Austrian pine was cultivated by seed-sowing in 2004. In the study year (2010), pine stand height was 1–1.5 m with a pronounced dominance of *Solidago gigantea* in the herb-layer vegetation.

(2) 15-year-old site (3.2 ha in area). Post-mining pine stand is located 3.5 km north of village Nagyegyháza, 9 km northwest of the city of Bicske, surrounded by sessile oak – Turkey oak forests and artificial black locust stands. The abandonment of bauxite mining was followed by the complex reclamation in 1995 (Novák, 2007). Prior to the seed-sowing of Austrian pine, organic fertilizer and chopped bark were added and mixed the cover layer to build soil organic matter. By the study year (2010), a 3–5 m high, dense pine stand (only with local natural thinning) developed in the area predominated by *Calamagrostis epigeios* in the herb layer.

(3) 20-year-old site (6.4 ha in area). The area is situated 1 km west of the 6-year-old study site. The neighbouring vegetation was formed mainly by sessile oak – Turkey oak forests except westward where a secondary scrubland was grown (invaded by the alien black locust). Establishment of Austrian pine stands was completed in 1990 by transplanting pine saplings after meliorating the cover layer with addition of sawdust and inorganic fertilizer (Kovács, 1998). In the study year, the 4–6 m high pine stand had great spatial variation in density due to the local decay of trees. Species-rich herb layer was observed especially in the glades of the stand.

For floristic survey, five 10 m by 10 m permanent quadrats were randomly located within each study site at least 20 m from the edge of pine stand then their centres were localized with GPS instrument (*Table 1*). In the quadrats, canopy cover of Austrian pine was visually estimated, afterwards the percentage cover of each herb-layer species (including low shrubs) were recorded three times during the vegetation season (May/June 2010, August/September 2010 and April 2011) in order to get a complete species inventory. Species names were used after Simon (2000). For data processing, each species was considered with its maximum cover value detected during the three sampling occasions. Total number of species, average number of species per quadrat as

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well as dominant and constant species were determined for each study site. Shannon diversity (H') of sample area was calculated by merging the data of the five quadrats and using natural logarithm. In addition, the evenness (E) of the vegetation and the diversity of every single quadrat was also calculated (rare species were considered with 0.1% cover value). Shannon diversity of study sites were compared statistically by performing Hutcheson's t-test (Hutcheson, 1970). Relationships between the cover of Austrian pine and the cover, species number and Shannon diversity of the herb-layer vegetation in quadrats were analysed by using Spearman's rank correlation. For characterizing the vegetation developed since the afforestation two types of plant traits were used: *(1)* social behaviour type (Borhidi, 1995) and *(2)* Raunkiaer life-form (Simon, 2000) of the species. Based upon the mean percentage cover of species (counted by averaging the data of the five quadrats) and their plant traits, group distributions were calculated with the application of the following categories:

(1) Social behaviour type: S (specialists); C (natural competitors); G (generalists); NP (natural pioneers); DT + W (disturbance-tolerants and indigenous weeds); RC (indigenous ruderal competitors); AC + I (alien competitors and introduced species). On group distribution graphs, categories are displayed in order of decreasing naturalness value (Borhidi, 1995).

(2) Raunkiaer life-form: MM + M (Phanerophyte); Ch (Chamaephyte); H (Hemikryptophyte); G (Geophyte); TH (Hemitherophyte); Th (Therophyte).

The quasi-mean naturalness value of vegetation was computed for each study site, based on the naturalness value of the constituting species weighted with their cover (according to Borhidi, 1995). Vegetation of study sites was compared by applying homogeneity test on the group distributions of *(1)* and *(2)*.

Comparative analyses of the studied vegetations were made in two ways:

(A) Vegetations of the reclaimed bauxite quarries were compared to mature stands of oak forests (sessile oak – Turkey oak ass. and pedunculate oak – Turkey oak ass.), the community types considered the natural climax associations of the territories surrounding the studied quarries (Zólyomi, 1989). This type of comparison was dedicated to assess how much the restored vegetations became similar to the theoretical final stage of the succession. In this way, the 15-year-old site was compared to sessile oak – Turkey oak stand relevés from the southern part of Gerecse Mountains (unpubl. data by Z. Barina), whereas the 6- and 20-year-old reclaimed sites were compared to pedunculate oak – Turkey oak stand relevés from the Bakony Mountains (Szodfridt and Tallós, 1964). Since latter authors applied Braun–Blanquet scale in their work, data were converted into percentage cover by van der Maarel transformation (Maarel, 2007).

(B) Vegetations of the reclaimed quarries were compared to successional stages of the natural vegetation with comparable age. The idea of this type of comparison was to assess how much slower (or faster) the succession is in the restored vegetation sites than in sites where a spontaneous regenerative succession takes place. Csontos (2010) studied regeneration cycle of sessile oak – Turkey oak forests and identified four stages along time since clear-cutting of the stands. These stages were: I= 1–3 years old; II= 4–11 years; III= 12–21 years and IV= 22–28 years. Relevés from stage II were compared to the 6-year-old reclaimed vegetation, whereas the 15 and 20 years old reclaimed quarries were compared to relevés from stage III of the oak forest regeneration succession.

The comparative analyses *(A)* and *(B)* were based on distributions of plant functional groups, namely social behaviour types and Raunkiaer life-forms of the vegetation, since this approach is often provides an excellent supplementation to the analysis of vegetation based only on species composition (Woźniak et al., 2011). *Statistica* software package (ver 9, StatSoft Inc., OK, USA) was used for the statistical analyses.

Results

Altogether 170 vascular plant species were listed in the reclaimed areas (for detailed floristic data, see Cseresnyés and Csontos, 2012b). The total number of species increased with the age of pine stand, as it was the lowest (83) in the 6-year-old stand and the highest (108) in the 20-year-old stand (Table 2). Average number of species per quadrat ranged from 48.3 (15-year-old stand) to 55.2 (20-year-old stand), while the herb layer cover varied between 39% and 95% in the 15- and 6-year-old stand, respectively. The Shannon diversity (H') and also the evenness (E) increased along with the increasing age of pine stand: both index proved to be the lowest in the 6-year-old stand (H'= 2.220; E= 0.502) and the highest in the 20-year-old forest (H'= 2.914; E= 0.622). Hutcheson's *t*-test showed significant ($p < 0.05$) differences in diversities only between the youngest and the oldest sampling areas, while the 15-year-old study site had an intermediate position (*Table 2*). Number of constant species varied considerably from 6 to 19 in the 15- and 20-year-old site, respectively.

Table 2. *Characteristics of the vegetations developed in bauxite quarries reclaimed with Austrian pine 6, 15 and 20 years ago, in Hungary. Superscript letters after diversity values indicate groups distinguished by Hutcheson's t-test.*

	6-year-old	**15-year-old**	**20-year-old**
Total number of species	83	106	108
Average number of species per quadrat	50.8	43.8	55.2
Average cover of herb layer (%)	95	39	73
Shannon-diversity (H')	2.220^{a}	2.538^{ab}	2.914^{b}
Evenness (E)	0.502	0.544	0.622
Number of constant species	19	6	17

Characterization of the herb layer of study sites in association with dominant and constant species (*Table 3*):

(1) 6-year-old stand: Vegetation of the former waste-rock pile was dominated by the abundant *Solidago gigantea* (48%), *Calamagrostis epigeios* (12%) and *Erigeron annuus* (4.4%). Besides, the flora was determined by the composition of the leguminous *Trifolium pretense*, *T. repens*, *T. alpestre* and *Medicago lupulina* (4–6% cover by each) as well as the grasses *Holcus lanatus* and *Dactylis glomerata*. The high spatial homogeneity and the high number of constant species of the herb-layer vegetation observed in the area was due to the combined effect of the evenly low (10–15%) canopy cover of Austrian pine and the young age of the stand. Each of the nine species

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Table 3. *Mean percentage cover of dominant and constant species in the vegetation of reclaimed bauxite quarries of different ages.*

	Dominant species	**%**	**Constant species**	**%**
6-year-old stand	*Solidago gigantea*	48	*Solidago gigantea*	48
	Calamagrostis epigeios	12	*Calamagrostis epigeios*	12
	Trifolium pratense	6.2	*Trifolium pratense*	6.2
	Medicago lupulina	5	*Medicago lupulina*	5
	Trifolium repens	5	*Trifolium repens*	5
	Trifolium alpestre	4.6	*Trifolium alpestre*	4.6
	Erigeron annuus	4.4	*Erigeron annuus*	4.4
	Holcus lanatus	1.8	*Holcus lanatus*	1.8
	Dactylis glomerata	1.64	*Dactylis glomerata*	1.64
	Fragaria vesca	1	*Fragaria vesca*	1
			Galium verum	0.64
			Achillea collina	0.46
			Astragalus glycyphyllos	0.46
			Tussilago farfara	0.46
			Cirsium arvense	0.28
			Lotus corniculatus	0.28
			Prunella vulgaris	0.1
			Sanguisorba minor	0.1
			Scabiosa ochroleuca	0.1
15-year-old stand	*Calamagrostis epigeios*	20	*Calamagrostis epigeios*	20
	Solidago gigantea	3.8	*Erigeron annuus*	1.02
	Erigeron annuus	1.02	*Achillea collina*	0.84
	Achillea collina	0.84	*Daucus carota*	0.82
	Daucus carota	0.82	*Crataegus monogyna*	0.68
	Fraxinus ornus	0.82	*Medicago lupulina*	0.66
	Festuca rupicola	0.8		
	Crataegus monogyna	0.68		
	Medicago lupulina	0.66		
	Asclepias syriaca	0.64		
20-year-old stand	*Fragaria vesca*	16	*Fragaria vesca*	16
	Brachypodium sylvaticum	13.2	*Brachypodium sylvaticum*	13.2
	Calamagrostis epigeios	10	*Calamagrostis epigeios*	10
	Solidago gigantea	6.2	*Trifolium alpestre*	4.82
	Trifolium alpestre	4.82	*Securigera varia*	2.6
	Securigera varia	2.6	*Clinopodium vulgare*	2.24
	Clinopodium vulgare	2.24	*Dactylis glomerata*	1.82
	Dactylis glomerata	1.82	*Crataegus monogyna*	1.8
	Crataegus monogyna	1.8	*Medicago lupulina*	1.6
	Salix caprea	1.8	*Trifolium repens*	1.42
			Agrimonia eupatoria	1.26
			Galium mollugo	1.24
			Daucus carota	0.86
			Achillea collina	0.66
			Picris hieracioides	0.28
			Sanguisorba minor	0.28
			Rosa canina	0.1

mentioned above with high cover values were also members of the constant species group (see *Table 3*).

(2) 15-year-old stand: Flora developed in the reclaimed mine-pit was dominated by disturbance-tolerant and invasive plants. The ubiquitous *C. epigeios* and the alien *S. gigantea* represented 20% and 3.8% mean cover, respectively, and their joint cover exceeded 50% in some places. Among woody species, saplings of *Fraxinus ornus* and *Crataegus monogyna* appeared densely in the area. As regards for constant species *Erigeron annuus, Achillea collina*, *Daucus carota, C. monogyna* and *Medicago lupulina* can be mentioned beside the dominant *C. epigeios*.

(3) 20-year-old stand: High spatial variability and species richness of the herb-layer vegetation was observed with the dominance of *Fragaria vesca*, *Brachypodium sylvaticum*, *C. epigeios* and *S. gigantea* mixtured with other 11 plant species exceeded 1% in coverage. Juvenile shrubs and trees (*C. monogyna*, *Salix caprea*, *Prunus spinosa*, *Ligustrum vulgare*, *Pyrus pyraster*) were also found sporadically. Altogether 17 constant species were encountered in this study site (*Table 3*).

Increasing canopy cover of Austrian pine had significant ($p< 0.05$) negative effect on the cover and the species number of herb layer (Spearman's $r_{rank}= -0.897$ and -0.662, respectively; *Figure 1*), whereas had no influence on Shannon diversity (Spearman's $r_{rank}= 0.113$).

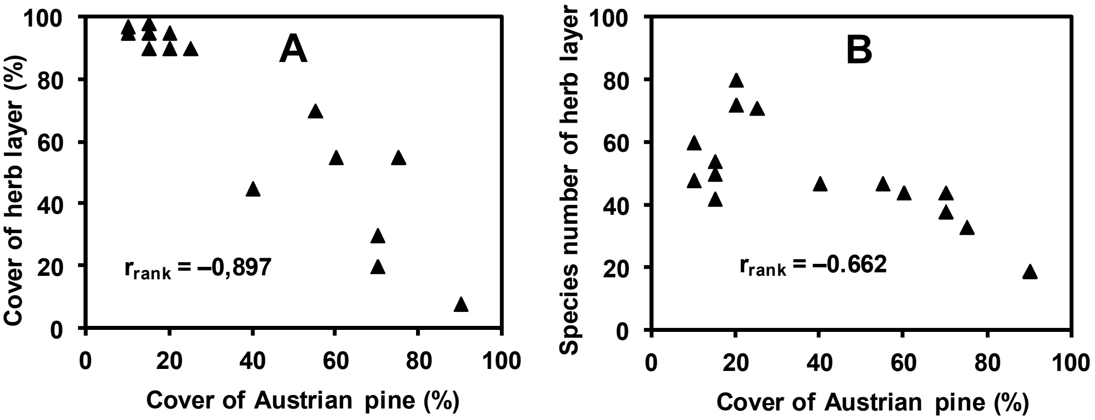

Figure 1. *Change of the cover **(A)** and the species number **(B)** per quadrats of the herb-layer vegetation related to the cover of Austrian pine in the reclaimed bauxite quarries.*
r_{rank} = Spearman's rank correlation coefficient

Results of homogeneity tests of group distributions based on social behaviour types and Raunkiaer life-forms for different study sites are summarized in *Table 4*.

In the analysis based on the social behaviour types, homogeneity test showed significant differences among the vegetation of the three bauxite quarries (*Figure 2/A, Table 4 `A`*). In the 6-year-old pine stand, alien plants became predominant (51.6%) mainly due to the strong invasion of *S. gigantea*, but *Erigeron annuus* and *Conyza canadensis* were present as well. High proportion of disturbance-tolerants and

Table 4. *Statistical evaluation of the vegetations developed in the reclaimed bauxite quarries. Data show χ^2 values and levels of significance obtained from the homogeneity test of group distributions based on the ecological attributes of species.*

	Compared study sites of different ages since pine stand establishment	**Ecological attribute**	
		Social behaviour type	**Raunkiaer life-form**
`A`	6-year-old vs. 15-year-old	43.39***	19.37**
	6-year-old vs. 20-year-old	60.76***	10.72^{NS}
	15-year-old vs. 20-year-old	74.01***	10.08^{NS}
`B`	15-year-old vs. Barina [*ined.*] (native oak forest)	119.2***	106.9***
`C`	6-year-old vs. Szodfridt and Tallós [1964] (native oak forest)	125.6***	45.54***
	20-year-old vs. Szodfridt and Tallós [1964] (native oak forest)	49.86***	24.36***
`D`	6-year-old vs. Csontos [1996] II stage (oak forest regeneration)	98.38***	18.01**
`E`	15-year-old vs. Csontos [1996] III stage (oak forest regeneration)	118.2***	23.12***
	20-year-old vs. Csontos [1996] III stage (oak forest regeneration)	52.08***	15.20**

****= p< 0.001; **= p< 0.01; NS= not significant*

weeds as well as of indigenous ruderal competitors (principally *C. epigeios*) were detected. From among the 27 generalist species, *Trifolium alpestre*, *Holcus lanatus* and *Fragaria vesca* attained considerable cover. On the 15-year-old study site, herb-layer vegetation was featured by indigenous ruderal competitors (46.9%) owing to primarily the propagation of *C. epigeios* and secondly the abundance of *Cirsium arvense*. Alien invaders (*S. gigantea*, *Asclepias syriaca* and *Ambrosia artemisiifolia*) as well as 62 species classified as native disturbance-tolerants or weeds also formed important groups with 23.2% and 18.4% proportion, respectively. Natural competitors (9 species) and generalists (20 species) formed relatively small groups in cover. The flora of the 20-year-old bauxite quarry was mainly composed by generalist colonizers (35 species with 51.1% group mass) with the predominance of *Fragaria vesca* and *Brachypodium sylvaticum* and the relatively high frequencies of *Trifolium alpestre*, *Clinopodium vulgare* and *C. monogyna*. However, the disturbance-tolerants and weeds (48 species, *e.g. Securigera varia*, *Dactylis glomerata*, *Salix caprea*) and also the indigenous ruderal species (*C. epigeios*, *Cirsium arvense*) and adventive plants (*S. gigantea*, *Erigeron annuus*, *Robinia pseudoacacia*) were still abundant. Small percentage contribution (below 0.3%) of both natural pioneers and specialists were obtained for each of the three reclaimed areas. The quasi-mean naturalness value calculated for the vegetations of the 6-year-old and the 15-year-old plantations proved to be very low (–0.87 and –0.55, respectively) clearly due to the high cover degree of species having small naturalness value, while for the 20-year-old study site considerable higher (2.18) naturalness value was obtained.

Considering Raunkiaer life-form distributions significant difference was shown only between the herb-layer vegetations of the 6 and the 15 years old stands (*Figure 2/B, Table 4 `A`*). Outstanding percentage ratio (68.5–85.2%) of hemicryptophytes was found in each study site. Phanerophytes represented notable group mass (17.8%) in the 15-year-old site owing to the dense regrowth of *Fraxinus ornus*, *C. monogyna* and *Quercus petraea*. Not more than 4.6–11.2% relative coverage of therophytes was found, notwithstanding their relatively high species number (16–25) in each site. Each reclaimed site was characterized by the low ratio of chamaephyte, geophyte and hemitherophyte life-forms; although from the latter group *Picris hieracioides* and *Centaurea biebersteinii* were relatively abundant.

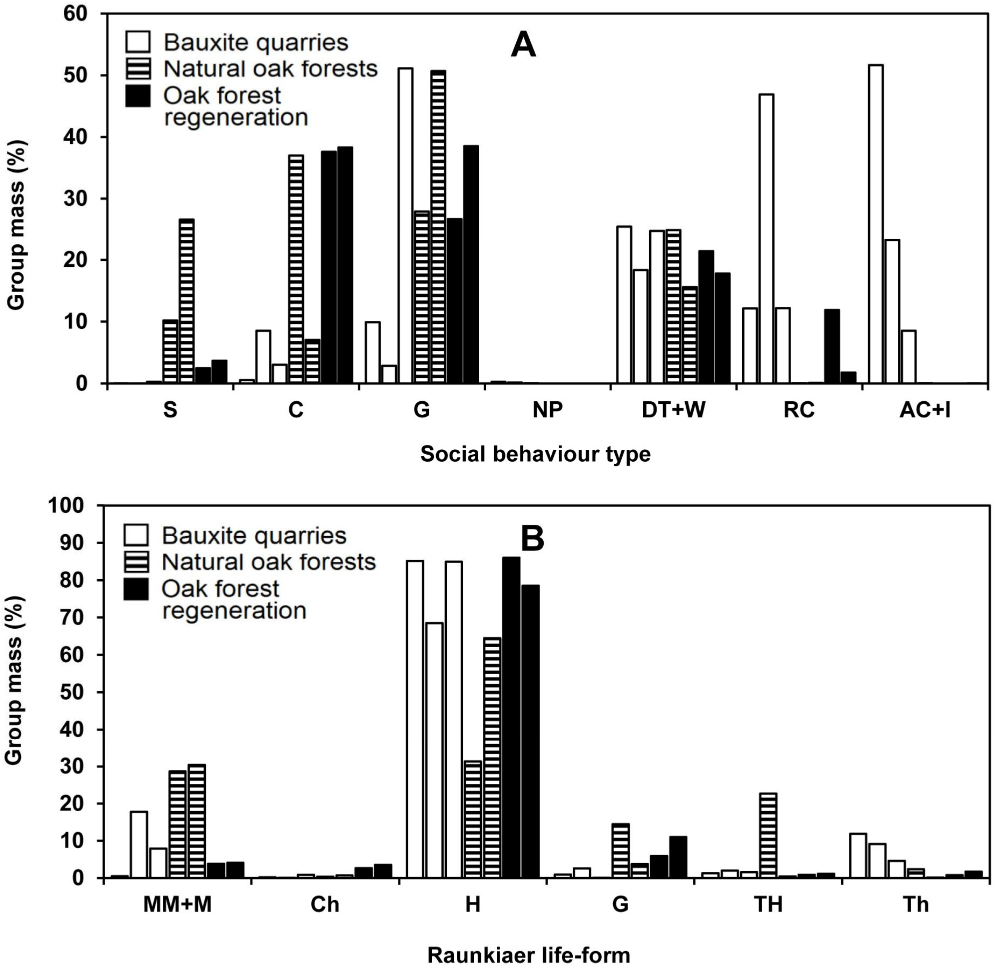

Figure 2. *Group distributions of social behaviour types* (A) and Raunkiaer life-forms (B) for the herb-layer vegetation of the (i) reclaimed bauxite quarries of different ages (empty bars from left to right: 6, 15 and 20 years old stands), (ii) the natural oak forests (striped bars from left to right: Gerecse and Bakony Mts.) and (iii) oak forests regenerated after clear-cutting (filled bars from left to right: regeneration stage II and III).* S= specialists; C= natural competitors; G= generalists; NP= natural pioneers; DT= disturbance-tolerants; W= indigenous weeds; RC= indigenous ruderal competitors; AC= alien competitors; I= introduced species*

Further comparative evaluation of the vegetation formed in the reclamation areas was completed by employing the published (Szodfridt and Tallós, 1964; Csontos, 2010) and unpublished (Barina, *ined.*) data described earlier. Homogeneity tests showed significant differences in each case when group distributions calculated on the post-mining flora was compared with those of generated on the basis of literature data derived from native or regenerated forest associations.

Distributions concerning the herb layer of native sessile oak – Turkey oak forests were examined at first (*Figure 2*). Considerable differences appeared between the flora

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of the 15-year-old reclamation area and the herb layer of *Quercetum petraeae–cerris* stands from Gerecse Mountains (*Table 4 `B`*). As regards the reclaimed bauxite quarry, the distribution of social behaviour types shifted markedly towards species groups having lower naturalness values. Oak forests were dominated by natural competitors and generalists and characterized by a remarkably higher percentage cover of specialists, whereas plenty of indigenous ruderal competitors and alien species as well as several natural pioneers featured the flora on the reclaimed site. Concerning the Raunkiaer life-forms, lower proportion of phanerophytes and geophytes as well as higher ratio of hemicryptophytes and therophytes were observed in the reclaimed areas than in the native sessile oak – Turkey oak stands. (The outstanding proportion of hemitherophytes in the oak forests of Gerecse Mts was due to the high cover of *Alliaria petiolata*, a species with ambiguous classification between TH and H life forms.)

The flora developed on the 6- and 20-year-old study sites was compared with the herb-layer of the native oak forest in Bakony Mountains (*Table 4 `C`; Fig. 2*). Specialist species (having high naturalness value) constituted an insignificant part of coverage on the reclaimed areas, moreover here the natural competitors also represented a smaller ratio than in native oak forest. Conversely, post-mining Austrian pine stands were colonized by several natural pioneer plants as well as by the crowds of disturbance-tolerant, indigenous ruderal competitor and adventive species. Phanerophyte and geophyte life-forms proved to be less frequent on reclaimed sites than in oak forest, whereas hemikryptophyte, hemitherophyte and therophyte species had higher proportion in the reclaimed sites.

In a subsequent analysis, we compared the herb layer vegetation of the reclaimed areas to the herb layer of oak forests' clearings regenerated after clear-cutting (*Figure 2; Table 4 'D'*). Vegetation of the 6-year-old study site was compared with the 4–11 years old successional stage of oak forest regeneration (stage-II in Csontos, 2010). Group distributions of social behaviour types showed just slight differences in proportion of either the disturbance-tolerants or the indigenous ruderal competitors. On the other hand, post-mining vegetation was predominated by adventive plants, but the regenerated forest clearings were mainly characterized by the intensive propagation of natural competitors and generalists as well as the relatively high cover degree of specialists. Apart from the uniformly high ratio of hemikrytophytes, some fundamental disparities appeared between the graphs of Raunkiaer life-forms: the reclaimed bauxite quarry seemed to be abound in therophytes and hemitherophytes, whereas was rather poor in phanerophytes, chamaephytes and geophytes.

Regarding the 15- and 20-year-old pine stands, comparison was made with the 12–21 years old successional stage of oak forest regeneration (stage-III in Csontos, 2010), (*Table 4 `E`*). According to the distribution of social behaviour types, species groups with low naturalness values were expressing considerable cover ratios in the reclaimed areas. In the same-aged successional stage of oak forest regeneration, natural competitors and specialists accompanied the generalist species; alien plants played an insignificant role and natural pioneers were absent. Graph of Raunkiaer life-forms (*Figure 2/B*) shows the higher proportion of phanerophytes, hemitherophytes and therophytes in the reclaimed bauxite quarries, whereas the higher abundance of chamaephytes and geophytes was observed in the regenerating oak forests.

Discussion

Post-mining vegetations of different ages were clearly distinguished by the dominant species and the group distributions of social behaviour types and Raunkiaer life-forms. Herb layers of the 6, 15 and 20 years old Austrian pine stands could be considered as a successional chronoseries. In the youngest (6-year-old) stand, the small species number, low Shannon diversity and evenness values were mainly caused by the dominance of *S. gigantea* and *C. epigeios* that formed altogether 60% of the vegetation cover. The remaining part of the species-pool belonged principally to disturbance-tolerants and ruderal competitors, similarly to the findings of other successional studies in the literature (Kompala-Baba and Baba, 2013). Consequently, the calculated quasi-mean naturalness value (–0.87) was the lowest among the study sites. In the 15-year-old pine stand, a smaller cover ratio of alien plants were observed, but the vegetation was still predominated by disturbance-tolerants and indigenous ruderal competitors, leading to a similarly low naturalness value (–0.55). The species number, diversity and evenness of the herb layer proved to be the highest in the 20-year-old pine plantation, where the role of native broad-leaved forests' species was already important. Indigenous and alien ruderal competitors were still present, but generalists prevailed over other social behaviour types, resulting in a more favourable spectrum of the naturalness value (with the quasi-mean of 2.18). The increasing pine canopy cover reduced the cover and species number of the herb layer but no change in Shannon diversity was detected. The independence observed between pine cover and herb layer diversity can be explained by the excessive spreading of *C. epigeios* and *S. gigantea* in the open quadrats, causing a reduction of both evenness and diversity. Rapid propagation of *C. epigeios* through the early stage of regenerative succession, as well as its penetration to various forest communities were also observed in Central Europe (Sierka and Chmura, 2005; Mudrák et al., 2010). Házi et al. (2011) suggested an artificial control of *C. epigeios* by regularly repeated mowing in order to promote the appropriate course of succession. Negative effect of Austrian pine cover on species number and fine-scale structural complexity of the herb-layer vegetation was reported earlier as well (Bartha et al., 2004; Mudrák et al., 2010).

Indigenous ruderal and alien competitors as well as natural pioneers were nearly absent from the native sessile oak – Turkey oak associations, furthermore habitat-indifferent plants were substituted by species of broad-leaved forests. According to the social behaviour types, these species mainly belonged to the specialists, natural competitors and generalists, contributing to a much higher naturalness of sessile oak – Turkey oak forest. As for Raunkiaer life-forms, both the species number and relative coverage of phanerophytes exceeded the values determined for the reclamation areas and the proportion of therophytes were lower in oak forests due to the subordinate role of weeds. Conspicuous difference appeared between group masses of geophytes as well. Geophytes amounted to 5–15% cover ratio in the native oak forests, whereas they were absent from the reclaimed sites. Extreme low colonization ability and slow propagation of geophytes was also demonstrated during various secondary succession studies (Schmidt, 1988; Hayashi, 1991).

Herb-layer vegetation in the reclaimed areas expressed characteristic changes along with the aging of Austrian pine stands. Cover ratio of indigenous ruderal and alien competitors decreased gradually in parallel with the increasing role of the botanically more valuable generalists, natural competitors and specialists. An upward trend for the accompanying forest species group is also established on the reclaimed areas, as their initial 2.5% cover rate (at the 6-year-old stand) increased to 29.5% (at the 20-year-old stand). However, even the latter proportion falls far behind the 73% relative cover of this group found in the herb layer of natural sessile oak – Turkey oak forests (Szodfridt and Tallós, 1964; Kevey, 2008). Hemitherophytes and therophytes become repressed gradually parallel to the ageing of the reclaimed areas but the recurrence of geophytes did not occur. Such long-time changes in life-form and phytosociological spectra of the vegetation proved to be characteristic successional trends observed in different types of primary and secondary successions in infertile substrates, as confirmed by several field studies (Schmidt, 1988; Bartha et al., 2004; Frouz et al., 2008). Protected plants were absent from the reclaimed areas although several of them were frequently detected in the surrounding natural associations (Barina, *ined.*; Barina, 2006; Szodfridt and Tallós, 1964; Kevey, 2008).

Vegetation regenerated after the clear-cutting of sessile oak – Turkey oak forests (Csontos, 2010) also differed significantly from the flora formed on reclamation areas. Natural pioneers and adventive species were missing in stage II (4–11 years old) of the regenerative succession, but the latter group contributed nearly 52% of the cover in the 6-year-old reclaimed site. A particularly great inequality was shown for natural competitors representing about 0.5% proportion in the 6-year-old reclaimed site, whereas the herb layer of regenerating forest clearings was predominated (37.6%) by this social behaviour type. The graph of Raunkiaer life-forms clearly shows both the notably higher ratio of geophytes and the low cover degree of therophytes on the regenerating forest clearings. Additionally, vegetation of this early successional stage of clearings was already featured chiefly by the mixture of accompanying species (62%) instead of disturbance-tolerants and ruderal plants which were characteristic on the reclaimed sites.

By comparing the flora of the 15 and 20 years old post-mining pine stands with the successional stage III (12–21 years old) of oak forest regeneration (Csontos, 2010), differences proved to be similar in many respects, and a more pronounced disparity were obtained for the middle aged than for the older reclaimed stand. The herb layer of the regenerating oak forest clearings was richer in specialists and natural competitors, but conversely poorer in indigenous ruderal competitors than the reclaimed areas. Distribution of Raunkiaer life-forms displays the considerable cover rate of geophytes (11%) and the lower proportion of hemitherophytes and therophytes, in relation to reclamation areas. Oak forest clearings were dominated by the accompanying species of broad-leaved forests in this successional stage. Several protected species (*e.g. Doronicum hungaricum*, *Iris graminea*, *Lilium martagon*, *Lychnis coronaria*) were already established in the successional stage II of oak forest regeneration, followed by others (*e.g. Cephalanthera longifolia*, *Epipactis helleborine*, *Neottia nidus-avis*) in stage III (Horváth and Csontos, 1992; Csontos, 2010), whereas protected species were absent from the vegetation of the reclaimed sites.

We can conclude that the herb layer of post-mining Austrian pine stands passes through similar processes of regenerative succession than does the vegetation developed on clearings of formerly clear-cut native sessile oak – Turkey oak forests. However, the revegetation by spontaneous succession takes place more slowly or just partially in Austrian pine stands, thus the naturalness of the post-mining vegetation is always falls behind the same-aged stage of oak forest's regeneration. It corresponds to the characteristic differences between regeneration succession and secondary succession (Maarel, 1988), as the former takes place on oak forest clearings but the latter is the governing process on the reclaimed sites. In the reclaimed quarries, adventive plants may become abundant quickly, while the proportion of natural broad-leaved forests' species increases their share slowly. In addition, the major part of the constant oak forest species (particularly the specialists) does not occupy the reclaimed areas even after 20 years. Weak soil ameliorative effect of Austrian pine and its negative influence on the recolonization of native vegetation was formerly observed and silvicultural interventions, mainly small-scale and diffuse gap-opening in pine canopy was proposed for promoting natural succession (Barčić et al., 2006; Jonášová et al., 2006). However, it should be taken into consideration that plant recolonization in the post-mining areas can be strongly prevented by the deficiency of propagules which were presumably available on clearings regenerated after clear-cutting (surviving specimens, soil seed bank, bulbs, rhizomes *etc.*), but obviously absent from the top layer of reclaimed quarries. Spreading of propagules was found to be a crucial factor for plant migration and recolonization in disturbed areas that could strongly determined the course of regenerative succession (Matlack, 1994; Lanta and Leps 2009). For better understanding the consequences of Austrian pine application, long-term ecological monitoring of several reclamation areas should be initiated with involving unforested control sites receiving technical reclamation only. Nevertheless, presented results suggest the obstructive effect of Austrian pine on secondary succession, thus further establishment of pine stands can be reasonable just in case when the unfavourable habitat conditions (water management, nutrient supplying ability) do not make possible the utilization of other tree species in the spoil area. Hodačová and Prach (2003) realized that spontaneous succession could be an advantageous alternative to technical reclamation, leading to more natural vegetation with much higher species diversity. Consequently, existing pine stands have been created for silvicultural reclamation should not be maintained for decades. These plantations can be provisionally effective for preventing both water and wind erosion, but their conversion to the locally native vegetation types should be made as early as possible to achieve a perfect restoration goal.

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SURVIVAL OF ALKANE-DEGRADING MICROORGANISMS IN BIOGAS DIGESTATE COMPOST IN MICROCOSM EXPERIMENTS

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Abstract. The objective of the study was to investigate composted biogas digestate as a suitable carrier for the microorganisms to be used in bioremediation of petroleum hydrocarbon contaminated soils. The survival kinetics of alkane-degrading microorganisms was examined in microcosm experiments by two means: measuring the number of colony forming units and their ability to produce CO_2 from alkanes. The viability of the cells of *Rhodococcus* strains in the compost lasted longer than those of the tested *Brevundimonas*, *Chryseobacterium*, *Pseudomonas* strains. The decimal reduction time (D-value) used to express the survival of the microorganisms depended on their cell structure and biological features. Their survival was also affected by some abiotic factors: higher temperature (26 °C) decreased the survival of the microbes while the addition of burned perlite to the compost medium increased it. All of the tested microorganisms were able to use diesel oil as a sole carbon source both in the pure, unamended compost and in the compost-perlite mixture. Moreover, the addition of perlite increased the alkane-degrading activity for the tested *Rhodococcus* and fungal strains. These results indicate that biogas digestate composts can be suitable carriers for microorganisms used in bioremediation.

Keywords: *bacteria, fungi, viability, hydrocarbon, bioremediation*

Introduction

During the composting processes (mainly in their thermophilic phase), the majority of the starting organic materials become degraded due to microbial activity, pathogens and parasites perish as a consequence of the high temperature. Humus materials developing in the course of oxidative reconstruction can be utilized only minimally by microorganisms, and their chemical structure becomes stable during the maturation phase (de Bertoldi et al., 1983; Haug, 1993). The composts control the inner microbial populations due to a permanent antimicrobial effect. The level and the ratio of the different microbial populations in the chemically stable compost are determined by the different reduction of their survival abilities. The amount of microorganisms belonging to different species and taxa is stabilized at various levels. Autochthonous microorganism species are permanently present in compost media. However, the majority of the microorganisms found in the environment, and even those that play a role in the initial phase of composting belonging to allochthonous species, cannot be detected in matured compost (Insam and de Bertoldi, 2007). The composts originated from traditional starting materials (communal solid wastes, agricultural/horticultural wastes) do not contain pathogens or parasites. Moreover, they resist secondary colonization by these organisms. On the other hand, they are not suitable for making the carrier material of microbes for storage and inoculation. Meanwhile, the demand for soil-compatible organic carrier materials has been growing in numerous soil-bioremediation areas.

Biogas digestate, a by-product of anaerobic fermentation, is extremely rich in nitrogen and is microbiologically stable (Elfering et al., 1998; Maurer and Mueller, 2012). Its direct agricultural use as fertilizer is widespread, especially in the European Union where the amount of biomass degraded anaerobically exceeds 1500 million tons per year (Weiland, 2010). Recently, digestate can be composted in order to remove compounds that are chemically harmful or hygienically unacceptable for human and the environment (Bustamante et al., 2012; Philipp and Hoelzle, 2012; Walker et al., 2009). These composts may contain some advantageous residual components, such as stabilizing colloidal factors of biogas digestate but they have not been tested for their effect on allochthonous microorganisms.

The group of microorganisms that are able to degrade alkanes is extremely diverse. Bacterial species belonging to various divisions and fungal species belonging to various taxa can assimilate alkanes as sole carbon source (Das and Chandran, 2010). The quantitative relation of microbial population is markedly influenced by temperature among the physical factors (Farrell and Rose, 1967). The viability of steady-state cells decreases at higher temperature. The integrity of cell membrane can be protected by some inorganic materials with large surfaces, porous and chemically indifferent structures, the application of burnt silica rocks. Recently, perlite became particularly well-known in the field of maintenance of bacterial cells (Daza et al, 2000). Perlite may also be suitable for the extension of the viability of fungal cells (Homolka et al., 2007, Homolka and Lisá, 2008).

The objective of this study was to determine the effect of the compost processed from biogas digestate sourced from the mixture of mycelium residue of oyster mushroom production and swine slurry on the survival of alkane degrading microorganisms belonging to different groups. The survival of microbes was followed not only by direct testing of their activity but also by re-culturing their living cells. The cell quantity of alkane degrading microorganism was compared at room temperature and at a lower, storing temperature in the presence or absence of perlite.

Materials and methods

Compost and microorganisms

The compost medium used in this study was processed in an aerated static pile composting system from the mixture of biogas digestate (60% dw), vinasse (20% dw), wheat straw (15% dw), poultry manure (4% dw) and microbial inoculants (1% dw), 6 months before the beginning of the experiments. Physical and chemical parameters of the applied biogas digestate compost were the following: electric conductivity, 5.62 μS cm^{-1}; pH, 7.2; total organic matter, 59.66%; total nitrogen, 1.6%; and carbon:nitrogen ratio 16:1. The biogas digestate compost used in the experiments was previously sterilized by chloroform fumigation.

Four Gram-negative (*Brevundimonas vesicularis* SM-0625, *Chryseobacterium hungaricum* CHB-20p, *Pseudomonas putida* NCP-3, *P. putida* NC-1494), seven Gram-positive bacterial strains (*Rhodococcus aetherivorans* AK-44, *R. globerulus* AK-36, *R. gordoniae* AK-38, *R. pyridinivorans* AK-37, *R. pyridinivorans* CHB-15p, *R. rhodochrous* AK-40, *R. erythropolis* AK-35) and five fungal strains (*Cladosporium cladosporoides* CP3, *Paecilomyces variotii* Bd41, *Rhodotorula minuta* CP2, *Talaromyces wortmannii* KO24, *Yarrowia lipolytica* Bd22) were used as test microbes. They originated from different hydrocarbon polluted soils in Hungary.

Microcosm-1 for the examination of survival kinetics

Aliquots of 500 g of the incubation mixtures were placed in glass jars with a bottom area of 176 cm^2 and volume of 3500 cm^3. The incubation mixtures consisted of chloroform-fumigated biogas digestate compost and the cell mass of different alkane-degrading microbes with population densities of 10^9 CFU g^{-1} for Gram-negative bacterial strains and 10^8 CFU g^{-1} for *Rhodococcus* and fungal strains, respectively. The Microcosm-1 systems were incubated for 256 days at 4 °C and 26 °C with permanent provision of sufficient air supply and moisture content (55%). In parallel experiments, microcosms with the same media containing burnt perlite of 5% dw were also investigated.

Reisolation of alkane-degrading bacteria and fungi from the prepared compost samples

The survival of the investigated hydrocarbon-degrading microorganisms in the compost samples was monitored with reisolation of their cells in order to determine the decimal reduction time (D-value), the interval of time during which 90% of the microorganisms are inactivated at a defined temperature. The populations of Gram-negative and Gram-positive bacteria were checked 7 times in the 256 day long incubation period (at days 2, 4, 8, 16, 32, 64 and 128). In order to recover the actual CFU of the different alkane-degrading bacteria or fungi, three different microbiological nutrient media were used. Gram-negative bacteria were cultured on King's B agar (King et al., 1954), whilst Rhodococcus strains (Gram-positive, acid-fast bacteria with highly specific colony morphology) on CAZ-NB agar (von Graevenitz and Pünter-Streit, 1995). The number of fungal elements in the incubation mixtures was followed with the use of neomycin containing potato-dextrose agar (Merck).

Measurement of alkane degradation by microorganisms

The alkane-degrading activity of the tested microorganisms was examined by the OxiTop® Control (WTW) manometric system (Microcosm-2). Measurements were carried out in closed vessels at 26 °C for 96 hours. Each microcosm contained 20 g experimental mixture, 1% (w:w) diesel oil and the tested microorganism in quantities of 10^6 CFU g^{-1} in the case of Gram-negative bacteria, and 10^7 CFU g^{-1} for rhodococci and fungi. Simultaneously, microcosms supplemented with 5% (w:w) burnt perlite were also examined. The carbon dioxide production during the degradation of diesel oil compounds was constantly measured. Incubation mixtures without microorganism were applied as controls. To measure the diesel oil originated carbon dioxide, the control carbon dioxide values were subtracted.

Statistical Analyses

All of the experiments were carried out in triplicates. Data were presented as mean values and standard deviations. Differences between the treatments were determined by one-way analysis of variance. The D-values for the determination of survival of different alkane-degrading microorganisms at 26 °C were determined from the negative reciprocal of the slopes of the regression lines, using the linear portions of the survivor curves (CFU g^{-1} versus time of incubation).

Results and discussions

In vitro survival kinetics of alkane-degrading bacteria in biogas digestate compost

The effect of the chemical factors involved in the compost medium were adequately investigated by the incubation at 4 °C. The initial cell counts (10^9 CFU g^{-1}) of the applied Gram-negative alkane-degrading bacterial strains (*Brevundimonas vesicularis* SM-0625*,* *Chryseobacterium hungaricum* CHB-20p*,* *Pseudomonas putida* NCP-3) gradually decreased to the value 10^8 CFU g^{-1} during the incubation period of 128 days in the examination system Microcosm-1. Following the process of culturing with enumeration, the quantity of the cells decreased by 8-28% until day 2 and 32-49% until day 4 (*Fig. 1a*).

The examined *Chryseobacterium hungaricum* strain (CHB-20p) proved to be the most tolerant towards the physical and chemical factors reducing the quantity of cells. It was present in the compost in the amount of 9.3 x 10^8, 6.2 x 10^8 and 5.1 x 10^8 CFU g^{-1} on day 2, 8 and 32, respectively. The survivability of *Brevundimonas vesicularis* SM-0625 was lower than that of *Chryseobacterium hungaricum* CHB-20p during the examination; the amount of its reculturable cells was 9.2 x 10^8, 6.8 x 10^8 but 1.7 x 10^8 CFU g^{-1} on day 2, 4 and 64, respectively. *Pseudomonas putida* NCP-3 survived the least between the three examined alkane-degrading Gram-negative bacterial strains. Its cells remained reculturable in the rate of 20.7% and 12.6% on day 32 and 64, respectively.

The survival of the three examined alkane-degrading Gram-negative bacterial strains showed similar kinetics, the quantity of their cells decreased continuously in the compost mixture. Nevertheless, approximately 10% (10^8 CFU g^{-1}) of the initial cells were reculturable until the end of the experiment.

In contrast, the survival of cells of the tested alkane-degrading Gram-negative bacteria belonging to the division Gammaproteobacteria is very limited since these bacteria do not form endospores or any other durable cells. However, their metabolism including their respiration level is relatively high. They can minimize the harmful effects of contaminants by their motility and extremely broad spectrum of catabolic enzymes that make a remarkably fast adaptation possible. These bacteria are not responsible for composting and they usually do not live in compost. Their long-lasting survival can be explained by their resistance to the antimicrobial substances of compost or by the presence of metabolizable residues of the compost mixture.

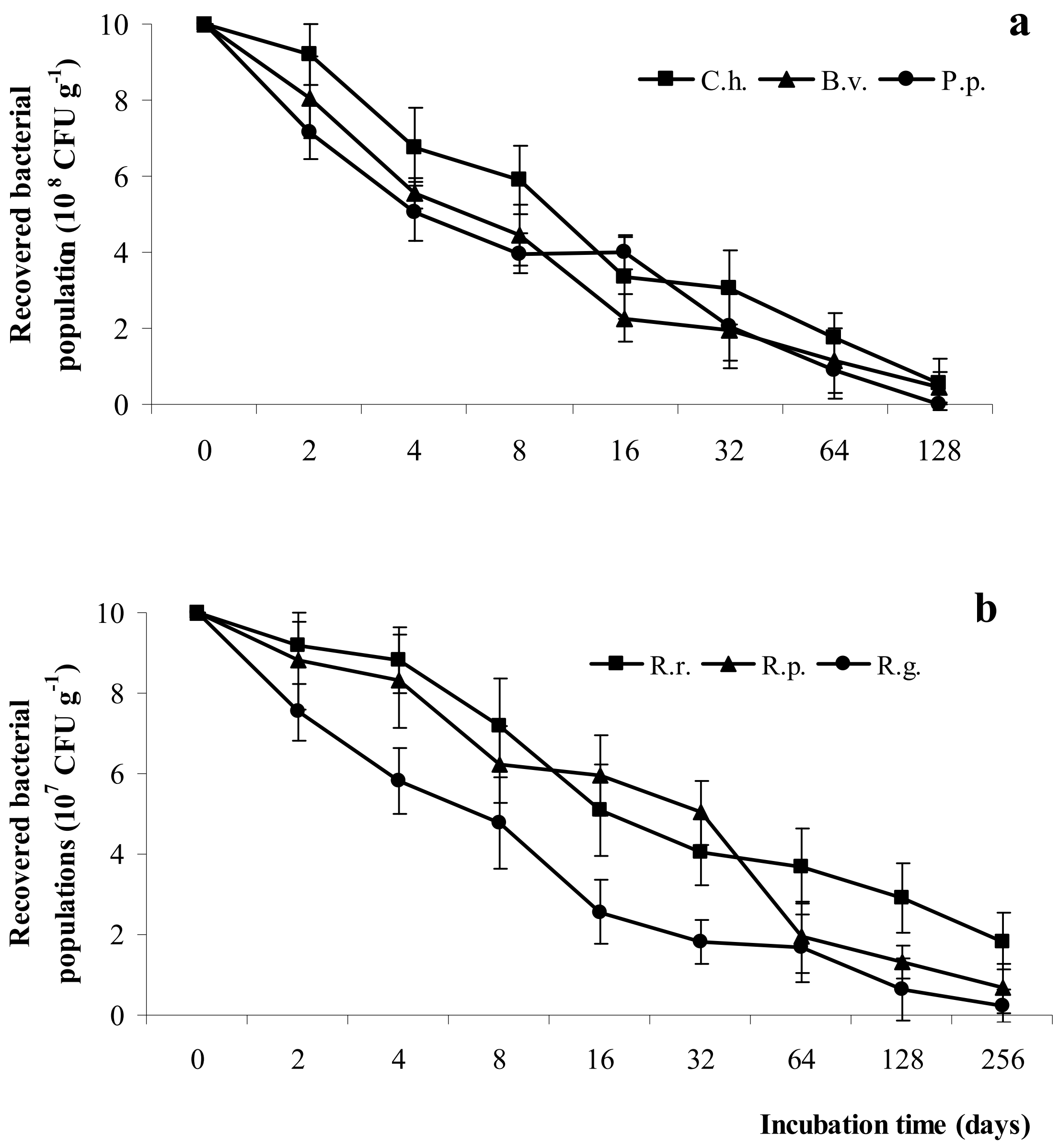

Figure 1. *In vitro survival of alkane-degrading Gram-negative (a) and Gram-positive bacteria (b) in biogas digestate compost at 4 °C.*
(B.v.: Brevundimonas vesicularis SM-0625, C.h.: Chryseobacterium hungaricum CHB-20p, P.p.: Pseudomonas putida NCP-3, R.p.: R. pyridinivorans AK-37, R.g.: R. gordoniae AK-38, R.r.: R. rhodochrous AK-40)

Colloidal compatibility of glycocalyx covering the Gram-negative bacterial cells with biogas digestate residues can also be a factor in their survival. The fundamental biological reason of the very similar survival kinetics of the examined bacterial strains in the biogas digestate compost can be explained by their close taxonomic relations to each other and by their similar phenotypic features, especially regarding their cell structure.

The initial cell count (10^8 CFU g^{-1}) of all the three alkane-degrading *Rhodococcus* strains, representing Gram-positive prokaryotes in the experiment (Microcosm-1) decreased moderately to the level of 10^7 CFU g^{-1} during the 256 day long incubation period at 4 °C. Nevertheless, the survival kinetics of the *Rhodococcus* strains slightly differed from each other (*Fig. 1b*).

The first type of the survival kinetics of the *Rhodococcus* strains were represented by *R. rhodochrous* AK-40 and *R. pyridinivorans* CHB-15p: 83-88% of their cells could be recultured on day 4. The cell count of the *R. rhodochrous* AK-40 decreased by half during the first 16 days but it was still 1.8 x 10^7 CFU g^{-1} after the 256 day long incubation period. The survival kinetics of *R. pyridinivorans* CHB-15p slightly differed from the previously mentioned AK-40 strain since its cell density halved after day 32 but 0.7 x 10^7 living cell was found on day 256. The survival kinetics of *R. gordoniae* AK-38 represents the second type. The cells of the strain AK-38 are apparently less viable in the compost: its survival kinetics show a fast decline until day 4, and only the half and the quarter of the amount of the cells could be recultured on day 8 and day 16, respectively. Later on, the fast decline slowed down and the level of 10^7 CFU g^{-1} was still present on day 256.

The longer survival of rhodococci can be connected with the bigger size of their cells and genomes, the thicker cell wall consisting of more layers and their wide range catabolic activity. In general, more than 20% of the cells of the *Rhodococcus* strains were present at a viable state in the incubation mixture on day 64. Therefore, we suggest that they contribute to thehydrocarbon degrading activity.

Reduction of the viability of different alkane-degrading bacteria and fungi

In vitro survival of the different alkane-degrading bacteria and fungi during the long-term incubation in microcosms (Microcosm-1) was characterized by the decimal reduction time (D-value) in chloroform-fumigated biogas digestate compost (*Table 1*).

The reduction rate of the cell counts of 4 alkane-degrading strains of Gram-negative bacteria was examined in the pure compost media stored at 4 °C. The *Chryseobacterium hungaricum* CHB-20p strain proved to be the most tolerant (with a D-value of 128.1 days) while *Pseudomonas putida* NC-1494 strain (D-value: 49.7 days) was the least tolerant among the examined strains. The effect of incubation temperature on the survival kinetics of all Gram-negative bacteria was significant. At 26 °C, the D-values of the examined strains ranged from 3.1 to 5.5 with an average of 3.9 days. The D-values for all tested Gram-negative bacteria indicated that their survival in biogas digestate compost were moderate to weak.

Based on their D-values, the tested Gram-positive strains, namely the examined species of genus *Rhodococcus* were the most tolerant microorganisms among the alkane-degrading ones of our collection. The majority of them could tolerate the pure compost up to the survival ratio of 10 % for approximately 100-250 days (with an average of 174.6) at 4 °C. The strain *R. erythropolis* AK-35 was the least tolerant (D-value: 105.1 days) while the strain *R. pyridinivorans* AK-37 was the most tolerant (D-

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value: 248.6 days) organism in the examined group. An extraordinary D-value (>256 days) was shown by the strain *R. rhodochrous* AK-40 due to its unusual survival kinetics. The effect of incubation temperature on the survival of all examined *Rhodococcus* strains was also significant. At 26 °C, D-values for rhodococci ranged from 6.3 to 12.8 days with an average of 9.7.

The D-values for the 5 alkane-degrading fungal strains ranged from 64.9 to 151.7 days with an average of 109.3 at 4 °C. They were found to be more tolerant to the ambient compost than the strains of the examined Gram-negative species but less tolerant than the *Rhodococcus* strains. The effect of incubation temperature was the least pronounced on the survival of the tested fungal strains. D-values determined on the basis of their colony-forming units ranged from 24.4 days (*Rhodotorula minuta* CP2) to 40.3 days (*Talaromyces wortmannii* KO24) at 26 °C.

Table 1. *Decimal reduction time (D-value) for different alkane-degrading bacteria and fungi within a long-term incubation in mixtures with biogas digestate compost*

Strains	**D-value (days)**			
	Pure compost		**Compost-perlite mixture**	
	4 °C	**26 °C**	**4 °C**	**26 °C**
Gram-negative bacteria				
Brevundimonas vesicularis SM-0625	110.6	3.5	93.8	33.5
Chryseobacterium hungaricum CHB-20p	128.1	5.5	124.6	48.0
Pseudomonas putida NCP-3	96.3	3.1	68.9	21.5
P. putida NC-1494	49.7	3.6	57.5	23.9
Gram-positive bacteria				
Rhodococcus aetherivorans AK-44	176.9	6.3	258.0	187.4
R. globerulus AK-36	124.6	12.4	147.9	121.6
R. gordoniae AK-38	146.8	7.5	203.5	189.5
R. pyridinivorans AK-37	248.6	9.2	>256	>256
R. pyridinivorans CHB-15p	164.5	8.4	197.5	175.0
R. rhodochrous AK-40	>256	11.3	>256	>256
R. erythropolis AK-35	105.1	12.8	137.3	126.7
Fungi				
Cladosporium cladosporoides CP3	64.9	27.9	94.7	33.1
Paecilomyces variotii Bd41	96.5	32.7	208.5	182.7
Rhodotorula minuta CP2	83.8	24.4	199.2	50.6
Talaromyces wortmannii KO24	149.4	40.3	196.8	49.0
Yarrowia lipolytica Bd22	151.7	38.4	182.3	68.9

During composting, the easily biodegradable materials are converted to more stable organic materials,e.g. humic and fulvic acids. These acids are not only slowly metabolizable compounds but they also have some antimicrobial effect. The tolerance of the effect of humic materials was a major factor in the selection of our alkane-degrading test microorganisms. Based on the results, the chosen strains were able to survive easier for a shorter or a longer period of time in compost. We also suppose that some special colloid compounds in the biogas digestate compost increased the survival rate of the tested microorganisms.

The Gram-negative bacteria with smaller cells and thinner cell wall were more sensitive to different abiotic effects and their survival was shorter than that of the rhodococci with larger cell size, more extended genome and multilayered cell wall. The survival of the fungi can be explained by their big cells, thick cell wall and their the eukaryote cell structure.

The survival of the alkane-degrading bacteria in the biogas digestate compost containing burnt perlite was generally longer than in the pure compost. The survival kinetics of the tested Gram-negative strains in the perlite-containing compost at 4 °C slightly differed from that in the pure compost at the same temperature. In the perlite-containing compost media, D-values for the tested Gram-negative bacteria varied between 57.5 (*Pseudomonas putida* NC-1494) and 124.6 (*Chryseobacterium hungaricum* CHB-20p) days with an average of 86.2 days. While the effect of perlite was not significant at 4 °C, the perlite contentmarkedly increased the D-values of Gram-negative bacteria at 26 °C. They ranged between 21.5 and 48.0 days with an average of 31.7 days.

The presence of perlite in compost medium caused a moderately increased survival of the tested *Rhodococcus* strains at 4 °C. For 5 isolates, the D-values ranged between 137.3 and 240.0, and in the case of 2 strains the determined value exceeded the experimental period of 256 days. The most outstanding effect of the perlite content was found in the case of the survival of rhodococci at 26 °C. D-values for 5 of them varied between 121.6 and 232.0 with an average of 168.9 and the cell count of the further 2 *Rhodococcus* strains (*R. pyridinivorans* AK-37 and *R. rhodochrous* AK-40) did not decrease to 10% in the perlite-containing compost during the 256-day long incubation.

In the case of fungal strains, a significant elongation of the survival could be observed in the compost containing perlite at 4 °C. The D-values of fungal survival increased with the average of 60% in the presence of perlite in the compost medium. The effect of perlite in the medium was remarkable at 26 °C, too. Colony forming units of 4 fungal strains increased in a similar rate in spite of the fact that they are not strongly related taxonomically. The blastoconidia of *Cladosporium cladosporoides* CP3, the phialoconidia of *Talaromyces wortmannii* KO24 and the budding cells of *Rhodotorula minuta* CP2 and *Yarrowia lipolytica* Bd22 showed an average 65% longer survival in the presence of perlite than in the pure compost at 26 °C. The survival of the phialoconidia of *Paecilomyces variotii* Bd41 in the perlite-containing compost was also improved by perlite in the compost compared to pure compost at the same temperature.

The increase of the survival of different alkane-degrading microbe strains in biogas digestate compost containing burnt perlite at proportion of 5% can be considered as a complex phenomenon. As for the effect on the survival of Gram-negative bacterial strains, burnt perlite can provide a chemically inert large surface for the immobilisation of biologically intact cells. Besides, especially for rhodococci and fungi, perlite stabilizes the optimal humidity and aeration of compost medium.

Carbon dioxide production of the prepared hydrocarbon-containing mixtures

During the degradation of components of the diesel oil added to the biogas digestate compost, carbon-dioxide produced by the alkane-degrading microorganisms was constantly measured in microcosm experiments (Microcosm-2). Results are summarized in *Table 2*. In the examined aerobic microcosms, high level respiration (9.2-14.6 μg CO_2 g^{-1} h^{-1}) was found in the case of Gram-negative bacterial strains with the initial cell count of 10^7 CFU g^{-1}. The highest carbon dioxide production was showed by the strain *Pseudomonas putida* NCP-3 while the carbon dioxide production of *Chryseobacterium hungaricum* CHB-20p was considerable, too. The respiration level of *Rhodococcus* strains introduced into the system at the amount of 10^6 CFU g^{-1} was also relatively high

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(5.8-9.6 µg CO_2 g^{-1} h^{-1}). The highest carbon dioxide production of rhodococci was found for the strain *R. pyridinivorans* AK-37. The respiration level of hydrocarbon degrading fungal strains in the presence of diesel oil was also remarkable. The carbon dioxide production (9.1 µg CO_2 g^{-1} h^{-1}) of the well known hydrocarbon degrader *Yarrowia lipolytica* Bd22 is followed by that of the strain *Rhodotorula minuta* CP2 (6.0 µg CO_2 g^{-1} h^{-1}). It can be concluded that the different hydrocarbon degrading strains not only can survive as viable cells in the biogas digestate compost but they can also degrade the alkanes of diesel oil added to the incubation mixture.

Table 2. *Carbon dioxide production of alkane-degrading microbial strains in compost or compost-perlite composition with the addition of diesel oil*

Strains	**Carbon-dioxide production (µg CO_2 g^{-1} h^{-1}) by alkane degradation**	
	Compost	**Compost-perlite mixture**
Gram-negative bacteria		
Brevundimonas vesicularis SM-0625	9.2 ± 0.9	10.7 ± 0.9
Chryseobacterium hungaricum CHB-20p	11.9 ± 1.1	9.2 ± 0.8
Pseudomonas putida NCP-3	14.6 ± 1.2	14.9 ± 1.0
P. putida NC-1494	10.3 ± 0.9	12.3 ± 1.1
Gram-positive bacteria		
Rhodococcus aetherivorans AK-44	7.5 ± 0.4	7.1 ± 0.5
R. globerulus AK-36	8.4 ± 0.6	8.2 ± 0.5
R. gordoniae AK-38	6.7 ± 0.4	10.5 ± 0.7
R. pyridinivorans AK-37	9.6 ± 0.7	14.8 ± 0.9
R. pyridinivorans CHB-15p	7.4 ± 0.5	13.6 ± 0.9
R. rhodochrous AK-40	5.8 ± 0.3	12.4 ± 0.7
R. erythropolis AK-35	6.2 ± 0.4	11.7 ± 0.7
Fungi		
Cladosporium cladosporoides CP3	4.4 ± 0.8	7.9 ± 1.4
Paecilomyces variotii Bd41	5.1 ± 0.4	8.0 ± 0.5
Rhodotorula minuta CP2	6.0 ± 0.8	7.9 ± 0.9
Talaromyces wortmannii KO24	4.8 ± 1.1	5.2 ± 0.8
Yarrowia lipolytica Bd22	9.1 ± 1.4	9.3 ± 0.9

All representatives of the three examined taxonomic groups of the alkane-degrading microbial strains produced carbon dioxide during the degradation of diesel oil added to the compost mixture even in the presence of 5% perlite. The respiration of the applied Gram-negative bacterial strains incubated in the compost-perlite mixture did not significantly differ from that incubated in pure compost. However, the majority of the applied *Rhodococcus* strains produced significantly more carbon dioxide in the perlite-containing incubation medium. Similarly to rhodococci, the majority of the applied fungi showed an increased activity due to the effect of perlite in the compost mixture.

The presence of perlite in the incubation mixture increased the respiration activity of the applied *Rhodococcus* strains outstandingly. It was moderately increased for applied fungal strains while no effect was found concerning that of the Gram-negative strains. Several physical and chemical factors can be considered as the natural background of the effect of perlite application. One of the most important factors may be the water activity stabilizing effect of perlite being advantageous for actinobacteria and fungi.

Adaptability of the applied bacteria to hydrocarbons

During the 96-hour long incubation period of previously fumigated and diesel oil containing experimental mixtures inoculated with alkane-degrading bacteria, carbon dioxide evolved in two different phases (Microcosm-2). At 12-24 hours of the incubation, the average rate of carbon dioxide production of the cells of the 3 alkane-degrading Gram-negative bacterial strains (*Brevundimonas vesicularis* SM-0625, *Cryseobacterium hungaricum* CHB-20p, *Pseudomonas putida* NCP-3) was 8.2, 10.4 and 11.7 CO_2 g^{-1} h^{-1}, respectively. At 84-96 hours the values of 10.1, 12.8 and 15.5 μg CO_2 g^{-1} h^{-1} were measured as maximal respiration intensity of these strains. The average carbon dioxide production of the tested 3 *Rhodococcus* strains (*R. pyridinivorans* AK-37, *R. gordoniae* AK-38, *R. rhodochrous* AK-40) was only 1.4, 1.2 and 1.8 CO_2 g^{-1} h^{-1} at 12-24 hours. In contrast, at 84-96 hours, the determined values were 10.4, 7.6 and 6.7 CO_2 g^{-1} h^{-1}, respectively (*Fig. 2*).

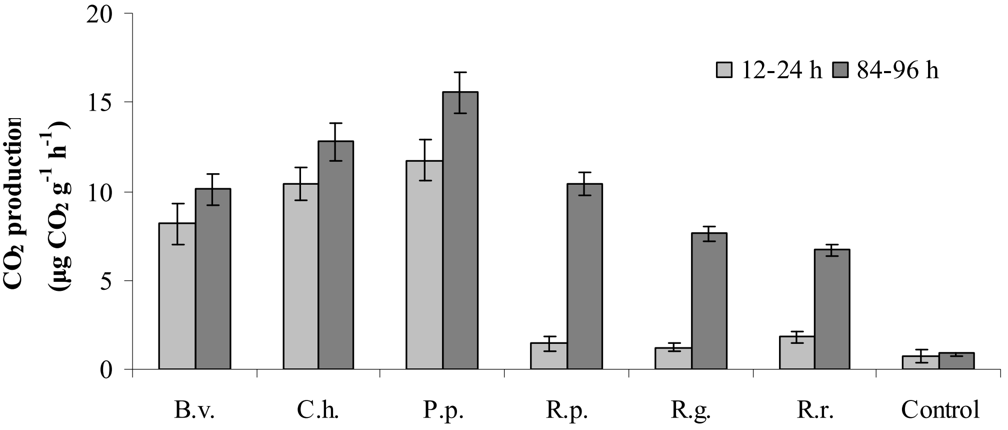

Figure 2. *Carbon-dioxide production of the experimental mixtures prepared with different alkane-degrading bacteria at 12-24 and 72-96 hours of incubation. (B.v.: Brevundimonas vesicularis SM-0625, C.h.: Chryseobacterium hungaricum CHB-20p, P.p.: Pseudomonas putida NCP-3, R.p.: R. pyridinivorans AK-37, R.g.: R. gordoniae AK-38, R.r.: R. rhodochrous AK-40, Control: non inoculated experimental mixtures)*

In the first phase, the respiration intensity of the Gram-negative strains reached quickly 75.5-81.5% of their maximum while *Rhodococcus* strains were able to reach only 13.7-27.4% of their maximum value at the same time. The difference in the carbon dioxide production between the examined two groups of bacteria suggests that they differ markedly in the adaptability to the presence of hydrocarbons.

Conclusions

Based on our results, the cell quantity of the tested 3 alkane-degrading Gram-negative bacterial strains gradually decreased from the initial 10^9 CFU g^{-1} to a minimum value in the compost during the 128-day long incubation at 4°C. Furthermore, 10% of the cells survived for approximately 50-90 days (D value). The survival kinetics of the population of the 3 alkane-degrading *Rhodococcus* strains was longer; it took 100-200 days to decrease to 10% of the initial cell quantity (10^8 CFU g^{-1}) at 4 °C. Moreover, nearly 20% of the cells of the strain *R. rhodochrous* AK-40 were present in the compost even at the end of the 256 days long incubation period. Interestingly, *R. gordoniae* of rhodococci represented a different type of survival kinetics: it rapidly decreased its number in the first week. Furthermore, ten percent of the initial cell quantity (10^8 CFU g^{-1}) of the fungi, representing eukaryote microorganisms with a chitinous cell wall, has survived for 65-105 days.

The results also indicate that the survival of both the bacterial and fungal strains depended considerably on the temperature of the compost. At 26 °C, the survival of Gram-negative bacteria and rhodococci was 94% lower than at 4 °C while the survival of fungi was 70% lower at 4 °C than at 26 °C. Burnt perlite added to the system in the volume of 5% to stabilize the porosity and the water activity of the compost increased the survival of bacteria by 31% and that of fungi by 61% in average.

Based on the carbon dioxide production experiments, we can conclude that the alkane-degrading microbes not only survived in the compost previously fumigated with chloroform but also could utilize the components of the diesel oil added to the system. The Gram-negative bacterial cells of the quantity of 10^7 CFU g^{-1} produced 9.2-14.6 µg h^{-1} carbon dioxide by metabolizing the diesel oil. The rhodococci and the fungal strains, with the cell count of 10^6 CFU g^{-1}, produced carbon dioxide in less quantity.

While the addition of 5% perlite to the compost-microorganism-diesel oil incubation mixture did not change significantly the carbon dioxide production of the Gram-negative strains, rhodococci and fungal strains markedly increased their activity.

The adaptability of Gram-negative bacteria and rhodococci to hydrocarbons was found to be markedly different. In the phase of 12-24 hours of the incubation, the quantity of the carbon dioxide produced by Gram-negative bacteria in the course of the metabolism of diesel oil has already reached 75.5-81.5% of the experienced maximum of the 84-96 hours experiment, while during the same period, *Rhodococcus* strains reached only 13.7-27.4% of their maximum values.

In summary, we suggest further investigations of the survival kinetics of each microorganism strain in the adequate media, both for experimental and technological applications.

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FEEDING ECOLOGY OF MANGROVE CRABS IN CAMEROON

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Abstract. The feeding ecology of several species of mangrove crabs was studied in Cameroon mangrove forest through tethering experiments and gut analyses from September 2010 to March 2011. Field observations were two-fold: to investigate mangrove propagules predation and feeding preferences of mangrove leaves. Feeding preference was determined on fresh and senescent leaves of Laguncularia racemosa, Avicennia germinans and Rhizophora species. Propagule predation was investigated on Rhizophora mangle, Rhizophora harrisonii and Rhizophora racemosa. In the laboratory, gut content analysis was done on Metagrapsus curvatus, Sesarma huzardi, Sesarma elegans, Sesarma alberti, Goniopsis pelii and Grapsus grapsus to assess their natural diets. The field observations suggest that 65.9% of the propagules studied was predated, 71.3% of the leaves was damaged. Mangrove material was the main component of crabs diet, it constituted 45.4% of Metagrapsus curvatus diet, 55% Sesarma elegans diet, 62.5% Sesarma alberti, 65.9% Goniopsis pelii diet, 47.8% Sesarma huzardi diet and 37.3% Grapsus grapsus diet. Damage on young mangrove trees was highly correlated to the number of crabs on trees (r2 = 0.75). The high damage rate of mangrove materials indicate that crabs play a significant ecological role in the functioning of the mangrove ecosystem, by affecting mangrove recruitment and biogeochemical functioning.

Keywords: *Feeding Behaviour, Mangrove leaf species, Mangrove Crabs, Cameroon*

Introduction

Mangrove crabs are among the most common and abundant faunal groups in mangrove forests (Smith et al. 1991). They play a vital ecological role in these ecosystems (Lee 1998). However, they are often viewed as threats to the successful regeneration or restoration of mangrove forests through their predation of propagules (Dahdouh-Guebas et al. 1997, Islam et al. 2010). In Cameroon, intense human development within mangrove ecosystems, coupled with the rapid urbanization of adjacent towns and the excessive utilization of trees for the production of fuelwood, charcoal and clearing for agricultural purposes, has further gradually degraded these ecosystems (Karen 2000). This degradation is expected to worsen in future years, largely as a result of climate-change induced sea level rise and salt water intrusion (Karen 2000).

Mangrove propagules or seedlings are an important food source for crabs. Majority of propagules are damaged within days of their release from the tree (Hogarth 1999). Crabs show distinct preference for different propagule species. *Avicennia* propagules are the most preferred because they contain simple sugars, low tannin, fibre and protein (Smith 1987). Propagule predation might also affect its mode of dispersal. Many mangrove species exhibit viviparity, a phenomenon where juvenile plants further develop after fertilization while attached to parent trees. When a propagule falls from a

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tree at low tide, it might establish itself upright in the mud; a phenomenon termed the planting strategy (Van Spreybroeck 1992, Dahdouh-Guebas et al. 2011). Alternatively, if the propagule falls during high tide, it might float in water and develop in another site, usually lying horizontally, a phenomenon termed the stranding strategy (loc. cit.). Stranding propagules are more predated, and this may play a critical role in limiting seedling establishment and subsequent recruitment (Van Spreybroeck 1992, Bosire 2005)

Propagule predation is an important source of mangrove mortality (Osborne and Smith 1990, Mckee 1995), suggesting a possibility of predator-controlled recruitment. Earlier studies of predation on mangrove propagules have focused on predation in relation to tree dominance. In reference to the dominance-predation hypothesis proposed by Smith (1987), tested on the Australian north coast (McGuinness 1997), in Malaysia (Smith et al. 1989), and in Belize (McKee, 1995). All these studies provided partial support for this hypothesis, although other studies have supported the hypothesis (Smith et al. 1989, Dahdouh-Guebas et al. 2011).

Leaf-eating mangrove crabs are extremely dependent on the mangrove litter. Leaf materials however, are an inadequate diet given that, irrespective of their stage of senescence and decomposition, the nitrogen content is low. Therefore, leaf-eating crabs must supplement their diet with nitrogen from other resources (Skov 2002). These include algae, bacteria, ingestion of their own faeces colonized by microorganisms, or grazing on surface sediments (Andersen 2002). Previous studies have suggested that crabs show food selective preferences depending on the food nutritional values, varieties and accessibility (Meziane et al. 2002, Dahdouh-Guebas et al. 2011). The critical value of 17: 1 for the C:N ratio has often been used as a point of comparison to determine the nutritional value of food resources (Russell-Hunter 1970). Dietary C:N ratios above 17:1 are considered to be under the nutritional requirement. However, crabs were reported to consume plant litter with C:N ratios ranging from 25 : 1 to 183.5 : 1 (Linton et al. 2007). Therefore, crab choice in the consumption of mangrove litter is unclear.

Although a great deal is known about the biology, behaviour and ecology of mangrove crabs in the past in a variety of geographic locations, very little is known about their feeding ecology in the west coast of Africa.

The goal of the present study is to improve understanding of the feeding ecology of mangrove crabs in West Africa. The objectives are to quantify (1) the propagule consumption rates and preference, (2) the leaf consumption rates and preference, (3) the diet of Sesarmidae and Grapsidae under natural conditions.

Materials and methods

Study area

This study was carried out from September 2010 to March 2011 in the Cameroon Estuary mangrove (*Figure 1*), located in the South–Western part of Cameroon between latitudes 3^{o} 83' - 4 ^{o} 10' N and longitudes 9^{o}25' – 10^{o} 00' E. It is estimated that mangrove forest covers 1,750 km^2 in this estuary. The area has an equatorial climate characterised by two main seasons: the rainy season from March to October (8 months) and the dry season from November to February (4 months). It has an annual average precipitation of 1,500mm to 3,000mm and high temperatures ranging from 24^{o}C to 27^{o}C (Letouzey1985). Strong tidal influences on rivers (Wouri, Dibanda and Sanaga) and

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freshwater influxes enable mangroves to growth as far as 100 kilometres inland (UNEP 2007, Nfotabong et al. 2009).

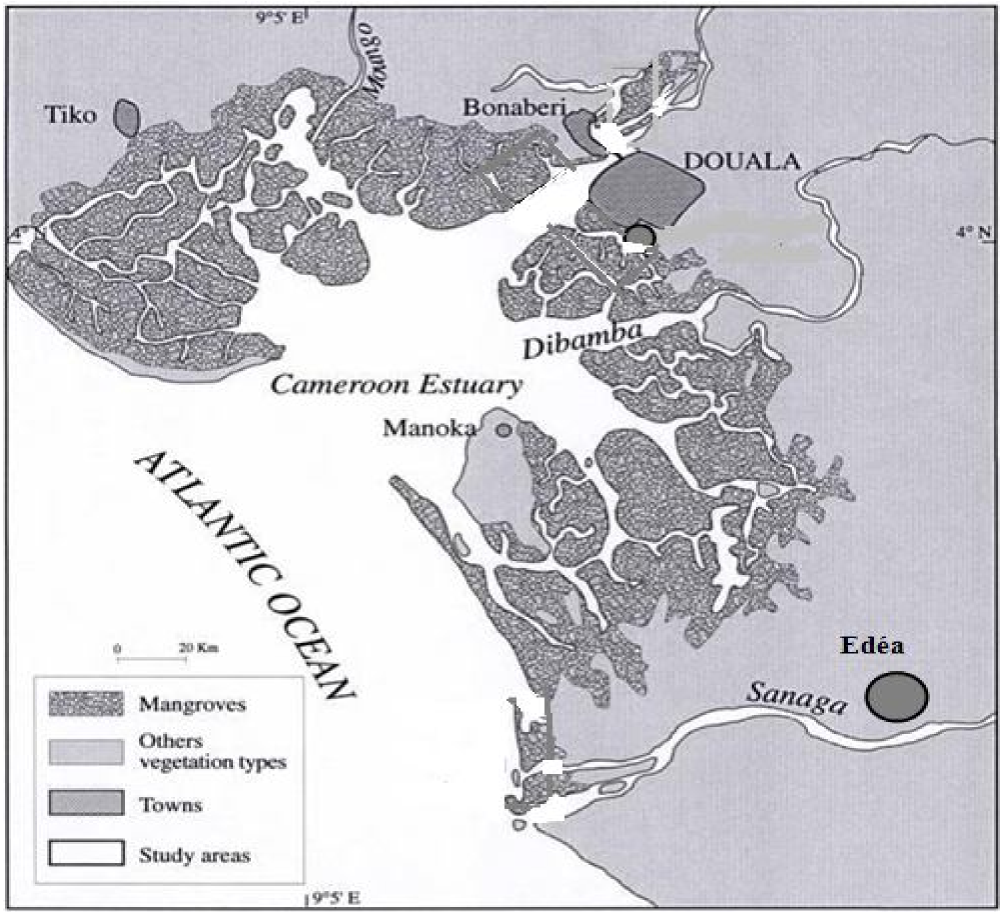

Figure 1: *Mangrove of the Cameroon Estuary (Adapted from Nfotabong et al. 2009)*

The floristic composition of Cameroon mangrove is characteristic of the Atlantic mangroves of West Africa. It is dominated by *Rhizophora mangle, Rhizophora harrisonii and Rhizophora racemosa.* Other species include *Avicennia germinans, Laguncularia racemosa, Conocarpus erectus, Acrostichum aureum, Pandanus candelabrum and Nypa fruticans* (Spalding et al. 1997).

Propagule predation

This experiment was carried out in an extensive gap within the forest. A transect of 100 m was established, and 5 evenly dispersed plots of 5m x 5m were set. Each plot was placed 15 m apart, they were located on opposite sides along the transect. The vegetation in each plot was recorded. *Rhizophora* (*R. mangle, R. harrisonii* and *R. racemosa*) were the dominant species in the plots, and only *Rhizophora* species were used in this study because of their elongated propagule. Thirty fresh, mature, similar-sized propagules were collected, ten from each mangrove species and planted either vertically (50%) or laid horizontally (50%) on the forest floor haphazardly. Six propagules were planted in each plot. The vertical planting represents a dispersal

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strategy when the propagule plants itself into the mud when dropping from the adult tree, whilst the horizontal represents the circumstance where the propagule is dispersed by floating on the water at high tide (Dahdouh-Guedas et al. 1999). Each propagule was tethered independently with a 50 cm length of nylon twine, the other end of which was tied to a piece of wood on the forest floor. The propagules were spaced far apart so that the tethers could not get tangled. The length of each propagule was measured and propagules individually tagged. The propagules were checked from a distance using binoculars over a 6 hour period, after which they were checked once a day for one week. All observations were carried out during low tide when the crabs are very active.

Predation status was recorded following Smith (1987b): (1) when the epicotyl was eaten (2) when 50 % of the hypocotyl was lost (3) when the propagule was pulled into the burrow of the crab. Each propagule was classified as viable (capable of growth, i.e. ≤ 50% of propagule eaten), non-viable (incapable of growth, i.e. >50% of propagule eaten) and missing (when lost). Signs of snail predation were also recorded.

Leaf predation

This study was conducted in 10m x10m quadrats. The vegetation of the quadrat was a mixture of *Laguncularia racemosa*, *Avicennia germinans* and *Rhizophora* (*R. mangle, R. harrisonii* and *R. racemosa*) the main species in Cameroon mangroves. Leaves of *Laguncularia racemosa*, *Avicennia germinans* and *Rhizophora* species were used; both fresh and senescent leaves were gathered. Fresh leaves were harvested from trees, while senescent leaves (yellow and easily abscised) were either picked from the forest floor or harvested from the tree. Ten replicate leaves (fresh and senescent) of each species were spread across the quadrat, each tethered with a nylon string 50cm in length with the other end tied to a piece of wood on the forest floor and tagged. The leaves were tied randomly and far apart to avoid tangling. The leaf surface was measured by tracing the edge on graph paper. The Leaves were checked after 24 hours, and percentage predation damage and leaf position was recorded (whether the leaves were found on the surface or in a crab burrow). Leaves that were in crab burrows were removed by gently pulling on the attached string. The experimental quadrat was not isolated from falling leaves. The forest floor was clean, probably due to the fact that the fallen leaves were swept by tidal movements.

Additional data on leaf predation were gathered from crabs predating within the canopy. Crabs were seen residing on tree trunks, branches and prop roots. They were observed climbing mangrove trees; usually early in the morning to feed on leaves and by midday they descended, and climbed again at twilight then descended by night fall. An average of 5 crabs was found on a single tree. Two young trees (1.5m to 2m tall, dbh 2.3cm to 5cm) of each species *Laguncularia racemosa*, *Avicennia germinans* and *Rhizophora* species were observed from a close distance for about 5 hours and crab feeding activities and leaf damage recorded.

Gut contents analysis

Adult grapsid [*Metagrapsus curvatus*, *Sesarma (Perisesarma) huzardi*, *Sesarma (Chiromanthes) elegans*, *Sesarma (Perisesarma) alberti*] and sesarmid[*Goniopsis pelii*, *Grapsus grapsus*] crabs of similar size 20-35mm carapace width were sampled for gut content analysis. They were collected during the most active period, at low and receding tides, when the foregut would be most likely to be full and the contents most easily recognised. The crabs were sedated in ice for a few minutes, washed and placed in 70% alcohol for later analysis. The crabs were dissected, stomaches removed and the contents stirred with 10-15ml of distilled water in a petri dish and examined under a light microscope at X20 magnification. The percentage composition of different items within each gut was determined over 10 cells within a 100 cell grid. The degree of fullness of the stomach was estimated as follows: 0 for an empty stomach, 1 for a half-full stomach, 2 for a full stomach. The contribution of each food item to the total diet was expressed in terms of the percentage of the field occupied by the different items. The items were classed as plant material (not identified to mangrove species level), animal material, algae, sand, detritus and unidentified debris.

Data analyses

Descriptive statistics were used to analyze gut contents, propagules and leaves predation. To test whether or not there was a significant difference between propagule predation amongst plots, the non parametric Kruskal-Wallis ANOVA was performed. Differences in leaf damage among mangrove species were also compared using parametric ANOVA test.

Non parametric tests (Friedman, Tukey) were employed to test for differences in propagules planting strategies, propagules viability (viable and non-viable) and leaf consumption preference (fresh and senescent). Correlation between tree leaf damage and the number of crabs on trees was analysed. All of the above analyses were performed using SPSS.

Results

Propagule predation

Propagule predation by crabs occurred in all of the five plots, ranging from 34% to 80%. An average of 69.8% of *Rhizophora racemosa* , 66.3% of *Rhizophora mangle*, 61.6% of *Rhizophora harrisonii* was predated. The effect of crab predation on propagules did not differ amongst mangrove species in all the plots, except for plot 4 where *Rhizophora harrisonii* was substantially reduced (*Figure 2*). There were no significant differences of propagule predation between mangrove species in all plots (F=0.16, df=2, P=0.86) .Majority of the propagules were found to be non-viable after predation and some were lost by being washed away by high tide (*Figure 3*). There was a significant difference between the number of non viable and viable propagule (T= 2.13, df = 4, P = 0.002) with majority being non viable. In general, crabs prefer horizontally laid propagules, irrespective of mangrove species or plot position (*Figure 4*). Crabs were aggregated around horizontal propagules, whereas vertical propagules close by were neglected, and only horizontally planted propagules were dragged to crab burrows (F = 7.11, df = 2, P = 0.05). Some propagules were predated by gastropods, but the extent of this was minimal.

Leaf Predation

The percentage of leaves consumed by crabs varied amongst mangrove species (*Figure 5*). *Rhizophora* species was the most consumed and *Avicennia* was the least, although this was not significant between species (ANOVA, F = 2.3, P = 0.24).

Senescent leaves were preferred more than fresh leaves for all species (*Figure 6*), and there was a significant difference in the percentage consumed of fresh and senescent leaves (T = 4.3, df = 2, P = 0.02). The majority of leaves were taken into burrows *Laguncularia racemosa* 60% *Avicennia germinans* 55%*Rhizophora* species 75%, and they were substantially eaten when recovered from the burrows. No leaf breakage was identified during the recovering process.

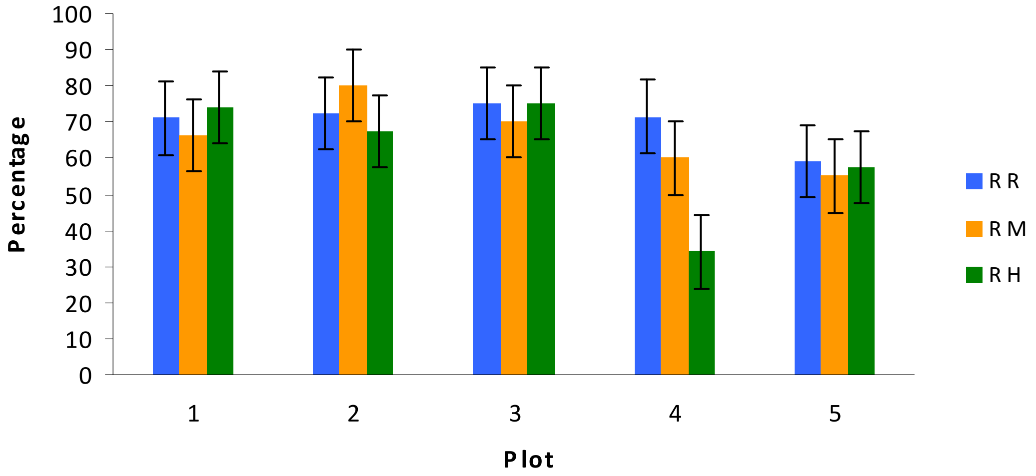

Figure 2. *Percentage of propagule predation within each plot. RR= Rhizophora racemosa, RM= Rhizophora mangle, RH = Rhizophora harrisonii (Error bars represent standard errors)*

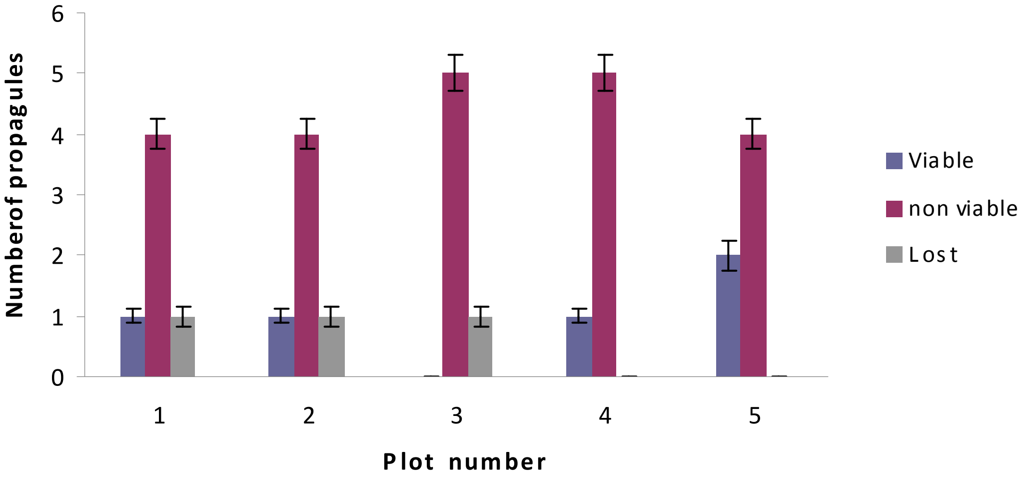

Figure 3: *Number of propagules per plot killed by crabs, lost or still viable after predation (Error bars represent standard errors)*

A total of 195 attached leaves were examined in 6 young trees for the presence of crab damage. Thirty four leaves were found damaged on the sampled trees, fifty three leaves were examined in *Laguncularia racemosa* species, 4(7.6%) were damaged, sixty four leaves were examined in *Avicennia germinans* species, 7(10.9%) were damaged

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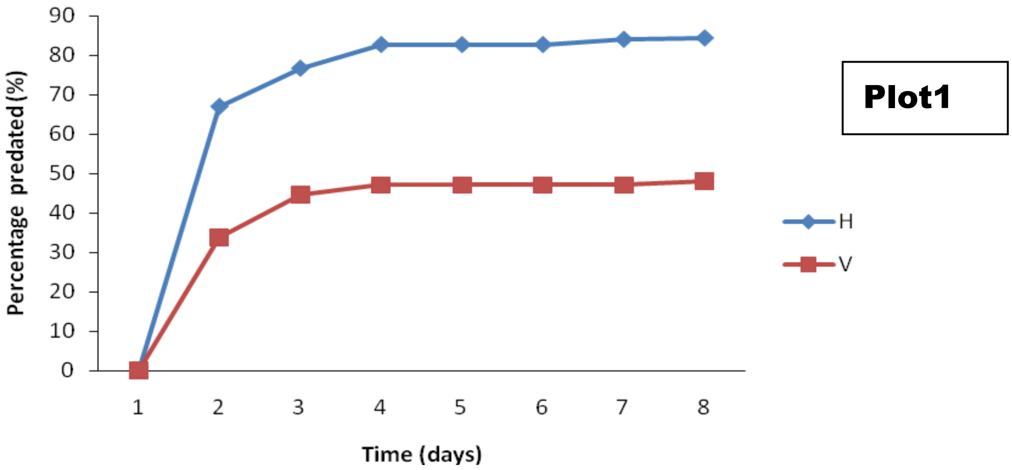
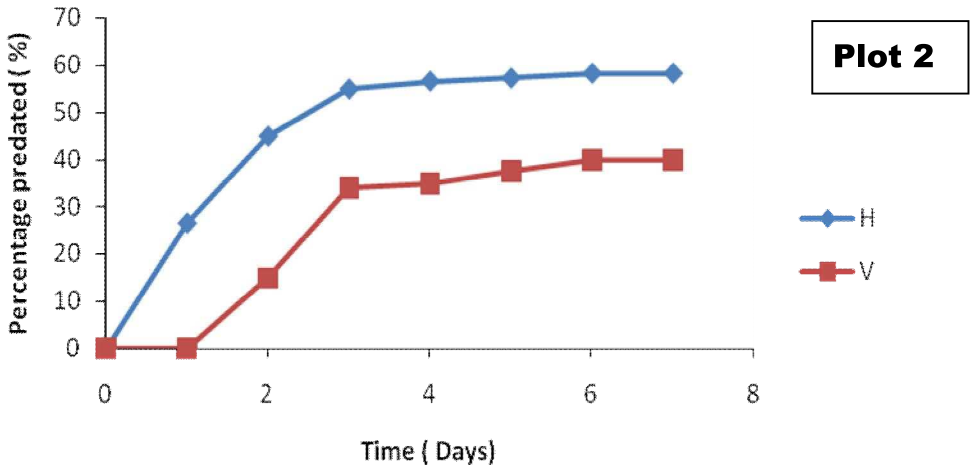
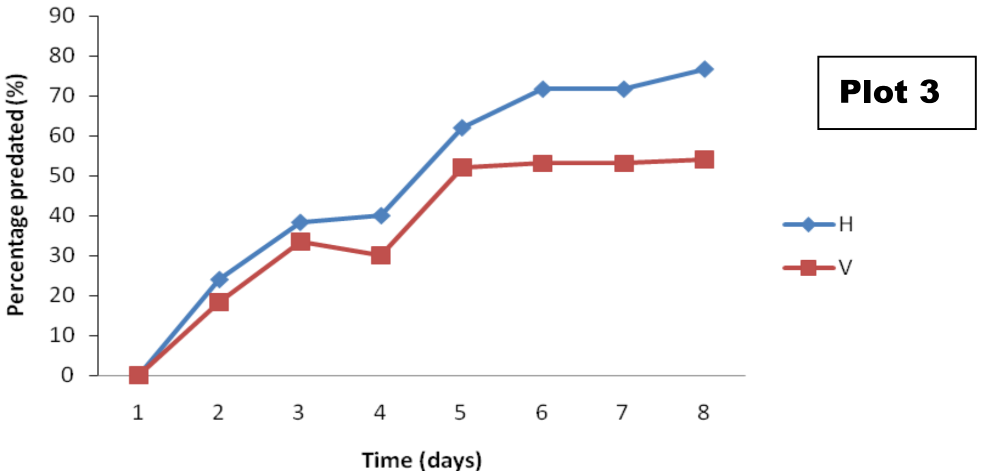
and seventy eight leaves were examined in *Rhizophora* species, 23 (29.5%) were damaged. All the crabs recorded were from family grapsidae; *Goniopsis pelii (G. cruentata)* (37), *Grapsus grapsus* (8) and sesarmidae; *Sesarma* spp (21), *Metagrapsus curvatus* (12). The estimated damaged leaves were highly correlated to the number of crabs on trees ($r^2 = 0.75$) (*Figure 7*).







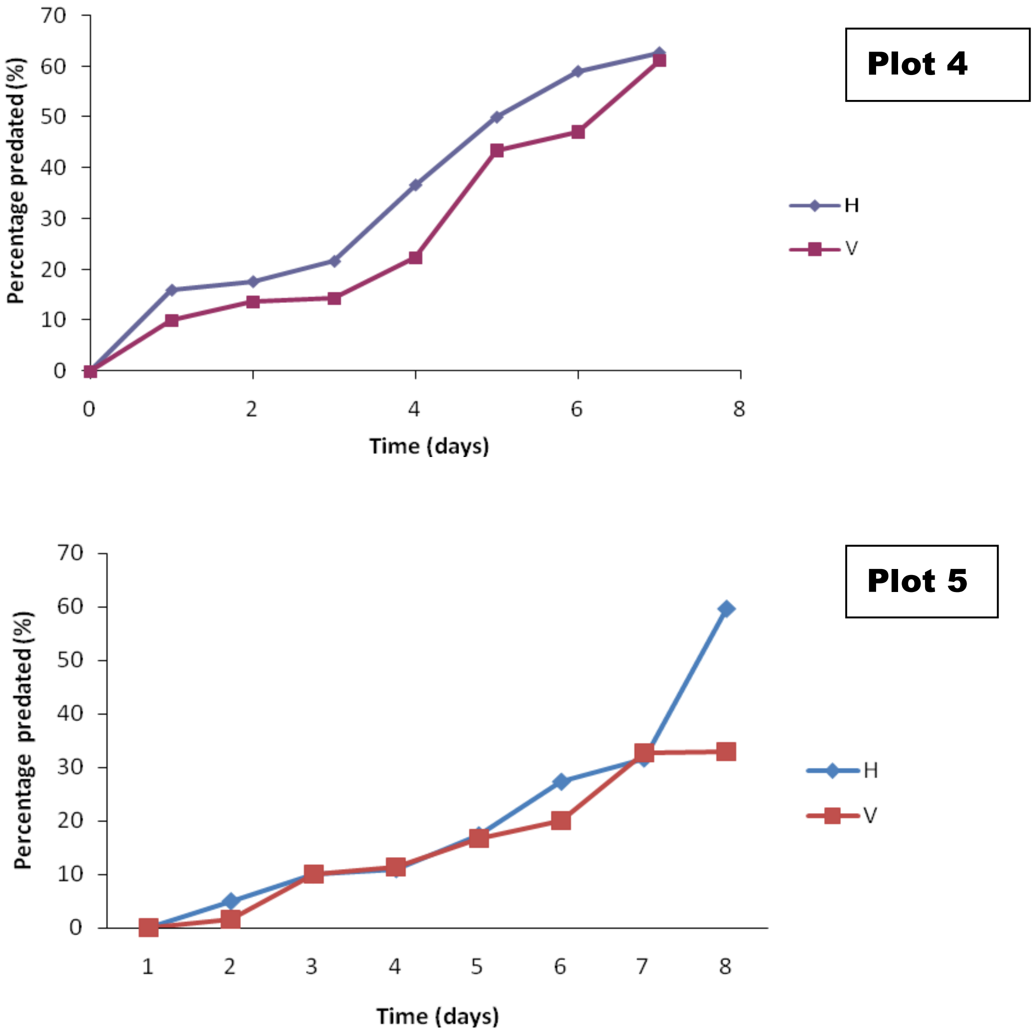


Figure 4: *Rate of propagule predation V= Vertical, H = Horizontal.*

Gut content analysis

A total of 43 crabs were dissected. Plant material was identified as principally mangrove leaves based on stomatal features and constituted 45.4% of *Metagrapsus curvatus* diet, 55% *Sesarma (Chiromantes) elegans* diet, 62.5% *Sesarma (Perisesarma) alberti*, 65.9% *Goniopsis pelii (G. cruentata)* diet, 47.8% *Sesarma (Perisesarma) huzardi* diet and 37.3% *Grapsus grapsus* diet (*Figure 8*). It is clear that plant material (mangrove and algae) is a major component of the diet of grapsid and sesarmid crabs in Cameroon mangrove, with animal materials of minor importance.

Discussion

Numerous species of mangrove macrofauna are known to consume plant materials, including crabs (Smith 1987a, Micheli 1993, Steinke et al. 1993) and snails (Slim et al. 1997) and insects (Robertson and Duke 1987). Amongst these, crabs are thought to be

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major consumers and a key source of leaf and seedling mortality in mangroves (Clarke and Kerrigan 2002). Amongst mangrove macrobenthos, crabs are one of the most significant groups in terms of species numbers and total biomass. It is therefore important to understand their feeding habits and position in the food web (Macintosh 1988, Cannicci et al. 2008). Many species of mangrove crabs are known to consume a large proportion of the annual primary production of mangrove (leaves and propagules) (Lee 1998). For example, in Malaysia *Chiromanthes onychophorum* de Man (Malley 1978), in Sri Lanka *Neosarmatium malabaricum* (Henderson), *N. meinerti* (de Man), *N. smithii* (H. Milne-Edwards) and *Episesarma tetragonum* (Fabricius)(Dahdouh-Guebas et al. 2011), in South Africa and Kenya *Neosarmatium meinerti* (Emmerson and McGwynne 1992, Dahdouh-Guebas et al. 1997, 1998), *Goniopsis cruenata* (Latreille) (Von Hagen 1977).

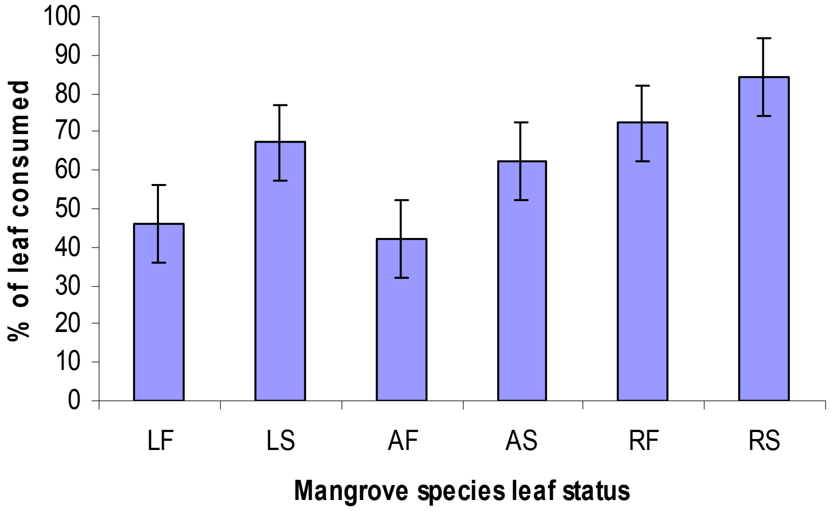

Figure 5: *Percentage of leaf material consumed by crabs for each mangrove species. LF=Laguncularia fresh, LS= Laguncularia senescent, AF = Avicennia fresh, AS = Avicennia senescent, RF = Rhizophora fresh, RS = Rhizophora senescent (Error bars represent standard errors)*

Leaves consumed by herbivorous crabs vary in size, state of composition, physical toughness, nutritional value and palatability (Robertson 1986, Micheli 1993). Fresh mangrove leaves that have fallen from the tree are poor in nitrogen but rich in tannin, but most grapsid crabs have been reported feeding on fresh leaves on the forest floor or take entire leaves into their burrows (caching) (Robertson 1986, Micheli 1993). According to Giddins et al. (1986), crabs prefer decaying leaves to senescent or fresh leaves when given the choice. They also suggested that crabs allow leaves to decompose inside their burrows for many weeks before eating them, during this time tannins are lost from the leaves through leaching, whilst nitrogen concentration increases through bacterial action, resulting in a higher nutritional content. Other advantages of taking leaves into the burrow is to prevent them from being carried away by the tides (Robertson 1986) It creates a safe environment for crabs to eat without fear of predators, tidal inundation, high temperatures and low humidity (Wolcott and O'Connor 1992).

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Therefore caching might be frequent in areas with shortage of food supply and greater predator pressure.

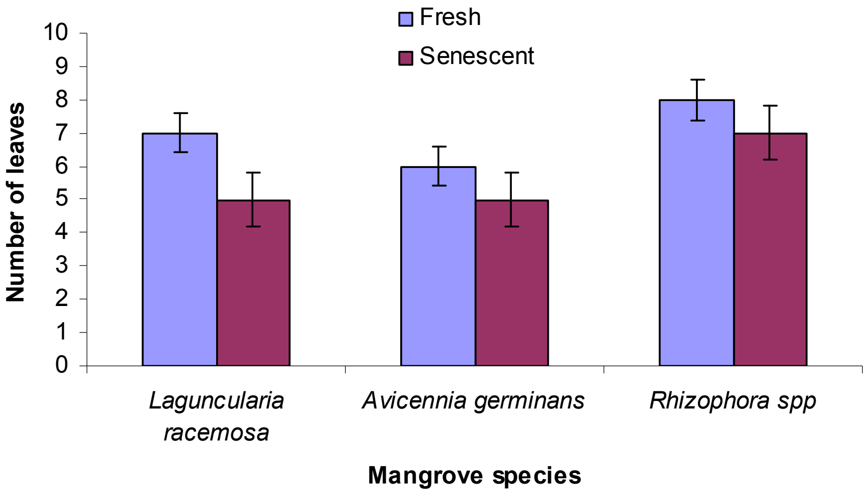


Figure 6: *Number of leaves taken down crab burrows (Error bars represent standard errors)*

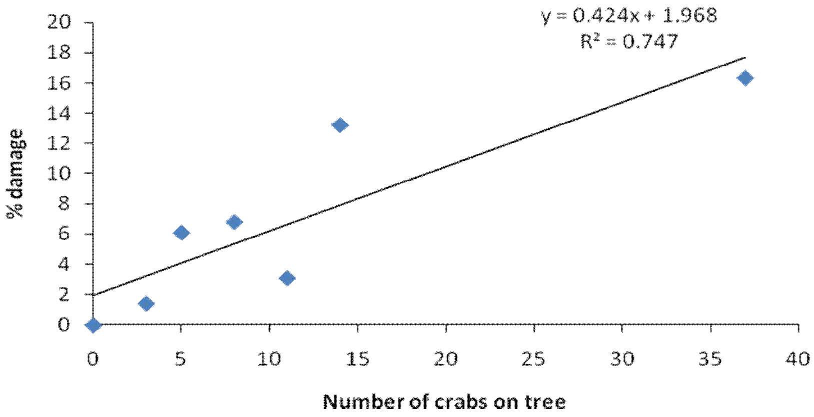


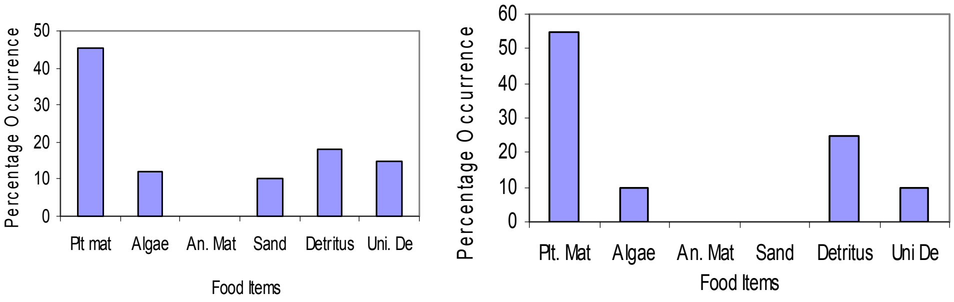
Figure 7: *Relationship between number of crabs and leaf damage*

Many studies have examined gut contents or leaf and propagule damage to determine herbivore diet exclusively. This study is unique in that it might be the first on propagules and leaf feeding on the central Africa mangrove and it is combination of three methods to assess the potential ecological threat to forest health of mangrove ecosystems; gut content analysis, propagule and leaf predation assessment and standing tree assessment. Combination of the results suggested that mangrove crabs damage large amounts of mangrove plant material, both leaves and propagules, and this may have significant ecological consequences for ecosystem structure and function.

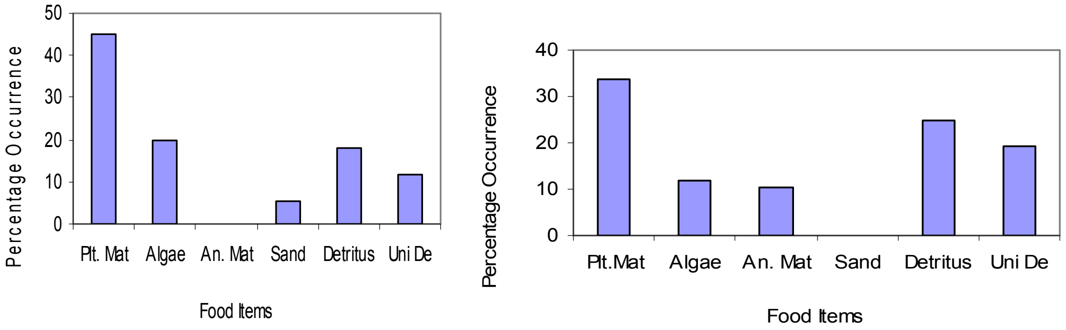
Propagule predation

Grapsid and sesarmid crabs are palpable predators of mangrove propagules. *Sesarma* and *Metapograpsus* spp have been reported predating Rhizophoraceae and *Avicennia* propagules (Smith et al. 1989, Robertson et al. 1990), and *Aratus pisonii* and *Goniopsis cruentata* in the Caribbean fed on *Rhizophora* propagules (Warner 1967). According to Dahdouh-Guebas et al (1997), in Mida Creek Kenya 50% of propagules were predated within 2 hrs and 85% within 24 hrs. In the present study, 66.7% of the propagules were predated leaving 50% non-viable. This high predation pressure could affect natural restoration of mangrove forest.

Metagrapsus curvatus (n = 10) *Sesarma (Perisesarma) huzardi* (= 5)



Sesarma (Chiromantes) elegans (n=6) *Sesarma (Perisesarma) alberti* (n=5)



Goniopsis pelii (n=10) *Grapsus grapsus* (n= 7)

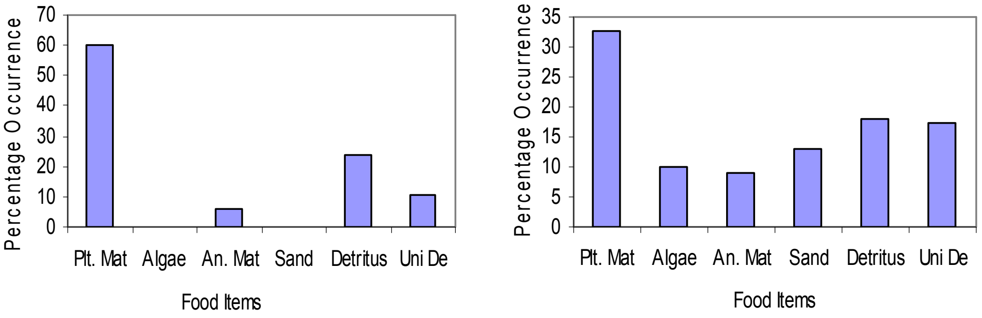


Figure 8. *Percentage occurrence of gut content items. Plt. Mat = Plant material (mangrove leaves), An. Mat = Animal materials (crustaceans, annelids), Uni-De (unidentified debris), n = number of crabs examined.*

Seedling establishment (i.e. type of planting strategy, horizontal or vertical) may also influence predation rate. In Kenya, the crab *Neosarmatium meinerti* preferred horizontally planted propagules (Dahdouh-Guebas et al. 1997), and in the present study, horizontally planted propagules were more predated (Figure 4). This might be due to the fact that crabs face difficulties handling vertical propagule because of their size and weight. Although seedling establishment coupled with tidal action might influence mangrove recruitment, selectivity of crabs feeding habit has been reported altering natural restoration of mangrove seedlings in a species-specific way (Lee 1998). In the present study, propagule predation was not significantly different among crab species, though *Goniopsis pelii was* the dominant species foraging on plant material.

Leaf predation

Earlier studies have reported mangrove crab preference for *Rhizophora* species. In Florida *Rhizophora* was preferred to *Avicennia germinans* and *Laguncularia racemosa* (Wilson 1981). The present study similarly indicates preference for *Rhizophora* species to Avicennia *germinans* and *Laguncularia racemosa.*

One of the interesting observations of this study was almost complete lack of fallen leaves on forest floor. Most likely cause might be tidal inundation and high crab activity in the region. High leaf damage rates have been reported in Australia (Robertson 1986; Micheli 1993). The present study similarly indicates high leaf damage by crab (71.3%).

In the present study, there was a significant difference between the amount of fresh and senescent leaves consumed. This result is similar to those of other studies that support the fact that crabs preferred aged leaves (Neilson et al. 1986, Micheli 1993, Kwok and Lee 1995) because of the leaching out of tannins, reduction in leaf toughness and increase of nitrogen content. Mangrove leaf decomposition may affect the leaf quality for herbivores, with decomposed leaves having different tannin concentration and nutritional value compared to fresh leaves, high tannin concentrations inhibiting microbial activity (Neilsons et al. 1986, Steinke et al. 1990).

Physical toughness of leaves will also affect herbivore choice. *Rhizophora* decomposes slowly compared to *Avicennia* and *Laguncularia,* probably due to the thick cuticle and sclerids in the tissues (Camilleri 1989), and this might inhibit feeding of some crab species, but they are preferred by others, although chelae modification may limit the effect of physical conditions in determining the leaf preference.

The gut content analysis provided additional information about the diet of mangrove crabs in the west coast of Africa. Identifiable gut diet material of mangrove leaves is composed about 45.4% in *Metagrapsus curvatus*, 55% in *Sesarma (Chiromantes) elegans* , 62.5% in *Sesarma (Perisesarma) alberti*, 65.9% in *Goniopsis pelii (G. cruentata)* , 47.8% in *Sesarma (Perisesarma) huzardi* and 37.3% in *Grapsus grapsus*. Other ingested crab matters were animal materials (crustaceans, annelids), sand, and Uni-De (unidentified debris)

The results of the present study, while preliminary in nature, contribute to our understanding of the feeding ecology of mangrove crabs in the west coast of Africa and how mangrove crabs feeding habits could be a potential ecological threat to forest health of mangrove ecosystems.

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MONITORING OF THE SURFACE OZONE CONCENTRATIONS IN THE WESTERN BANAT REGION (SERBIA)

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Abstract. This paper presents the results of measuring the concentrations of ozone, VOCs (benzene, toluene, *m*- and *p*-xylene, *o*-xylene and ethylbenzene), nitrogen oxides (NO, NO_2 and NOx), CO, H_2S, SO_2 and PM_{10} in the ambient air in parallel with recording the meteorological parameters: temperature, solar radiation, relative humidity, barometric pressure, wind speed and direction during the year 2009. The measurements were performed at the measuring station located within an agricultural area near the city of Zrenjanin (Serbian Banat, Serbia). The results are presented in this paper as average values in winter and summer *vs*. time of day, and as average daily values *vs*. measurement date. Several correlations of the ozone concentration *vs*. atmospheric observables were made, together with Principal Component Analysis. The statistical analysis of the obtained data, based on Principal Component Analysis (PCA), led to result that 80.87 % of the variance in the measured values could be described with five factors. A high level of intercorrelation of VOCs, NOx and CO was determined. These pollutants were all grouped in factor 1, which described 42.85 % of variances of the measured values. According to the VOCs/NOx and VOCs/CO ratios (which were 0.26 and 0.029, respectively), it was determined that production of tropospheric ozone is a VOCs sensitive process for the investigated region.

Keywords: *pollutants, ozone, VOCs, nitrogen oxides*

Introduction

The occurrence of high levels of tropospheric ozone (O_3) is currently a matter of worldwide concern due to its harmful influence on human health, crops and ecosystems derived from its highly oxidative nature (WHO, 2000; EEA, 2003). Reports published by the European Environmental Agency (EEA) are indicating a global tendency of increasing ozone concentrations in urban regions, especially in the EU and USA (EPA, 2007). These reports also highlight the negative influence of increased ozone concentration on human health (EPA, 2009). The tendency of increasing ozone concentrations is also being reported in other parts of the world (Abdul-Wahab and Al-Alawi, 2002), gaining more and more attention from scientists and other investigators. Episodes of ozone concentration enhancement in the ambient air of rural zones are regularly being reported. This is increasing the risk of slower development of the agricultural production in these seemingly unpolluted areas (Gonzales et al., 2010), as well as in partially industrialized regions (Castell et al., 2010). An increased concentration of tropospheric ozone in ambient air is not only an environmental problem of a country; but it also presents a source of health problems of its population as well as increased costs to the economy. At the same time, enhancement of the tropospheric ozone concentration represents a global problem that demands local actions (Parnel, 2006).

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Ozone, however, is unique among pollutants because it is not emitted directly into the air. It is secondary pollutant that results from complex chemical reactions in the atmosphere. It is a well-known phenomenon that the ozone results from the complex chemical interaction of nitrogen oxides (NOx), carbon monoxide (CO) and volatile organic compounds (VOCs) in the presence of UV radiation (Jacob, 2000; Trainder et al., 2000; Lengual et al., 2004; Duan et al., 2008). Variation in ozone concentration is also related to some meteorological variables (temperature, surface wind direction, speed, and global radiation) (Klaus et al, 2001).

The mechanism of tropospheric ozone production is not yet fully understood. It is usually assumed that the NO_2/NO ratio and ozone concentration are correlated. In this manner, if the correlation is positive, it is considered that the process of ozone production is "NOx sensitive". In such a case, the VOCs/NOx ratio is large, leading to a tendency for NOx to produce ozone (Shao, et al., 2009). On the contrary, if the VOCs/NOx ratio is small, then NOx has a tendency to inhibit ozone production (Duan, et al., 2008). In such cases, the overall ozone production depends on the VOCs content, and the ozone concentration is regarded as being “VOCs sensitive (Lengyel et al., 2004; Shao et al., 2009). Some researchers reported the influence of SO_2 and $PM_{2.5}$ on the ozone concentration (Odman Talat et al., 2009), while others considered meteorological parameters (Abdul-Wahab and Al-Alawi, 2002; Languel et al, 2004) and other influences as important factors (Madronich and Flocke, 1999).

To enable efficient prediction of tropospheric ozone as functions of other pollutants, their concentrations and meteorological conditions, which influence the production of ozone in the ambient air, attempts at mathematical modeling, based on statistical methods, have been widely reported. These methods are applied to data obtained from real time – real conditions measurements of the ozone concentration in the ambient air. For statistical analysis of this type of data, the Multiple Linear Regression Analysis (MLRA) method could be used (Otto, 1999; Langyel et al., 2004; Živković et al., 2009a, 2009b; Mihajlovic et al, 2010; Djordjević et al., 2010). Other potential tools for mathematical modeling are: Principal Component Analysis/Absolute Principal Component Score (PCA/APCS) (Duan et al., 2008) and PCA modeling (Multiple Regression Analysis -MLR, Partial Least Squares - PLS as well as Principal Component Regression - PCR) (Lengyel et al., 2004, Djuric et al., 2010). For complex dependencies, the methods of nonlinear regression analysis are widely used, among which Artificial Neural Networks (ANNs) are frequently applied (Spellman, 1999; Elkamel et al., 2001; Abdul-Wahab and Al-Alawi, 2002; Sousa et al., 2007, Živković et al., 2010).

Measurements of the ozone concentration in the ambient air commenced in Serbia a few years ago. However, a comprehensive study concerning its genesis, level of concentration and possible risks that it presents to human health have not hitherto been performed, because information on ozone dependences in this region of Europe is limited. The aim of this study was to obtain an insight on ground-level ozone concentrations in the central region of the Serbian Banat, a major agro-industrial region in Serbia with an important production of crops, potentially sensitive to this pollutant (such as grape, vegetables, cereal and fruits) and where a large number of protected species are present. Therefore, the daily and seasonal variability of ozone were measured at a measuring site within the study area during the plant growing season and a number of factors that might influence its levels. A model of the chemical mechanism was applied to assess the sensitivity of ozone formation on NOx and VOCs in the region. Simultaneously, the repercussion that ozone concentration might have on air quality and its potential serious effects on human health as well as on vegetation were explored.

Materials and methods

Area of study

The locality where the measurements were facilitated, the Banat, is a region in southeastern Europe divided among three countries: the eastern part belongs to Romania, the western part to Serbia (the Serbian Banat, mostly located in Vojvodina, except for a small part that is in Central Serbia) and a small northern part in Hungary. It is the part of the Pannonian plain bordered by the Danube to the south, the Theiss to the west, the Mures to the north and the southern Carpathians to the east. The Serbian part of the Banat, *Figure 1*, is an area of 8,997 square kilometers located in the northeast of Serbia. Zrenjanin is the center of this region, occupying 1,326 square kilometers, with a population of 80,000. From the whole territory that belongs to this municipality, 82.5 % is covered by large-scale farmlands. This area is the part of a humid continental climate region; with the average annual temperature of 11.2 °C and 622 mm of rainfall per year. Wind directions are mostly from the east, southeast or northwest. The average number of sunny hours in this area is 2,000 to 2,200 per year.

The Banat is one of the most fertile regions of Europe. The main agricultural products of this region are all kinds of cereals. This region is also convenient for the growth of sugar beet and tobacco. Vegetables grown in this region include: pepper, tomato, cabbage, spinach, *etc*. Grape vines are grown in the southern part of the region. Large scale industrial facilities include: the agro industry, milling, the brewing industry, sugar production, the textile industry, and brick production. Moreover, oil and natural gas are exploited in the region. Most of the agricultural plants that are grown in Banat are vulnerable to ozone air pollution (Fumagalli, 2001). For this reason, the present research study considers the ozone air quality problem of this region.

Figure 1. *The investigated Serbian Banat region and its position in Europe*

Air quality Monitoring and Meteorological data

Continuous measurement of the air pollutants was facilitated in an automatic measuring station, located in the city of Zrenjanin. This station measures air pollution levels originating from exhaust gasses and other sources of pollution. The following air pollutants are continually measured at this location: BTEX (benzene, toluene, ethylbenzene and xylene) according to the EN 14662 method; ozone according to the EN 14625, ISO 13964 method; carbon monoxide according to the EN 14626, ISO 4224:2000 method; PM_{10} (Particulate matter) according to the EN 12341 method; $NO/NO_2/NOx$ (nitrogen oxides) according to the EN 14211 method and H_2S/SO_2 (sulfur compounds) according to the EN 14212, ISO 10498:2004 method. Measurements are repeated in 2-minute intervals and the hourly average value for each hour in 0 – 24 intervals are calculated. The results of the measurements are publicly available on-line. The measurements, calibration of the equipment, quality control and standardization are organized by the Regional Environmental Protection and Sustainable Development Committee, located in Novi Sad, the administrative capital of the Vojvodina region. The meteorological parameters: wind speed and wind direction, air temperature and humidity, atmospheric pressure, rainfall per year and solar radiation intensity are measured at the same measuring station as the air pollutants concentration, with the same dynamics.

The potential risk of ozone air pollution is obvious in this region, considering that the measured hourly ozone concentrations are in the range 80 – 130 $\mu g/m^3$, during the summer season. The limiting value prescribed by the EU are 80 $\mu g/m^3$ (Directive 2002/3/EC) using the 1-h values measured between 08.00 and 20.00 hours Central European Time (CET) each day. Furthermore, these directives prescribe a value of 18,000 $\mu g/m^3$ h, for the protection of the vegetation and this limiting value became mandatory at the beginning of 2010, according to the AOT40 index.

Data analysis method

For the analysis of the ozone concentration in the ambient air in the rural vicinity of the city of Zrenjanin (Serbia), the data obtained from the automated measuring station were used. The data were collected during the year 2009, in the time periods in which all the pollutants and all meteorological parameters were measured simultaneously.
These time periods were:
Winter: 1–8 and 23 – 28 February; 21 – 31 December
Spring: 5 – 15 May
Summer: 13 – 18 July and 1 – 20 September
Autumn: 1 – 22 October

During these 83 days, the measurements were conducted from 0.00 to 24.00 hours, and the hourly average values were calculated. In this way, a representative data base for credible statistical analysis was generated. A total of 1477 data sets remained after deleting error points. A Mann-Whitney U non-parametric test was used to compare between the NO_2/NO ratios registered at the Zrenjanin station during episodes of high concentrations of ozone and those recorded normally. A rotated principal component analysis (PCA) was also performed with the correlation matrix of the pollutant concentrations, in order to identify potential factors determining the concentration of the studied pollutants. Statistical analysis was performed using SPSS 18.0 (SPSS Inc, Chicago, USA).

Results

Meteorology

Temperature range recorded during above defined measuring period in 2009 was –12.5 °C to 35.1 °C, average statistical value was 15.1 °C. The registered wind speed ranged from negligible values, corresponding to periods of calm (minimum value 0.18 m/s) to a maximum value 5.57 m/s, the mean value for the measuring period was 1.68 m/s. The prevailing wind directions during the investigated period were: SE, 58 %; NW, 31 % and N, 11 %, in the SE–NW orientation of the Banat region. The atmospheric pressure ranged from 270 hPa to 1020 hPa, with an average value of 993 hPa. The intensity of the solar radiation varied during the year of the measurements; it ranged from 4 W/m^2 to 848 W/m^2, with a statistical average value of 136 W/m^2. The air humidity ranged from 17 % to 92 %, with an average value of 65 %. The total rainfall in the year was 622 mm, which was equally distributed throughout the year. Changes in the variances were within satisfactory borders for the entire statistical assembly, as was the value of standard deviation. This indicates that all variables behaved according to normal (Gaussian) distributions.

Ambient air concentrations of O3, NOx, VOCs, CO, SO2, H2S and PM10

Numerous investigations indicated that the ozone concentration in the ambient air is dependent on the concentrations of NO, NO_2 and NOx, as well as on the concentration of volatile organic compounds (VOCs) (Duan et al., 2008; Shao et al.,2009; Gonzales et al., 2010). The automatic measurements of the ozone concentrations during the year 2009 at the Zrenjanin measuring station revealed that its concentration ranged from 1.3 $\mu g/m^3$ to 162 $\mu g/m^3$ with years average of 70.1 $\mu g/m^3$ ± 34 $\mu g/m^3$ (with a statistical reliability of 2σ). The average value of ozone concentration during the winter season (December – March), was 25 ± 15 $\mu g/m^3$. During the period May – October, which is relevant for vegetation growth, the average ozone concentration was 100 ± 25 $\mu g/m^3$. The average daily values of the ozone during the year 2009 are presented in *Figure 2*.

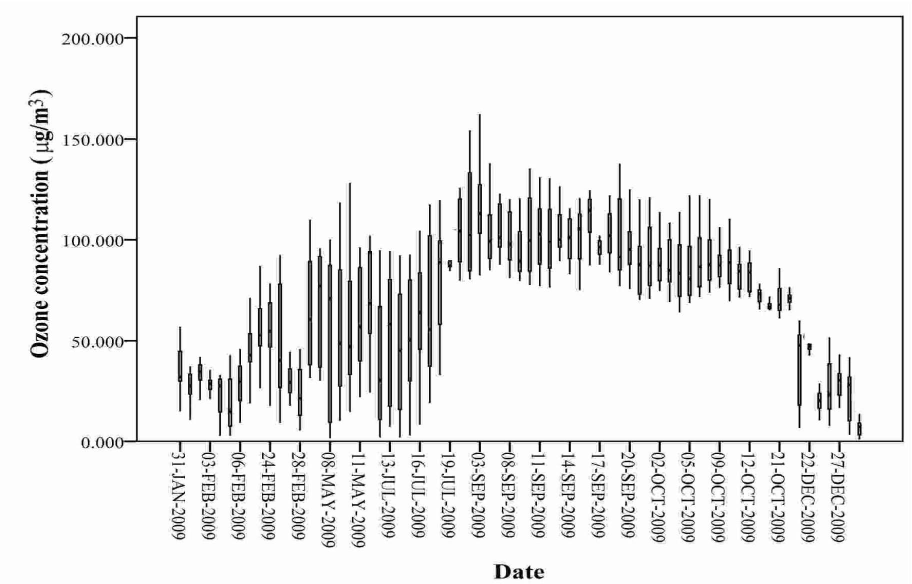

Figure 2. *Variation of ozone concentration in the air during the year 2009 (average daily values)*

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The daily changes in the ozone concentration exhibited the following pattern: from 7:00 the concentration started to increase, reaching a maximum value from 12:00 to 16:00 and then it decreased until 20:00, after which it remained constant until the following morning. This trend was the same throughout the year, as can be seen in *Figure 3*. The results presented in this figure indicate that two periods could be defined: the warm weather period (May to October) and the cold weather period (December to March), depending on the time of measurements during the day. It should be emphasized that episodes of increased ozone concentrations were recorded during the year 2009: 713 measured values above 80 μg/m^3 (48.27 %), 72 values above 120 μg/m^3 (8.87 %) and 9 values above 140 μg/m^3 (0.6 %).

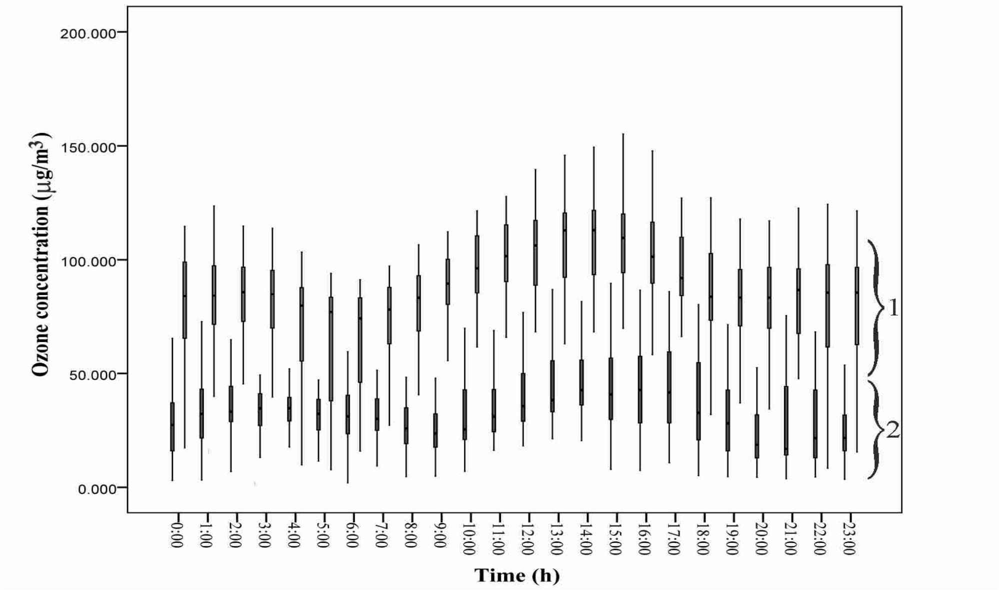

Figure 3. *Variations in the daily ozone concentrations during the warm (May to October = 1) and cold (December to March = 2) time periods*

The obtained results have the same trend as in the case of the Ebro Basin (Spain), with 25 to 30 % higher values measured in Zrenjanin during the spring and the summer seasons, which is important for vegetation growth. These values are in accordance with those measured in Virginia (USA) (Davis et al., 2010) and in towns located in the Pearl River Delta (China) (Shao et al., 2009). The average values of NO, NO_2 and NOx, for the referred time period were: 28.5 μg/m^3, 32.9 μg/m^3 and 76.5 μg/m^3, respectively. These concentrations are three times higher in case of NO, and five times higher in case of NO_2, compared to the values measured in a rural area of the Ebro Basin, Spain (Gonzales et al., 2010). The measured values are much higher than the prescribed limiting values for the protection of vegetation (30 μg/m^3) and human health (40 μg/m^3) (Martin et al., 2009). The VOCs concentrations (benzene, toluene, ethylbenzene, *m*-, *p*- and *o*-xylene - BTEX) were in the same range as in a rural area of the Ebro Basin, Spain (Gonzales et al.2010). The average measured values of the concentrations of the pollutants: CO, SO_2, H_2S and PM_{10} were 738 μg/m^3, 17.6 μg/m^3, 1.9 μg/m^3 and 42 μg/m^3, respectively. These values correspond to those measured in underdeveloped industrial regions with the same ozone concentrations (Abdul-Wahab and Al-Alawi, 2002; Lengyel, et al., 2004).

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The Pearson correlation coefficients between the air pollutants and the meteorological parameters measured at the Zrenjanin measuring station during the year 2009 are presented in *Table 1*. Statistical analysis revealed the existence of a significant positive correlation ($r = 0.687$; $p < 0.01$) between the ozone concentration and the radiation, as well as between the ozone concentration and the air temperature ($r = 0.473$; $p < 0.01$). A significant negative correlation ($p < -0.624$; $p < 0.01$) exists between the ozone concentration and relative humidity. The dependence of ozone concentration on the solar radiation, temperature and relative humidity (RH), as the most important predictors influencing the variation of its content in the ambient air, are presented in *Figure 4*. The obtained values of the coefficient of determination (R^2) for the dependences of the ozone concentration on solar radiation, temperature and relative humidity are 0.472; 0.224 and 0.390, respectively. These values indicate large catalytic influences of all three parameters on the tropospheric production of ozone. These results are within the same correlation range as those reported by Gonzales et al., (2010) and Martin et al., (2009).

The concentrations of NO; NO_2 and NOx are in negative correlation with the ozone concentration ($r = -0.380$ and $p < 0.01$; $r = -\ 0179$ and $p < 0.01$; $r = -0.340$ and $p < 0.01$, respectively). The obtained results are in accordance with the results reported by Lengyel et al., (2004). The concentrations of the VOC group of predictors are in negative correlations with the ozone concentration. The calculated values for benzene, toluene, *m*- and *p*-xylene, *o*-xylene and ethylbenzene are $r = -0.393$ and $p < 0.01$; $r = -0.327$ and $p < 0.01$; $r = -0.285$ and $p < 0.01$; $r = -\ 0.270$ and $p < 0.01$; $r = 0.267$ and $p < 0.01$, respectively. The CO concentration is in negative correlation with the concentration of ozone ($r = -0.184$ and $p < 0.01$). On the other hand, its correlations with NO, NO_2 and NOx are highly positive ($r = 0.748$ and $p < 0.01$; $r = 0.844$ and $p < 0.01$; $r = 0.844$ and $p < 0.01$, respectively), indicating that all these gases originate from the same source. The group of predictors containing sulfur (SO_2 and H_2S) are in high positive correlation with each other ($r = 0.552$ and $p < 0.01$). This also indicates their same source of origin. Their correlation with ozone concentration in the ambient air is positive ($r = 0.145$; $p < 0.01$ for SO_2 and $r = 0.207$; $p < 0.01$ for H_2S). Lower values of correlation were determined for the ozone concentration with PM_{10} and wind direction ($r = -0.132$ and $p < 0.01$; $r = 0.127$ and $p < 0.01$, respectively). The only predictor that does not have a statistically significant correlation with the ozone concentration is the wind speed. From the VOC group of pollutants, benzene has the largest negative correlation with the ozone concentration ($r = -0.393$ and $p < 0.01$). Its average concentration was 1.78 µg/m3 during the measuring period in this study area, which is in accordance with the results recorded in New York City during 2003 (Aleksic, et al., 2005).

The correlations between benzene and the other VOC predictors (toluene, *m*- and *p*-xylene, *o*-xylene and ethylbenzene) are also large ($r = 0.830$ and $p < 0.01$; $r = 0.794$ and $p < 0.01$; $r = 0.753$ and $p < 0.01$; $r = 0.806$ and $p < 0.01$, respectively), which reveals the same source of emission of all the VOC pollutants. Considering the climate conditions, there is a large positive correlation between air temperature and solar radiation on the one hand and its negative correlation to relative humidity on the other ($r = 0.595$ and $p < 0.01$; $r = -0.815$ and $p < 0.01$, respectively). The correlation between relative humidity and solar radiation ($r = -0.636$ and $p < 0.01$) is also large and negative. The high values of correlations between NOx and the VOC group ($r = 0.7 - 0.8$) also indicate that they all originate from the same source of emission.

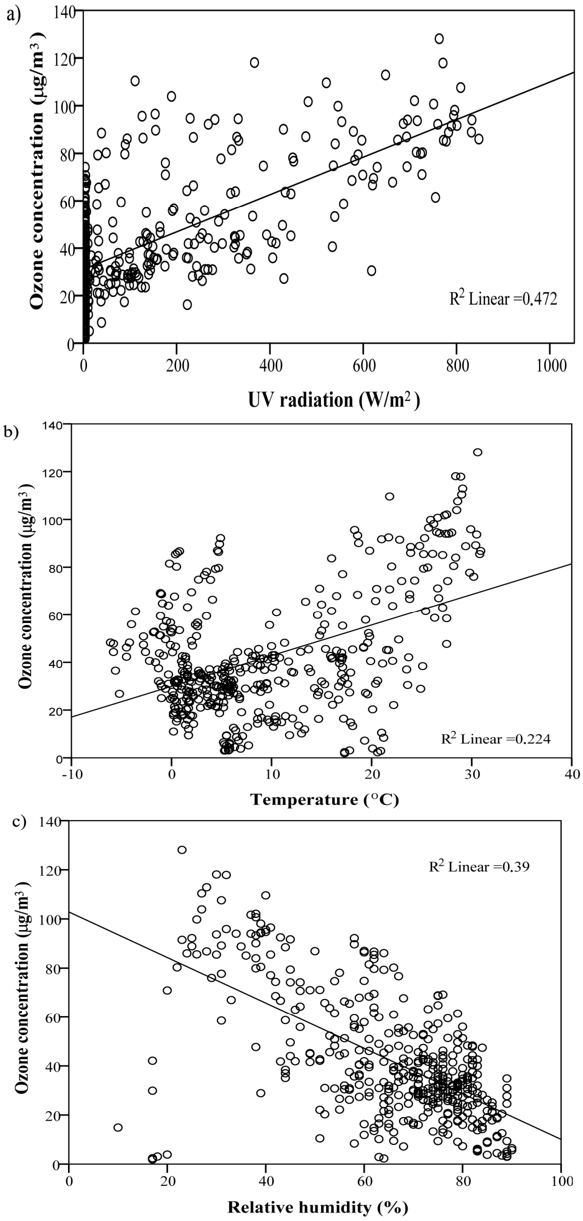

Figure 4. *Dependence of the ozone concentration on UV radiation (a); temperature (b) and relative humidity (c)*

Using the Principal Component Analysis (PCA) extraction method with Varimax rotation and Kaiser's normalization yielded five main factors that explained 80.87 % of total variance, (see *Table 2*). Factor 1 (42.85 % of the explained variance) consisted of all VOCs, CO and NOx, with positive intercorrelations. Factor 2 (17.69 % of the explained variance) groups the meteorological parameters: solar radiation, temperature and relative humidity (RH), which have the largest values of correlation coefficients with ozone. Factor 3 (9.25 % of the explained variance) indicates that the compounds containing sulfur are not emitted from the same source as the VOCs and NOx. Factor 4 groups air pressure and PM_{10} (5.55 % of the explained variance). Finally, factor 5 groups the wind speed and wind direction (5.54 % of the explained variance).

Table 1. Correlation coefficients between the pollutants and the meteorological parameters

	X1 Ozone	X2 SO_2	X3 NO	X4 NO_2	X5 NOx	X6 CO	X7 H_2S	X8 PM_{10}	X9 Benzene	X10 Toluene	X11 m-,p-Xylene	X12 o-Xylene	X13 Ethyl benzene	X14 Wind direction	X15 Wind speed	X16 Temp.	X17 Press.	X18 Rad.	X19 RH
X1	1																		
X2	.145**	1																	
X3	−.380**	.277**	1																
X4	−.179**	.208**	.689**	1															
X5	−.340**	.250**	.957**	.868**	1														
X6	−.189**	.126**	.748**	.844**	.844**	1													
X7	.207**	.552**	.064	.053	.059	.125*	1												
X8	−.132**	.265**	.500**	.591**	.580**	.442**	−.067	1											
X9	−.393**	.092	.645**	.711**	.735**	.729**	−.110*	.686**	1										
X10	−.527**	.070	.722**	.790**	.807**	.856**	−.003	.457**	.830**	1									
X11	−.285**	.084	.738**	.808**	.824**	.855**	.018	.449**	.794**	.985**	1								
X12	−.270**	.053	.652**	.720**	.718**	.724**	.000	.445**	.755**	.839**	.860**	1							
X13	−.267**	.090	.727**	.829**	.825**	.849**	.025	.480**	.806**	.969**	.985**	.875**	1						
X14	.127**	−.256**	−.153**	−.133**	−.161**	−.081	.065	−.180**	−.160**	−.134**	−.119*	−.034	−.104*	1					
X15	.074	.176**	−.120*	−.379**	−.233**	−.315**	−.006	−.231**	−.322**	−.259**	−.248**	−.279**	−.295**	−.079	1				
X16	.471**	−.016	.009	.184**	.069	.305**	.352**	−.277**	−.180**	.224**	.279**	.226**	.258**	.167**	−.067	1			
X17	−.126**	.062	.067	−.164**	−.018	−.143**	.042	.104*	−.060	−.180**	−.200**	−.164**	−.204**	.003	.117*	−.310**	1		
X18	.687**	−.005	−.112*	−.069	−.115*	.024	.052	−.152**	−.252**	−.085	−.053	−.058	−.057	.220**	.067	.595**	−.129**	1	
X19	−.624**	−.109*	−.078	−.267**	−.150**	−.328**	−.294**	.113*	.085	−.241**	−.300**	−.218**	−.283**	−.135**	.056	−.815**	.254**	−.636**	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

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Table 2. *Factors extracted by PCA on the pollutant concentration data within the studied area, which accounted for 80.87 % of the total explained variance*

Rotated component matrix **Total of explained variance**	**Factor 1** **(42.85 %)**	**Factor 2** **(17.69 %)**	**Factor 3** **(9.25 %)**	**Factor 4** **(5.55 %)**	**Factor 5** **(5.54 %)**
Ethylbenzene	.961				
***m*-, *p*-Xylene**	.959				
Toluene	.954				
NOx	.918				
CO	.906				
NO_2	.901				
***o*-Xylene**	.871				
Benzene	.862				
NO	.820				
Radiation		.886			
O_3		.873			
RH		–.828			
Temperature		.695			
H_2S			.928		
SO_2			.695		
PM_{10}	.598			.647	
Pressure				.635	
Wind direction					.795
Wind speed					–.568

Discussion

The mechanism of the photochemical production of ozone, the efficiency of tropospheric ozone production relative to the NOx concentrations, and the importance of NOx relative to VOCs and CO in the observed ozone levels were researched by Trainer, et al., 2000. As published by Klaus et al., 2001, the variation of ozone concentration is related to certain meteorological variables (temperature, surface wind direction, speed, and global radiation). The conditions for ozone production are determined by the VOCs/NOx ratio. Using this fact, the ratio of VOCs/NOx could be used to evaluate whether the production of the ozone is VOC-sensitive or NOx-sensitive (Duan et al., 2008). The values of the VOCs/NOx and VOCs/CO ratios obtained in this investigation were 0.26 ± 0.22 and 0.029 ± 0.02, respectively. Considering the low values of these ratios, the production of ozone in the investigated area could be regarded as VOC-sensitive. At the same time, CO is placed in Factor 1 together with the VOCs and NOx, as indicated in *Table 2*.

To confirm the local production of oxidants, the ambient ozone concentration was plotted against the variation of the NO_2/NO ratio at the measuring station, (see *Figure 5*). As shown in *Figure 5*, the ozone concentration and the ratio of NO_2/NO simultaneously increased, suggesting ozone formation in the process of NO_2 photolysis. The results presented in *Figure 5* indicate that an ozone concentration of 50–100 $\mu g/m^3$ is achieved when NO_2/NO ratio was in the range from 3 to 6. This suggests the existence of only a small influence of NOx on photochemical reaction of ozone

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production. In the case of a dominant influence of NOx on ozone production, this ratio would be in the range from 5 to 15 (Shao et al., 2009).

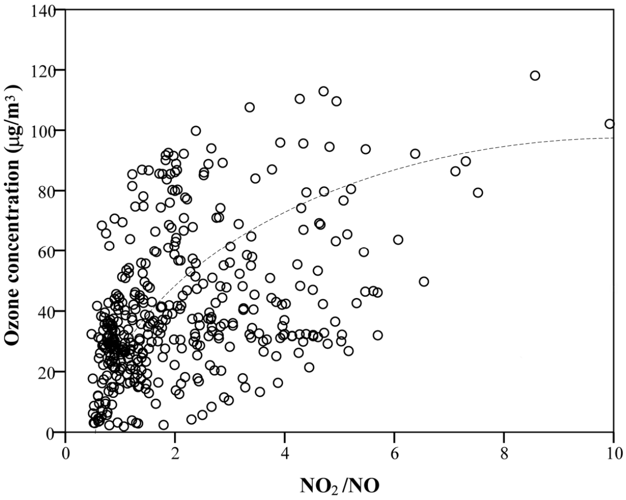

Figure 5. Relationship between the NO2/NO ratio and the O3 concentration at the Zrenjanin measuring station in 2009

The main photochemical reaction of ozone production in the troposphere can be presented as:

$$NO_2 + O_2 = NO + O_3 \qquad \text{(Eq. 1)}$$

Thus, the ozone concentration in the troposphere could be calculated using the following equation:

$$[O_3] = K \cdot [NO_2] \cdot [O_2] / [NO] \qquad \text{(Eq. 2)}$$

where K is the equilibrium constant.

Increasing the NO_2/NO ratio (*Figure 5*) indicate the possibility of ozone production according to reaction (1). However, when the NO_2/NO ratio achieves a value in the range from 3 to 6, the ozone concentration asymptotically tends to a value of 100 μg/m^3. Consequently, ozone produced by the mechanism defined by equation (1) is limited to this value.

The results obtained in this investigation also revealed that with increasing concentrations of VOCs and NOx, the ozone concentration decreased (see *Figure 6*). This indicates the complexity of the reactions occurring in the ambient air of the Banat region in Serbia.

Ozone production is certainly enhanced with increasing NOx level up to the point when the OH + NO_2 + M reaction competes against the OH attack on the hydrocarbon concentrations. Decreasing NOx concentrations can stimulate ozone production (AEAT/ENV/R/1029 Issue 2, 2002). This was also shown by the results obtained in this investigation, where the ozone concentration increased with decreasing NOx level, indicating that the production of ozone was VOC sensitive in this region. This conclusion is based on the fact that VOCs are involved in photochemical reactions and

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interaction with NOx from anthropogenic sources of emission (Jacob, 2000; Trainer et al., 2000; Atkinson, 2000). The VOCs, besides having anthropogenic origins: automobiles, industry (Habashi, 2009) and agricultural machinery, can also originate from vegetation. Considering that PCA analysis grouped VOCs, NOx and CO in the same factor (Factor 1), with 42.85 % of explained variance, it is not possible to clearly separate the sensitivity of ozone production to VOCs and NOx. It would be more accurate to consider their mixed influence, with only a little preference towards VOCs sensitivity.

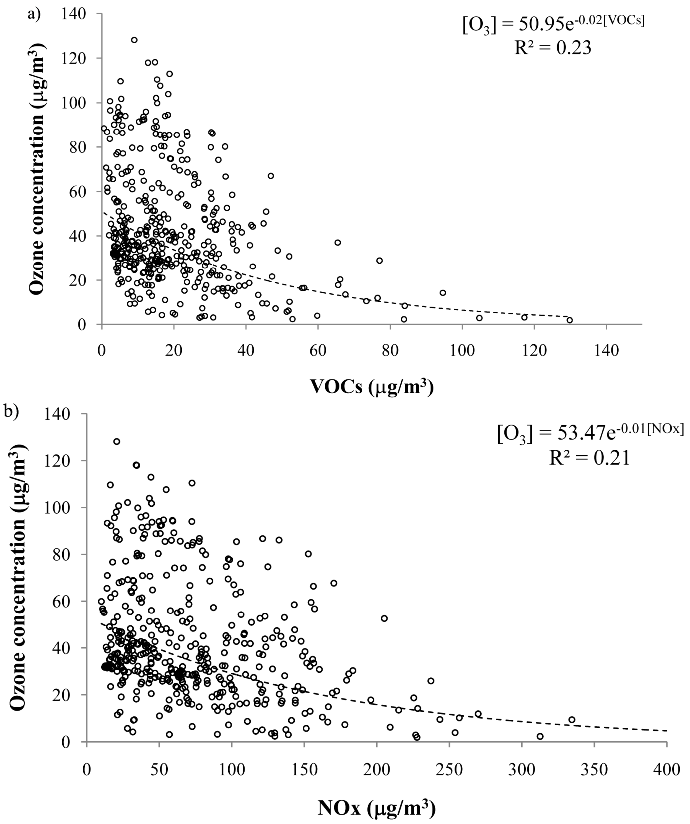

Figure 6. *The non-linear dependences of the ozone concentration on the concentrations of VOCs (a) and NOx (b).*

The episodes of high concentrations of ozone detected in this research were often associated with a noticeable increase in air temperature and significantly higher NO_2/NO ratios, which indicated an increase in the presence NO_2 non-attributable to traffic emissions within the area. This could be linked to an enhancement of ozone photoproduction, temperature itself being the likely surrogate of other meteorological parameters conditioning the formation of the pollutant (Gonzales et al., 2010).

Ozone concentrations in the ambient air above 18,000 $\mu g/m^3$ (obtained as the average value for a five-year period of measurement) represent a potential risk of a harmful influence of ozone on vegetation, according to the AOT40 index - defined by the European directive 2008/50/EC. Considering that continuous measurements of the concentration of air pollutants at this measuring station began during the year 2008, the AOT40 index could not be determined and included in this investigation. However, considering the cumulative nature of the AOT40 index, it is possible to estimate its value by comparing the concentration of ozone measured in the time period from May to July, with the results of other investigators obtained under similar conditions (Gonzales et al., 2010).

For average ozone concentrations ranging from 80 to 95 μg/m^3, the calculated AOT40 index was 57.147 ± 14.114 μg/m^3, as reported by Gonzales et al., (2010). For the ozone concentration measured during the investigations presented in this work (ranging from 100 to 120 μg/m^3), the AOT40 index should by all means be much higher. These data indicate that the average value of ozone in the investigated area was about 100 μg/m^3 (maximum limiting value for human health is 80 μg/m^3) and that the estimated AOT40, would be two to three times higher that the limiting value of 18,000 μg/m^3. This indicates that the concentration of tropospheric ozone in the investigated area represents a high risk for both human health and vegetation.

The main sources of VOCs and NOx emission in the investigated region of the Serbian Banat are mostly anthropogenic. These are gases emitted from automobile exhausts, which are mostly found with the older type of diesel engines. The number of automobiles with Euro 4 engines is still small in this part of Europe. On the other hand, the frequency of the traffic is becoming larger each year. In addition, considerable sources of pollution are agro-industrial complexes and the oil refinery in Pančevo, which is about 100 kilometers away from Zrenjanin. Natural gas and oil boreholes are also located in this region as well as a central underground reservoir for storing natural gas.. All of these sources can greatly influence an increase in the ozone concentration in the ambient air. The emission of natural VOCs and BTEX originate from vegetation, which is largely present in this region considering its agricultural tradition. The levels of VOCs and NOx and the tropospheric ozone concentration represent large potential risks for human health and vegetation in the investigated region. Considering the fact that this is mostly lowland terrain, this risk could be transferred to neighboring regions as well.

Conclusions

The high measured ozone concentrations are correlated with anthropogenic activities in the Banat region during the whole year, and especially in the period of vegetation growth. The measured ozone concentrations in the ambient air were above the thresholds prescribed by the EU standards for protection of the human health and vegetation. Thus, they present a risk for the region and its surroundings.

The results presented in this paper indicate the need for continuous recording of the episodes of increased ozone concentration and for undertaking the necessary precautions to lower the NOx and VOCs emission in this region. The NOx and VOCs concentrations influence the photochemical production of ozone which, in the increased range of concentrations, represents a potential risk to human health and vegetation.

Further investigations focused on an assessment of the actual effects of ozone on these receptors need to be performed in the area. Medium to long-range transport of pollutants could play an important role with respect to ozone levels in the area, which would also require further assessment.

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GENETIC EROSION IN THE FRESHWATER SNAIL *FILOPALUDINA MARTENSI* IS AFFECTED BY LEAD AND CADMIUM

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Abstract. This study aims to determine the lead (Pb) and cadmium (Cd) concentrations and genetic variability in the tissues of the freshwater snail *Filopaludina martensi*. These measures can be biomarkers of environmental quality. The samples were collected over three seasons from five sites around the Ubonrat Dam. The results showed that the average concentrations of Cd and Pb found in the tissue of fresh water snails during the rainy season (0.1040 and 0.0106 µg/g, respectively) were higher than in the winter (0.0566 and 0.0030, respectively) and summer (0.0654 and 0.0059 µg/g, respectively). Random amplified polymorphic DNA (RAPD) was successfully applied to produce 440 total bands used for dendrogram construction and analysis of genetic similarity (S). The dendrogram separated the studied samples by seasons, showing the highest S values of 0.59-0.93 in the Rainy S1-S5 and S2-S3, 0.62-0.83 in the Winter S1-S4 and S1-S2, and 0.64-0.84 in the Summer S1-S5 and S2-S3. The highest S values in the rainy season that are lower values compare to samples from other seasons are those associated with the highest average concentrations of Cd and Pb in the tissues sampled in this season. The metal concentrations are below the standard control level, but they can still affect the genetic material of freshwater snails.

Keywords: *Lead*, *cadmium*, *freshwater snail*, *genotoxicity*, *genetic erosion*

Introduction

Heavy metals are often contaminants in marine and freshwater environments, where they are generally found in low concentrations. However, human activities have increased the levels of metal ions in many of these natural water systems. Industrial and domestic effluents in particular have contributed to the increased metal loads in coastal waters and are ultimately deposited into aquatic sediments (Ansari *et al.*, 2004; Pandey *et al.*, 2010). These metals accumulate in organisms, and some may become biomagnified in food chains (Singh *et al.*, 2010). Many aquatic organisms uptake metals directly from the water, so tissue concentrations reflect metal concentrations in the water. Carnivores at the top of the food chain, such as aquatic birds and mammals (including humans), however, obtain most of their pollutant burden from aquatic ecosystems by ingestion, especially of fish, which have a considerable potential for biomagnification (Richter and Nagel, 2006).

The determination of total heavy metal contents in sediments has not been performed up to now because the behavior of heavy metals is closely related to their chemical forms, and only those with high bioavailability can be absorbed by organisms and do great harm to other organisms and human beings. Thus, the investigation of heavy metal

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contamination in organisms more directly reflects the hazards to human health and the potential heavy metal pollution in water. Lying in the second trophic level in the water ecosystem, mollusks have long been known to accumulate both essential and non-essential trace elements from aquatic ecosystems (Liang *et al.*, 2004; Wagner and Boman, 2004; Nϕrum *et al.*, 2005). Mollusks, including freshwater snails and bivalves, have long been regarded as promising bio-indicators and bio-monitoring subjects. They are abundant in many terrestrial and aquatic systems, making them easily available for collection. They are highly tolerant to many pollutants and exhibit high accumulation properties, particularly for heavy metals. Mollusks living in the sea can accumulate heavy metals and may serve as excellent passive bio-monitors (Wang *et al.*, 2005).

The Pong River is the principle river in Northeastern Thailand. The Moon River is the continuation of this waterway into the lower northwest of this region, thus any water pollution would have a combined effect. People living in the Loei province, the Nongbualamphu province and the Khonkaen province utilize these rivers for consumption and agriculture. Despite reports of water pollution in the Pong River since 1986, there has still been a continuing crisis of water pollution as reported in 1992 (Faculty of Engineering, 2003). Sources of pollution came from several industries, and noted a high impact on the Pong River. Examples of these industries include a paper mill factory, a combined gas turbine power plant, a distillery plant, a sugar factory and wood working plants. In addition, there are several smaller industries located within the vicinity of the Pong River. These are all likely major causes of heavy metal contamination in the Pong River along with traces from pesticides, chemical fertilizers and other wastes from the community.

The genotoxicity measurement process in living tissue at the DNA is advantageous because of its sensitivity and short response time (Gupta and Sarin, 2009). Heavy metals have an acute genotoxic potential in all living things. Immediately releasing these pollutants can cause morbidity and mortality in the exposed organism and can provoke order changes such as alterations to population dynamics and changes in biological diversity (An *et al.*, 2012). Random amplified polymorphic DNA (RAPD) is extremely efficient for DNA analysis in complex genomes as it is simple, inexpensive and minimally time-consuming. The resulting bands can be scored to evaluate genetic similarities and dissimilarities of samples through dendrogram construction. That is, RAPD bands are generally used to effectively indicate genetic relationships. The concept critical to cladistics is homology, which can be defined as a similarity resulting from common ancestry. Therefore, the cladogram designed depicts not only similarity but also evolutionary relatedness (Simpson, 2006).

The freshwater snail *Filopaludina martensi* Frauenfeld 1865 is a benthic fauna in the food chain ecosystem that lives and moves slowly to feed on the surface of sediments and thus accumulates heavy metals. This study aims to determine heavy metal accumulation in fresh water snail tissue and the genetic relationship shown by the similarity index, assessing other toxic contaminants through RAPD analysis.

Materials and methods

Sampling sites

The Pong River is the principle river in Northeastern Thailand, and the Moon River is the continuation of this waterway into the lower part of this region. These rivers support agricultural farms and many industries. The Bueng Jode reservoir receives a considerable amount of effluents from a paper mill factory before flowing into the Pong River. Five sampling sites were defined as the affected area of the Pong River (*Fig. 1*).

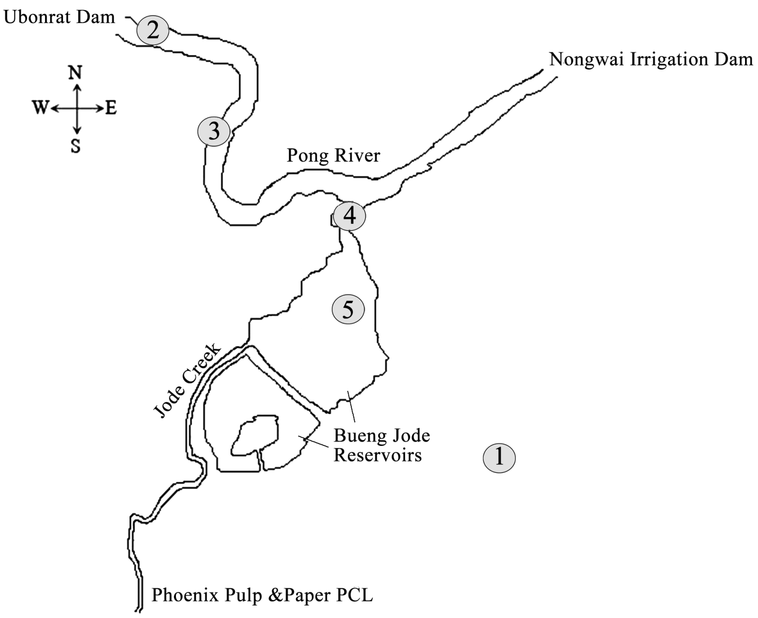

Figure 1. *Overview of the Pong River and locations of the five sampling sites as shown by numbers 1 to 5*

Sampling and sample analysis

Freshwater snails were collected from the five sites in three consecutive seasons in 2010. The concentrations of Cd and Pb were evaluated following these steps:

Three samples from each sampling site were homogenized, the soft tissues of freshwater snails were excised using a sterile stainless steel scalpel. These tissues were predigested with 3 ml of concentrated nitric acid overnight at 40 °C. After cooling, 2 ml of 30% hydrogen peroxide was added. There after the container was covered and placed in a high-pressure stainless steel bomb then put in an oven. The oven temperature was increased to160 °C and kept for 4 hrs. After cooling, the solution was diluted with Milli-Q water and transferred into a PET bottle to 50 g. Cd and Pb concentrations were determined with inductively coupled plasma-optical emission spectrometry (ICP-OES), PerkinElmer (Optima 4300 AV). For the wavelength analysis of ICP, wavelengths of Cd and Pb were 228.802 and 220.353 nm, respectively. The results are reported as dry weight. The accuracy of the results for heavy metal was evaluated with the certified reference material (CRM) using the 3111C method (APHA, 2005). Two aliquots of the CRM were spiked with a known amount of heavy metal spike standard, and one spike was analyzed according to 3111C method while the other was analyzed with the 3111B method (APHA, 2005). The metal recoveries were in the 96–100% range, which was acceptable (USEPA, 1994).

Statistical analysis

The seasonal variations and sampling sites were analyzed using SPSS V.19. All the statistical tests were conducted at a 95% confidence level. The data normality was tested using the Kruskal-Wallis test.

DNA extraction

DNA was extracted from kidney tissue using the Genomic DNA Extraction Kit (RBC Bioscience, Taiwan) following the manufacturer's instructions. The extracted DNA was checked by 0.8% agarose gel electrophoresis and then diluted to a final concentration of 20 ng/µl.

DNA fingerprinting and analysis by RAPD marker

Amplifications were carried out on each sample in 25 µl reactions consisting of GoTaq Green Master Mix (Promega), 0.5 µM primer and 5 ng DNA template. Several RAPD primers were screened and the 12 primers that successfully amplified clear bands are as follows (5′ to 3′): CATCCCCCTG, GGACCCTTAC, GTCCCGACGA, AAGCCTCGTC, CAGGCCCTTC, TGCCGAGCTG, AATCGGGCTG, GGGTAACGCC, CAATCGCCGT, CAAACGTCGG, GACCTACCAC and TCAGTCCGGG. The reaction mixture was incubated at 94 °C for 3 min and the amplification was performed with the following thermal cycles: 35 cycles of denaturation for 1 min at 94 °C, 2 min annealing temperature at 40 °C, 2 min at 72 °C, and 7 min final extension at 72 °C using a thermal cycler (Swift^{TM} Maxi Thermal Cycler, Esco Micro Pte. Ltd.). Amplification products were detected by 1.2% agarose gel electrophoresis in TAE buffer and visualized using ethidium bromide. The resulting RAPD bands were used for the dendrogram construction.

The RAPD bands from the primers that resulted in successful amplification were discerned on an agarose gel and were documented as diallele characters: present=1 and absent=0. These resulting bands were used to construct a dendrogram following NTSYSpc 2.10p (Rohlf, 1998). The dendrogram and genetic similarities were produced.

Results and Discussion

Cd and Pb concentrations

The Cd and Pb concentrations from the tissue of freshwater snail samples are shown in *Table 1*. The average concentration values of Cd and Pb in the winter, summer and rainy seasons are 0.0566, 0.0030; 0.0654, 0.0059; and 0.1040, 0.0106 µg/g of tissue, respectively. The contaminated values are higher in the rainy season than in the winter and summer. Statistical analysis shown in *Table 2* indicates that there are no significant differences between study sites or weather seasons with regard to Cd and Pb (p>0.05).

DNA fingerprinting of freshwater snail

The 12 successful RAPD primers generated clear and testable fingerprinting profiles. Examples of fingerprints are shown in *Figure 2-4*.

Table 1. *Concentrations of Cd and Pb in freshwater snail tissue (μg/g, dry weight)*

Parameter	Cd content (μg/g)	Pb content (μg/g)
Winter season		
Site 1	0.0290	0.0011
Site 2	0.0310	0.0009
Site 3	0.0390	0.0027
Site 4	0.1100	0.0050
Site 5	0.0740	0.0053
Average	0.0566	0.0030
SD	0.0349	0.0021
Summer season		
Site 1	0.0930	0.0073
Site 2	0.1100	0.0051
Site 3	0.0360	0.0170
Site 4	0.0570	0.0000
Site 5	0.0310	0.0000
Average	0.0654	0.0059
SD	0.0349	0.0070
Rainy season		
Site 1	0.0630	0.0160
Site 2	0.0700	0.0210
Site 3	0.0340	0.0043
Site 4	0.3200	0.0030
Site 5	0.0330	0.0086
Average	0.1040	0.0106
SD	0.1219	0.0077

Table 2. *Values obtained from SPSS test*

Source of variation	*p* value (Cd)	*p* value (Pb)
Sample sites	0.393	0.792
Seasons	0.826	0.248

Significant value with a confidence level of 95%

Countable bands ranged in size from 100 bp to 2,500 bp. Only bands present in at least two of three samples from each studied site in each season were counted. These resulted in 440 total bands, which were used for dendrogram construction. The dendrogram distinguished studied samples into three groups based on the samples from five different sites (S1-S5) and seasonal grouping.

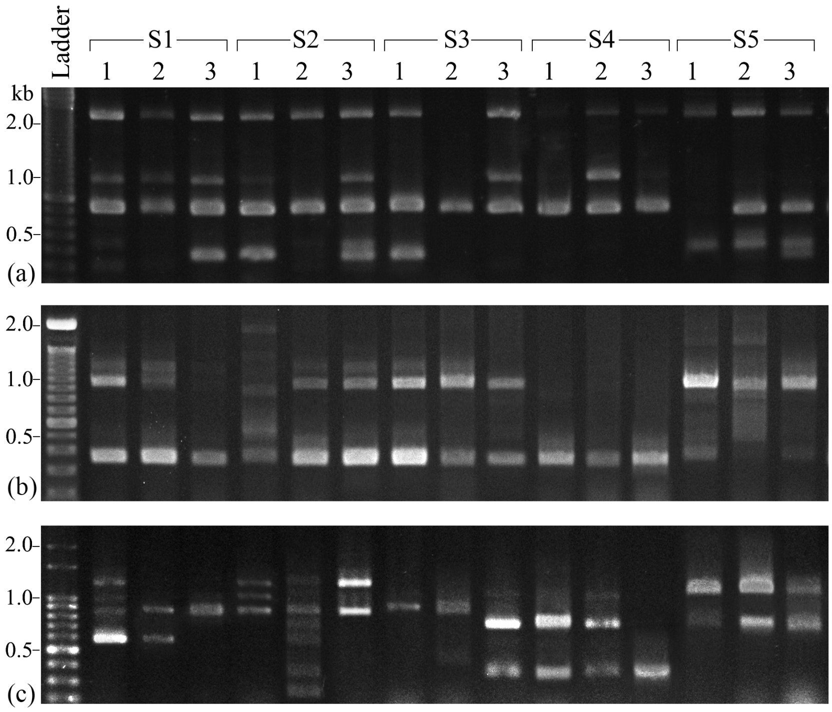

Figure 2. *Examples of RAPD fingerprints from all freshwater snail samples studied in the winter from primers CATCCCCCTG (a), CAATCGCCGT (b) and CAAACGTCGG (c)*

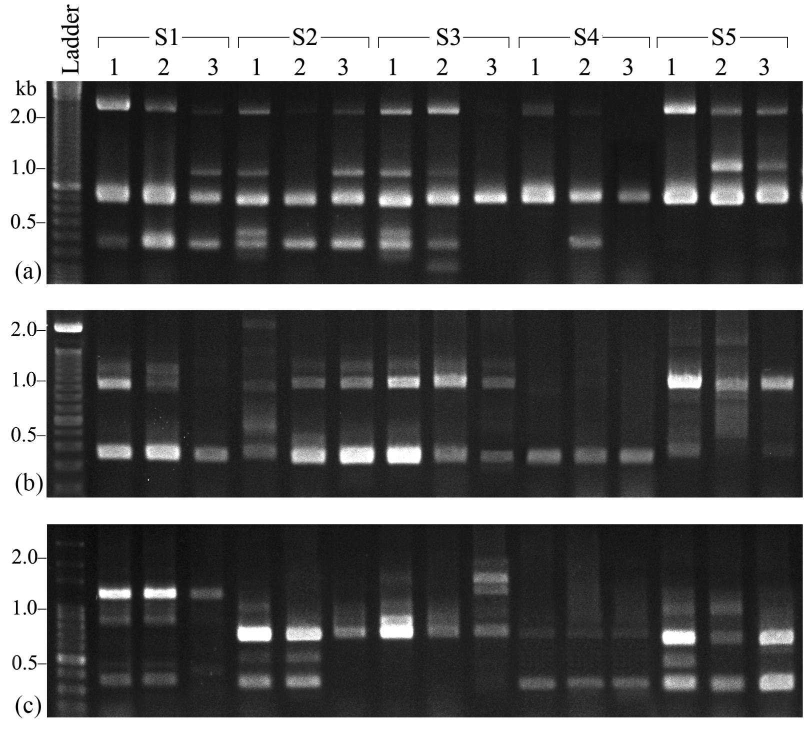

Figure 3. *Examples of RAPD fingerprints from all freshwater snail samples studied in the summer from primers CATCCCCCTG (a), CAATCGCCGT (b) and CAAACGTCGG (c)*

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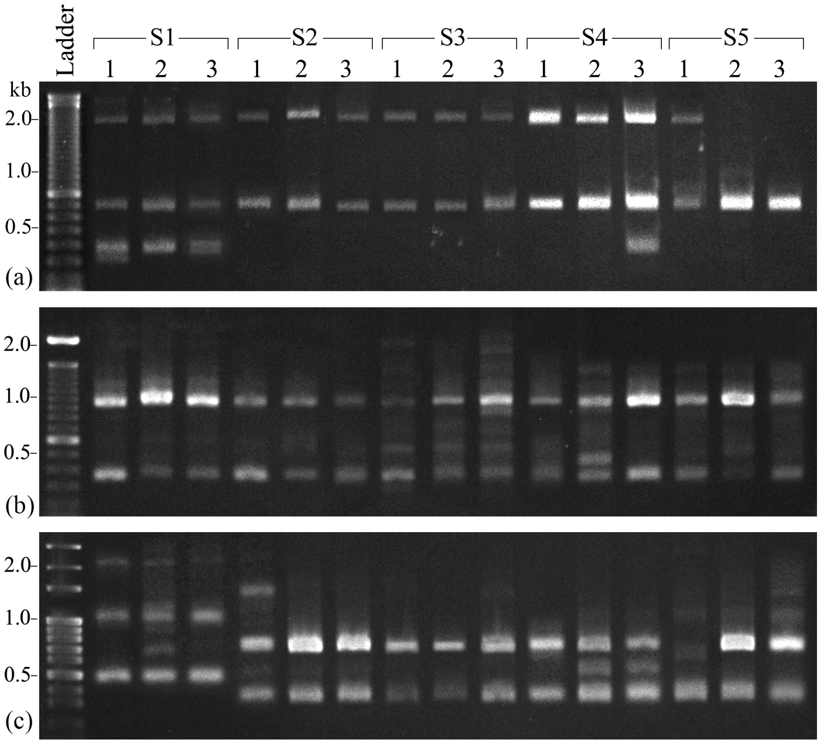

Figure 4. *Examples of RAPD fingerprints from all freshwater snail samples studied in the rainy season from primers CATCCCCCTG (a), CAATCGCCGT (b) and CAAACGTCGG (c)*

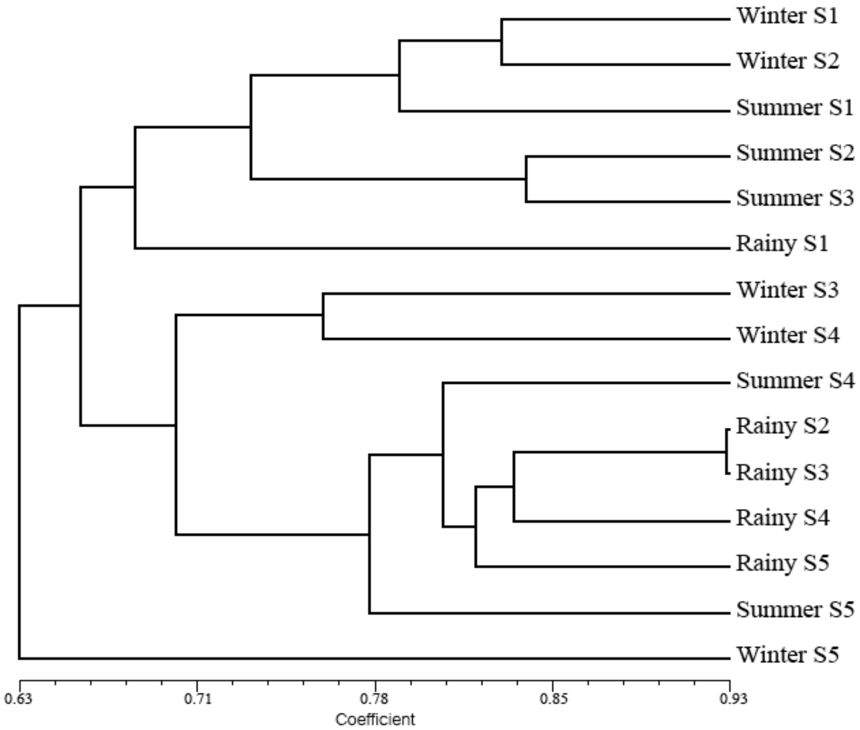

Figure 5. *The dendrogram constructed from twelve RAPD primers by the NTSYSpc 2.10p program showing the genetic relationships between the five studied sample sites at different seasons*

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Table 3. *Relationships of all pairwise studied samples at different seasons indicating genetic similarity values*

	Winter S1	Winter S2	Winter S3	Winter S4	Winter S5	Summer S1	Summer S2	Summer S3	Summer S4	Summer S5	Rainy S1	Rainy S2	Rainy S3	Rainy S4	Rainy S5
Winter S1	1.00														
Winter S2	0.83	1.00													
Winter S3	0.72	0.74	1.00												
Winter S4	0.62	0.64	0.76	1.00											
Winter S5	0.66	0.68	0.65	0.66	1.00										
Summer S1	0.76	0.82	0.68	0.65	0.63	1.00									
Summer S2	0.66	0.68	0.61	0.68	0.62	0.76	1.00								
Summer S3	0.69	0.76	0.64	0.67	0.67	0.81	0.84	1.00							
Summer S4	0.64	0.68	0.69	0.77	0.68	0.69	0.77	0.76	1.00						
Summer S5	0.61	0.61	0.66	0.76	0.69	0.64	0.74	0.66	0.80	1.00					
Rainy S1	0.62	0.68	0.61	0.58	0.52	0.65	0.73	0.72	0.64	0.61	1.00				
Rainy S2	0.60	0.64	0.63	0.77	0.60	0.65	0.75	0.72	0.83	0.80	0.62	1.00			
Rainy S3	0.57	0.59	0.64	0.76	0.61	0.62	0.76	0.71	0.82	0.81	0.63	0.93	1.00		
Rainy S4	0.67	0.65	0.60	0.72	0.61	0.66	0.78	0.77	0.78	0.75	0.67	0.84	0.83	1.00	
Rainy S5	0.53	0.57	0.62	0.74	0.55	0.58	0.67	0.64	0.80	0.73	0.59	0.86	0.81	0.79	1.00

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The first group is the Winter S5 only; the second group comprised the Rainy S2, Rainy S3, Rainy S4, Rainy S5, Summer S4, Summer S5, Winter S3 and Winter S4 samples; the third group comprised the Rainy S1, Summer S1, Summer S2, Summer S3, Winter S1 and Winter S2 samples. The dendrogram (*Fig. 5*) shows genetic similarity between all studied samples according to the pairwise genetic relationships shown in *Table 3*. The dendrogram separated the studied samples by seasons and showed the highest S (0.59-0.93) of the studied samples in the rainy season, Rainy S1-S5 and S2-S3. The other two sites are 0.62-0.83 in the Winter S1-S4 and S1-S2, and 0.64-0.84 in the Summer S1-S5 and S2-S3, respectively.

Toxin-free food is essential for people's wellbeing and animal health. Freshwater snails have long been used as an environmental indicator. However, there are no current studies addressing ecotoxicology of the Pong River or of the potentially harmful impacts of pollutants on food. This research is much needed for supporting agricultural farms and people's life styles in Northeastern Thailand, along with the many industries surrounding them. Consequently, these concerns were addressed in this study by measuring metal concentrations in tissues. The freshwater snail, *F. martensi* is a much-relished ingredient in Thailand's cuisine. To ensure quality food and a safe environment around the Pong River, this experiment examined the concentration of heavy metals Cd and Pb in the freshwater snail's tissues and determined the cellular biomarker as effective for these objective examinations. The study showed that the average concentrations of Cd and Pb found in freshwater snail tissue in the rainy season were 0.1040 and 0.0106 µg/g, respectively, and these concentrations are lower in the winter, at 0.0566 and 0.0030 µg/g, respectively, and in the summer, at 0.0654 and 0.0059 µg/g, respectively. However, there are no significant differences in heavy metal concentrations according to SPSS V.19 analysis (Kruskal-Wallis) for each site (p=0.393 for Cd, p=0.792 for Pb) or for each season (p=0.826 for Cd, p=0.248 for Pb). Accordingly, the dendrogram distinguished the studied samples into the three groups (the Winter S5; the Rainy S2, Rainy S3, Rainy S4, Rainy S5, Summer S4, Summer S5, Winter S3 and Winter S4; the Rainy S1, Summer S1, Summer S2, Summer S3, Winter S1 and Winter S2) from the different samples from different sites and different seasons, grouping them independently from the heavy metal concentrations. Fortunately, these metal concentration values are below both Thailand's standard control level (2.0 µg/g (Thailand Pollution Control Department, 1986)) and the Food and Agricultural Organization (FAO) (2.0 µg/g in Cd and 1.0 – 6.0 µg/g in Pb.) The rainy season has running water leaching heavy metals such as Cd and Pb from communities and industries into the Pong River, which are then deposited in sediments and accumulate in the tissue of freshwater snails. This likely accounts for the increased concentration of Cd and Pb during the rainy season. The freshwater snails in this area are affected by many industries, agricultural fertilizers and insecticides, but can still be of good quality. This animal can endure these two heavy metals, and survive in the environment around the Pong River, which is a moderately contaminated area. This study, in sum, is a field investigation, and there are other possible measures of toxicity from the environment beside these two heavy metals.

Understanding the effect of pollutants on genetic variability is fundamental for preservation. The evolutionary potential of natural populations, the effects of toxic contaminants on the environment, and the effect of Cd and Pb on the freshwater snail habitats were investigated at the molecular level using RAPD marker analysis. Genomic DNA modifications such as damage and structure variations were detected, and these modifications can be biomarkers. The highest S values (0.59-0.93) in the rainy season samples that were low compared to samples from other seasons are those associated with the highest average concentrations of Cd and Pb in the rainy season, suggesting an effect of the metals on the freshwater snail tissue. Although, the heavy metal concentrations are under the standard control level, they can have an effect on the genetic material of the freshwater snail, which is an important ingredient in the local food culture. This study suggests that the freshwater snail is a good indicator species, by which to measure the effectiveness of local waste-water treatment systems.

Note that actual conditions in the field are affected by several pollutants, such as fertilizers, heavy metals, chemicals, and insecticides, etc., in addition to Cd and Pb, and these pollutants can all have positive and negative effects on the ecosystem.

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GRADIENT ANALYSIS OF VEGETATION ON THE SOUTH SLOPE OF VITOSHA MOUNTAIN, SOUTHWEST BULGARIA

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Abstract. Detailed analysis of the relationship between vegetation and environment requires a thorough understanding of the environmental processes that influence vegetation. There is an intimate relationship between vegetation concepts' development, mathematical methods of analysis and knowledge of environmental processes. Traditionally most vegetation/environment studies have been done at a single scale of observation. It has been considered important to assess whether the processes that determine community structure and function are similar at different scales or whether the most important processes change with the scale of observation. This study aims to describe in a quantitative manner the vegetation pattern in a localized area and on that basis to test/verify statistically some previously proposed hypothesis and assumption concerning vegetation organization. Describing the coarsest vegetation pattern, ordination and classification analyses were used. The detailed relationships between environmental gradients and vegetation were examined using regression and correlation methods. Obtained results were tested with different statistical tests for their reliability. We found that the basic environmental gradients influencing the local vegetation are elevation and habitat moisture. The correlation between environmental gradients and vegetation increased with the increasing scale of observation. Environmental gradients influencing vegetation most strongly were the same at the different scales. With the increase of sampled area, correlation strength between environment and vegetation also increased. In the future, if possible, all studies of vegetation/environment relationships should be done at different spatial scales. Gathered quantitative information and verified hypothesis in the current study could be very successfully used in the management and conservation of the local and regional vegetation.
Keywords: *vegetation variables, ordination, correlation, regression, CCA, GAM*

Introduction

Detailed analysis of the relationship between vegetation and environment requires a thorough understanding of the environmental processes that influence vegetation (Austin 2005). There is an intimate relationship between vegetation concepts' development, mathematical methods of analysis and knowledge of environmental processes. Austin (2005) postulated tree fundamental questions which can account for these aspects: 1) Is vegetation pattern continuous or discontinuous and how is this pattern related to environment? 2) What theory and methods are most appropriate for investigating such patterns? 3) What is the relative importance of environment and factors intrinsic to the vegetation in determining the observed patterns?

Answering these questions requires appropriate methods for vegetation/environment analysis. Until now numerous such methods have been developed. For example, direct ordination or direct gradient analysis, is the analysis of species distributions (presence/absence or abundance data) and collective properties (e.g. species richness) in relation to environmental variables conventionally referred to as environmental gradients (Austin 2005). On the contrary, the indirect ordination or indirect gradient analysis, determines the major gradients of variation to be found in the vegetation data itself. A graphical representation of the variation in vegetation across all sites can be

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constructed by measuring the similarity between each site based on the species composition. Such an ordination diagram summarizes the major axes of variation in the vegetation data matrix (Austin 2005).

Studying the vegetation of Santa Catalina Mountains and using gradient analysis approach, Whittaker and Niering (1975) found some tendencies, concerning important vegetation variables, that can be observed and that can possibly imply the answers of the previous three questions: 1) within the tree layer, no correlation between the species richness and productivity was evident; 2) among perennial herbs both diversity and productivity showed bimodal distribution and weak correlation between them; 3) weak correlation of diversity and productivity was observed, when all shrub life forms were grouped together; 4) for the dominants' strata, there were no correlation between diversity and productivity in habitats where these layers were dominant; and 5) in temperate vegetation the tree layer was in general poorer on species than shrub and herb strata; herb and shrub layers were on average less rich under closed forest canopies than in open communities. The current study tried to verify all these hypothesis.

Traditionally most vegetation/environment studies have been done at a single scale of observation (Reed at al. 1993). It has been considered important to assess whether the processes that determine community structure and function are similar at different scales or whether the most important processes change with the scale of observation (Reed at al. 1993). Studying the understory vegetation of North Carolina at different spatial scales, Reed et al. (1993) proposed few hypothesis which we also try to test: 1) the correlation between vegetation composition and environment increases with increasing quadrat size; 2) the environmental factors most highly correlated with species composition and richness should be similar at all scales and should be among the environmental factors strongly correlated with species composition over the much larger extent; 3) with increased extent of sampling the correlation between environment and vegetation should also increase.

Structure, composition and post-disturbance forest development could be studied in the context of local environmental gradients. Investigations of most community and ecosystem properties including diversity, biomass and productivity need to be conducted within a multidimensional framework including both site condition and stand development. Similarly, studies of population change during succession need to be placed in an environmental context (Peet 1981). Resulting from his study, Peet (1981) proposed tree models of stand development, depending of the local site conditions: 1) favorable middle-elevation sites; 2) highest or middle elevation sites in very dry conditions; 3) lower elevation sites with episodic regeneration and favorable conditions. The current study tried to analyze the local stands, aiming to place them in the context of these tree models.

Explicit testing of vegetation patterns according to environmental gradients has yet to be achieved. Descriptive vegetation patterns resulting from gradient analyses combined with statistical modelling can be of exceptional benefit. Understanding these patterns is an essential ingredient in sustainable vegetation management, despite that there are still many questions to be answered in vegetation science (Austin 2005).

Material and Methods

Study area

Vitosha Mountain is located in Western Bulgaria. It is characterized with compactness and well expressed elevation gradient. The mountain has steep slopes and variable expositions. Its vegetation has variable species composition. The current study embraced the south slope of the mountain. GPS coordinates of that territory are between N42°32' E23°09' and N42°26' E23°21'. The area covers 118 km^2. For details about the studied area, refer to Dyakov (2012, 2013).

Sampling

Sampling strategy was based on gradsect method of Austin & Heyligers (1991). Its advantages are: 1) relatively cheap, 2) highly effective, 3) easy to apply on the field, but requiring experienced workers. During the summers of 2008 and 2009, a total of 159 (0.1 ha) samples were taken. Detailed information on the field techniques can be found in previous papers (Dyakov 2012; Dyakov 2013; Dyakov & Zhelev 2013).

Environmental gradients

Four indirect or factor-complex (*sensu* Whittaker 1978) environmental gradients were used – elevation, exposition, slope inclination and slope convexity. According to Austin & Smith (1989) and Austin (2005), indirect gradients influence direct gradients such as temperature and rainfall which have direct effect on plant growth. On a local scale, the author recommends measuring and using indirect gradients because they have much more meaningful influence on vegetation. For detailed information on gradient quantification methodology, refer to Dyakov (2012).

Scale

Traditionally, vegetation structure and correlation of vegetation and environment have been analyzed at a single scale of observation, using one quadrate size. However, different ecological processes are important at different spatial and temporal scales and dependent on the scale of observation (Reed et al. 1993). We analyzed vegetation patterns at two different spatial scales using two data sets. First data set was composed of 159 0.1 ha field plots. Second one comprises 843 1 m^2 field subplots.

Ordination and Classification

Using divisive classification algorithm of Two-Way INdicator SPecies ANalysis (TWINSPAN) (Hill 1979), all samples were clustered into compositional groups or community types. The cluster analysis was run with checked default program options. This method not only classifies the samples, but also constructs double-ordered table from the data matrix (samples × species). Combination of these characteristics made it one of the most popular programs in vegetation ecology nowadays.

The general picture of the vegetation pattern in the environmental space was outlined with direct gradient analysis, using Canonical Correspondence Analysis (CCA) (ter Braak 1986). Being a method for direct gradient analysis, CCA explains the variation in the data on the basis of preliminary chosen environmental gradients (Jongman et al. 2004). The main advantages of CCA are: 1) the simultaneous ordering of sites and species (if needed), 2) rapid computation, 3) very good performance when species have nonlinear and unimodal relationships to environmental gradients, and 4) robustness to violation of preliminary assumptions (Palmer 1993).

Trying to clarify the relationship between the environmental gradients and vegetation species composition we also used indirect gradient analysis – Detrended Correspondence Analysis (DCA). DCA constructs theoretical variables which best explain data variance without the necessity of preliminary introduced environmental variables. This is done by choosing most adequate sample scores, i.e. these ones which maximize the explained variance in species composition. When the result are plotted on the biplot, the two axes (first and second) are these theoretical variables which explain greatest percentage of data variance, being uncorrelated each other (Jongman et al. 2004). Two different DCA ordinations were carried out with two data sets at the two different spatial scales of observation.

Correlation and Regression

Trying to reveal the detailed relationships between the species distribution or abundance and the environmental gradients, we used multiple regression and correlation techniques. Because correlated variables were not normally distributed we used nonparametric correlation coefficient of Spearman, R_s (Spearman 1904).

The aim of regression analysis is to describe the dependent variable as a function of one or multiple independent ones. Using regression analyses, dependent variables can be predicted (described) with minimal or less statistical error (Jongman et al. 2004). General Additive Models (GAM) were used as a multiple regression method. The aim of GAM models is to maximize the quality of the dependent variable description, which may have various distributions. GAM does so by developing unspecified nonparametric functions of the independent variables, which are "connected" with the depended variable by link function (Thomas & Mitchell 1991).

Statistical tests

Obtained results were tested for significance with Monte Carlo Permutation test and F-test. In all statistical tests, the significance level was $P<0.05$ unless shown otherwise.

In all analyses the following specialized software products were used: STATISTICA, version 8.0 (StatSoft 2007); CANOCO for Windows, version 4.51 (ter Braak & Smilauer 2003); CanoDraw for Windows, version 4.1 (Smilauer 2003).

Results

General picture of local vegetation pattern

TWINSPAN classification resulted in 14 clusters, shown with different symbols in *Figure 1* (for the full names of plant communities see the *Legend* below the *Figure 1*), which represents the summarized picture of the studied vegetation, set in the context of the four preliminary chosen environmental gradients. The latter are considered as the most important local environmental gradients, playing fundamental role in vegetation organization on the south slope of Vitosha Mountain.

For the correct interpretation of CCA biplot, it should be noticed, that the longer the arrow of the corresponding environmental variable, the more highly correlated it is to the ordination axis in its close proximity. Moreover, the closer the sample (symbol) point of given plant community is to the arrow point (or to that part of the biplot, to

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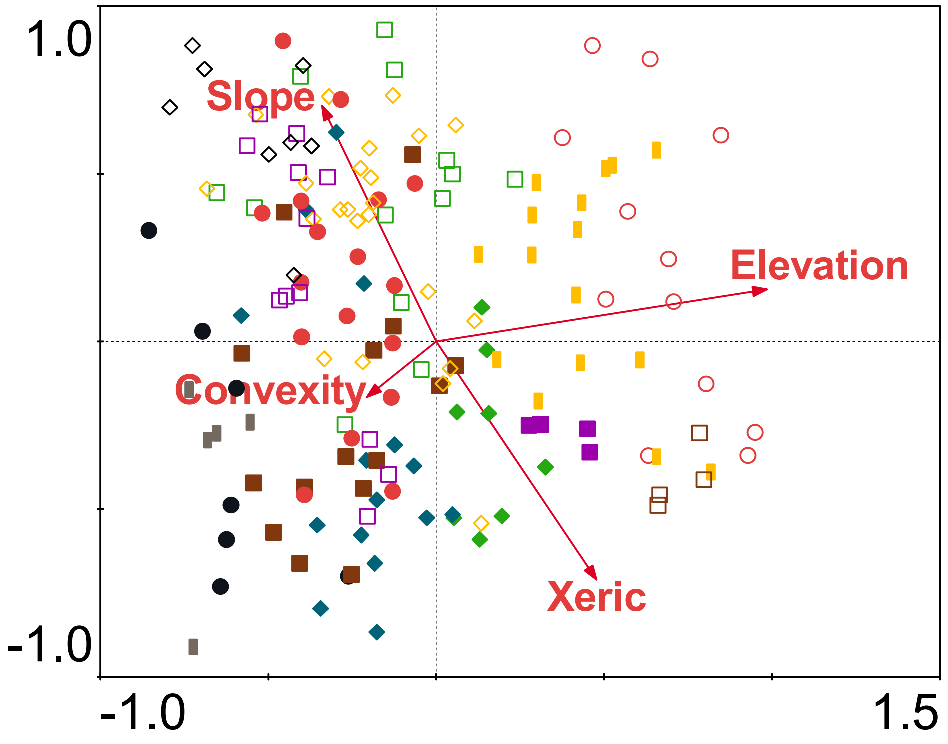

Legend:

◇ *Fagus sylvatica-Hepatica nobilis (n=8)*
□ *Fagus sylvatica-Physospermum cornubiense (n=12)*
□ *Fagus sylvatica-Galium odoratum (n=13)*
◇ *Fagus sylvatica-Luzula luzuloides (n=23)*
● *Fagus sylvatica-Festuca drymeja (n=17)*
■ *Fagus sylvatica-Corylus avellana-Brachypodium pinnatum (n=15)*
◆ *Pinus sylvestris-Fragaria vesca (n=15)*
▮ *Pinus nigra-Crataegus monogyna (n=5)*
● *Quercus cerris-Cornus mas (n=6)*
■ *Festuca pseudodalmatica-Chamaespartium sagittale (n=4)*
◆ *Galium verum-Thymus glabrescens (n=8)*
▮ *Juniperus sibirica-Vaccinium myrtillus (n=16)*
○ *Juniperus sibirica-Vaccinium myrtillus-Vaccinium vitis-idaea (n=12)*
□ *Juniperus sibirica-Sesleria comosa (n=4)*

Figure 1. *Ordination of the 159 samples (0.1 ha) in the context of local indirect environmental gradients. Community types are denoted with the Latin names of dominant plant species composing them. Elevation = altitude above sea level; Xeric = moisture gradient; Slope = slope inclination in degrees; Convexity = slope convexity (for details on the indirect gradient methodology see cited papers in Material and Methods section).*

which the arrow is pointed out) the greater is the importance of the corresponding environmental gradient for the vegetation in these habitats.

Statistical parameters of the CCA analysis (*Table 1*) show that first axis alone as well as all axes taken together at the two scales are highly statistically significant (*P=0.002*).

Table 1. *Statistical parameters of CCA ordination*

0.1 ha scale (*n=159*)					
CCA axes	1	2	3	4	Total inertia
Eigenvalues:	0.651	0.253	0.120	0.067	9.706
Monte Carlo test of the CCA axes (499 permutations)					
First canonical axis				*F*=11.067	*P*=0.002
All axes				*F*=4.875	*P*=0.002
1 m^2 scale (*n=843*)					
CCA axes	1	2	3	4	Total inertia
Eigenvalues:	0.676	0.315	0.263	0.097	52.863
Monte Carlo test of the CCA axes (499 permutations)					
First canonical axis				*F*=10.859	*P*=0.002
All axes				*F*=5.497	*P*=0.002

CCA ordination of the dominant species, composing local vegetation, i.e. these species, most highly correlated with the local environmental gradients, is represented on *Figure 2*. These plant species also have greatest importance for the formation of studied vegetation.

The open subalpine vegetation is located at the right part of the biplot, found only at relatively higher elevation (*Figure 1*). The more mesophytic plant communities (such as *Juniperus sibirica-Vaccinium myrtillus-Vaccinium vitis-idaea* and *Juniperus sibirica-Vaccinium myrtillus*) are positioned at the upper right part, while the subalpine shrub and herb plant communities, preferring more xeric, sunny slopes and ridges (*Juniperus sibirica-Sesleria comosa, Festuca pseudodalmatica-Chamaespartium sagittale* and *Galium verum-Thymus glabrescens*) are localized in the right lower biplot part. Similar positions have the plant species dominant in the corresponding vegetation. In the upper right part of *Figure 2* are found more mesophytic herb species like *Hypericum maculatum, Sesleria comosa, Vaccinium myrtillus, Vaccinium vitis-idaea, Rubus idaeus, Calamagrostis arundinacea* etc., while the lower right part is occupied by plant species preferring more xeric and poor habitats, like *Festuca nigrescens, Agrostis capillaris, Achillea millefolium, Thymus glabrescens, Chamaespartium sagittale, Trifolium alpestre, Galium verum* etc.

Forest vegetation and the species composing it are located at the left part of the CCA biplots (*Figure 1* and *Figure 2*). Forest vegetation on the south slope of Vitosha most often reaches its limit around 1600 m a.s.l. Forest communities exceed this altitudinal limit only at some isolated places. For example, it happens along some deep moist ravines with permanent or seasonal upland streams. Lower left part of *Figure 1* is occupied by subxeric and xeric forests, developed at lower elevation (including some pine plantations, still surviving after being planted there in the past). These are plant communities such as *Pinus nigra-Crataegus monogyna, Pinus sylvestris-Fragaria vesca, Quercus cerris-Cornus mas* and *Fagus sylvatica-Corylus avellana-Brachypodium pinnatum*. They are preferred by species like *Quercus cerris, Pinus sylvestris, Crataegus monogyna, Rosa canina, Corylus avellana, Brachypodium pinnatum, Fragaria vesca* and others (*Figure 2*). At the upper left part of the biplot

(*Figure 1*) are found more mesic forest communities, dominated completely by the common beech, occupying steeper mountainous slopes. These are *Fagus sylvatica-Hepatica nobilis, Fagus sylvatica-Physospermum cornubiense, Fagus sylvatica-Galium odoratum, Fagus sylvatica-Luzula luzuloides* and *Fagus sylvatica-Festuca drymeja* communities. These communities are dominated also by plant species like *Mycelis muralis, Lamium galeobdolon, Hepatica nobilis, Viola riviniana, Aremonia agrimonoides, Helleborus odorus* etc.

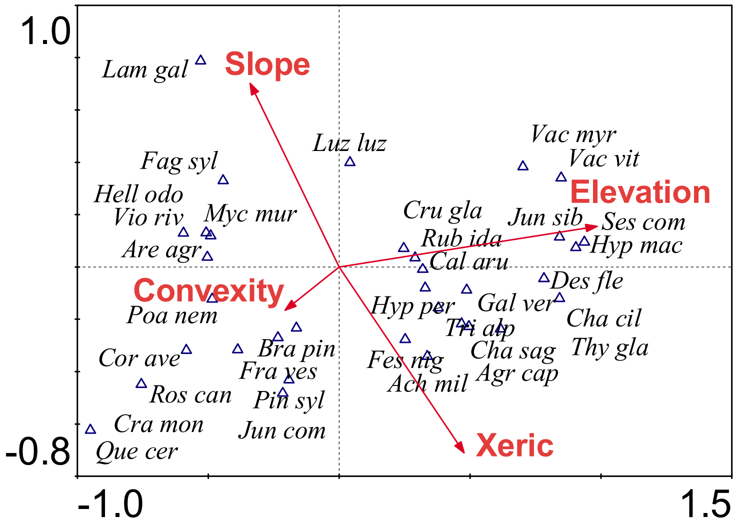

__Figure 2.__ Ordination of dominant plant species in the context of local indirect environmental gradients. Plant species are denoted with the first three or four letters of their genus and species name as follows: Lam gal = Lamium galeobdolon (L.) Ehrend. & Polatschek ; Fag syl = Fagus sylvatica L.; Hell odo = Helleborus odorus Waldst. & Kit. ex Willd. ; Vio riv = Viola riviniana Rchb.; Myc mur = Mycelis muralis Dumort.; Are agr = Aremonia agrimonoides (L.) DC.; Poa nem = Poa nemoralis L.; Co rave = Corylus avellana L.; Bra pin = Brachypodium pinnatum (L.) P. Beauv.; Fra ves = Fragaria vesca L.; Ros can = Rosa canina L.; Pin syl = Pinus sylvestris L.; Cra mon = Crataegus monogyna Jacq.; Jun com = Juniperus communis L.; Que cer = Quercus cerris L.; Cal aru = Calamagrostis arundinacea (L.) Roth; Des fle = Deschampsia flexuosa (L.) Trin.; Cal ver = Galium verum L.; Hyp per = Hypericum perforatum L.; Tri alp = Trifolium alpestre L.; Cha cil = Chamaecytisus ciliatus (Wahlenb.) Rothm.; Thy gla = Thymus glabrescens Wild.; Cha sag = Chamaespartium sagittale (L.) Gibbs; Fes nig = Festuca nigrescens Lam.; Agr cap = Agrostis capillaris L.; Ach mil = Achillea millefolium L.; Luz luz = Luzula luzuloides (Lam.) Dandy; Vac myr = Vaccinium myrtillus L.; Vac vit = Vaccinium vitis-idaea L.; Cru gla = Cruciata glabra (L.) Ehrend.; Jun sib = Juniperus sibirica Burgsd.; Ses com = Sesleria comosa Velen.; Hyp mac = Hypericum maculatum Crantz; Rub ida = Rubus idaeus L.

Correlation between the environmental gradients and vegetation

Gradient relationships

Correlation between some vegetation variables and the four DCA axes (surrogate of the underlying environmental gradients) at the two scales of measurement, are represented in *Table 2. Table 3* shows the correlations between the environmental gradients and vegetation variables themselves.

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It is obvious that the elevation is most highly positively correlated with the first DCA axes at the two scales. First DCA axis correlates highly positively with moisture gradient too, as well as highly negatively related with the slope inclination. Second DCA axis is most highly positively correlated to moisture gradient and negatively to the slope inclination at 0.1 ha scale. At the 1 m^2 scale it correlates strongly negatively with the elevation. First DCA axis is considered to be latent expression of elevation gradient and the second DCA axis – the change of microhabitat conditions, governed by the change of slope inclination, slope exposition and topography. It is hard to interpret third and fourth DCA axes despite the significant correlations found. One probable reason for this is the small number of environmental gradients used or their indirect (complex) influence on the vegetation. Anyway, elevation and moisture gradients explain the greatest part of data variation; therefore we consider them as variables with profound influence on vegetation composition and structure.

Vegetation cover

Strong negative correlation is found between tree layer cover and the first two DCA axes at the two scales of observation. This holds also for the correlation with elevation and moisture gradient (*Table 3*). It is considered as a proof for the correct interpretation of CCA biplot. The negative correlation in the second case can be explained with the more xeric habitat conditions at the slope ridges, where forests, if at all present, have more open character (these are most often pine plantations). Strong positive correlation is found between the slope inclination and tree layer cover. Steep slope bases in the close proximity to temporal or permanent springs are occupied predominantly by beech forests which are characterized with highly closed canopies.

We have found strong positive correlation between herb cover and first DCA axis at the two scales of measurement. This is true for the elevation gradient too. At the higher altitudes, due to lack of forest vegetation, the herb layer reaches its highest cover, approaching at some places 100%.

Shrub cover does not show clear tendency, except that it correlates highly positively with phanerophyte number. Herb cover correlates strongly positively with the elevation and xeric gradients and negatively with the slope inclination. In the second case, it is due to the more abundant herb layer, developed under the more open canopy of the xeric pine plantations and mixed oak forests, giving the opportunity to greater number of shrub and herb species to survive. These forests are distributed in drier habitats on more sloping places. On the other hand, beech forests, with some exceptions, are almost completely spared of shrub and herb layers. Shrub cover correlates significantly only with the elevation and it is negative relationship (*Table 3*).

Plant life forms

Strong positive correlation has been found between first DCA axis and the chamaephytes, hemicryptophytes and therophytes (*Table 3*) at the two scales of measurement. Strong positive correlation is also observed between the chamaephytes and the elevation as well as between the hemicryptophytes, elevation and xeric habitats. Cryptophytes correlate negatively with the two main environmental gradients – elevation and xeric habitats. Therophytes somehow repeat the tendency of chamaephytes and hemicryptophytes.

Table 2. *Correlation (nonparametric correlation coefficient of Spearman, R_s) between DCA axes, indirect environmental gradients and vegetation variables. Correlation coefficients greater than 0.4 are shown with* ***Bold*** *type; ns = not significant; *P<0.05; **P<0.01; ***P<0.001.*

	0.1 ha scale (*n=159*)					m² scale (*n=843*)				
	DCA axis 1	DCA axis 2	DCA axis 3	DCA axis 4		DCA axis 1	DCA axis 2	DCA axis 3	DCA axis 4	
Indirect gradients			R_s					R_s		
Elevation (m)	**0.591*****	*ns*	-0.174*	0.218**		**0.480*****	-0.259***	-0.246***	-0.128***	
Moisture gradient	**0.527*****	0.283***	*ns*	-0.349***		**0.564*****	*ns*	-0.175***	-0.173***	
Slope inclination (°)	**-0.489*****	-0.257***	0.288***	*ns*		**-0.430*****	*ns*	*ns*	*ns*	
Slope convexity	-0.164*	*ns*	0.267**	*ns*		*ns*	*ns*	*ns*	*ns*	
Cover (%)										
Trees (0.1 ha)	**-0.839*****	**-0.570*****	**0.495*****	*ns*		**-0.814*****	0.232***	*ns*	*ns*	
Shrubs (100 m²)‡	*ns*	*ns*	*ns*	-0.350***		*ns*	0.125***	*ns*	*ns*	
Herbs (1 m²)†	**0.809*****	0.387***	**-0.539*****	*ns*		**0.717*****	-0.232***	-0.102**	*ns*	
Plant life form (species number per 0,1 ha)										
Phanerophytes	0.201*	0.390***	**-0.511*****	**-0.434*****		0.181***	0.135***	0.121***	*ns*	
Chamaephytes	**0.735*****	0.369***	-0.279***	0.250**		**0.608*****	-0.321***	*ns*	0.187***	
Hemicryptophytes	**0.746*****	**0.540*****	**-0.647*****	-0.256**		**0.664*****	-0.138***	*ns*	0.106**	
Cryptophytes	-0.231**	*ns*	-0.251**	*ns*		-0.252***	*ns*	0.082*	0.124***	
Therophytes	**0.538*****	0.222**	-0.345***	-0.204*		**0.454*****	-0.087*	-0.092**	*ns*	
Alien species number per 0.1 ha	**0.531*****	0.340***	**-0.435*****	-0.332***		**0.456*****	*ns*	*ns*	*ns*	
Rare, protected and endemic species number per 0.1 ha	**0.454*****	0.241**	-0.273***	*ns*		**0.434*****	-0.205***	*ns*	0.084*	
DCA axes	1	2	3	4	Total inertia	1	2	3	4	Total inertia
Eigenvalues	0.779	0.380	0.264	0.184	9.706	0.937	0.895	0.770	0.733	52.863
Gradient length (in SD units)	5.146	3.700	3.490	2.540		9.782	3.240	-	4.952	

Note: ‡ means shrub cover per 100 m²; † mean herb cover per 1 m².

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Table 3. *Correlation (nonparametric correlation coefficient of Spearman, R_s) between indirect environmental gradients and vegetation variables. Correlation coefficients greater than 0.4 are shown with* ***Bold*** *type; ns = not significant; *P<0.05; **P<0.01; ***P<0.001.*

		Indirect environmental gradients				Cover (%)			Plant life form (species number per 0.1 ha)				
		Elevation (m)	Moisture gradient	Slope inclination (°)	Slope convexity	Trees (0.1 ha)	Shrubs (100 m^2)	Herbs (1 m^2)	Phanerophytes	Chamaephytes	Hemicryptophytes	Cryptophytes	Therophytes
Indirect environmental gradients	Elevation (m)	-	0.383***	-0.182*	ns	**-0.515*** **	-0.343***	**0.593*** **	-0.399***	**0.625*** **	0.324***	-0.185*	0.282***
	Moisture gradient		-	-0.341***	ns	**-0.453*** **	ns	0.375***	ns	0.259**	0.287***	-0.249**	0.352***
	Slope inclination (°)			-	ns	**0.412*** **	ns	**-0.456*** **	-0.190*	-0.348***	-0.399***	0.266**	-0.309***
	Slope convexity				-	ns	ns	-0.184*	-0.214*	ns	-0.193*	ns	ns
Cover (%)	Trees (0.1 ha)					-	ns	**-0.778*** **	-0.203**	**-0.675*** **	**-0.740*** **	ns	**-0.469*** **
	Shrubs (100 m^2)						-	ns	**0.423*** **	ns	0.204*	ns	0.174*
	Herbs (1 m^2)							-	ns	**0.692*** **	**0.734*** **	ns	**0.470*** **
Plant life form (species number per 0.1 ha)	Phanerophytes								-	ns	**0.414*** **	0.170*	0.187*
	Chamaephytes									-	**0.575*** **	-0.170*	0.383***
	Hemicryptophytes										-	ns	**0.562*** **
	Cryptophytes											-	ns
	Therophytes												-

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These results can be explained with the greater number of life forms in the open subalpine communities compared to the forest ones. With the exception of cryptophytes, all other life forms correlate negatively with the slope inclination and the greater part of them do not correlate significantly with the slope topography.

The positive correlation between first DCA axis and most life forms at the two measurement scales can be explained with the more open pattern of the xerophilic forests where greater numbers of species from most life forms are present. They are also more saturated with ruderals which most often belong to the therophyte life form. Richer on species (mainly chamaephytes and hemicryptophytes) are the open subalpine pastures, distributed above the tree line.

Significant positive correlations have been found between the cover of vegetation layers and most life forms (*Table 3*). For example, chamaephytes, therophytes and hemicryptophytes show similar relationship with the vegetation cover. These life forms correlate negatively with closed canopy forests and positively with the open grassy communities which, most often, have high herb coverage. On the other hand, phanerophyte number correlates positively with the shrub layer cover and negatively with the canopy closure. This is explained with the greater share of shrubs in that life form and the greater number of phanerophytes occupying the more open mixed oak forests and pine plantations. Cryptophytes do not show any significant correlation with vegetation layers. The correlation analysis of life forms themselves shows that hemicryptophytes correlate positively with the phanerophytes and chamaephytes as well as hemicryptophytes with therophytes.

Alien and rare plant species

Strong positive correlation between the number of alien species and first DCA axis have been found at the two measurement scales, implying that the xeric habitats, located at low and high elevation, are more prone to alien species invasion (*Table 2*). Concerning the open subalpine communities, this is probably connected to the high species richness as a whole, whereas the more xerophilic forests, being distributed at lower altitude, closer to human settlement, are more highly affected by the negative anthropogenic activities like logging, fires, grazing of livestock, etc.

The open subalpine pastures are occupied by greater number of rare, protected and endemic species per unit area (*Table 2*). This is confirmed by the high positive correlation between the first DCA axis and the number of rare plant species.

Scale

The number and power of the correlations between the DCA axes and the environmental variables increases with the scale extension (*Table 2*). This is most clearly demonstrated for the second and higher DCA axes. Explained variation is much greater at the coarsest scale which is probably due to the greater sample number at the finer scale and the greater data heterogeneity at that level. However, obtained results confirm the first tested hypothesis that the correlation between vegetation composition and environmental gradients increases with the increasing size of the sampling plots.

It is also obvious that the correlations between the first tree environmental gradients and the DCA axes are statistically significant at the two levels of measurement which is confirmation of the second tested hypothesis that environmental gradients correlated most strongly with the vegetation composition should be similar at the different scales.

The correlation between the environmental gradients is stronger at that scale which covers greater area of the studied territory (*Table 2*). This is the database with the 0.1 ha sampling plots which covers totally 15.9 ha, versus the smaller scale database, covering only 834 m^2. This unambiguously supports the third tested hypothesis that with the increasing area covered by the sampling plots, the correlation between environment and vegetation should also increase.

Regression

Cover

The detailed picture of the relationship between vegetation and local indirect environmental gradients on the south slope of Vitosha Mountain is revealed with the multiple regression analysis. Response surfaces of the vegetation layer cover as a function of main environmental gradients are represented on *Figure 3*. The four environmental gradients are denoted with arrows and shortened names (for the full names, see *Figure 1*). Isolines represent the predicted layer cover (%) in the abstract ecological space, designed by the used regression model. Statistical parameters of the models are shown in *Table 4*.

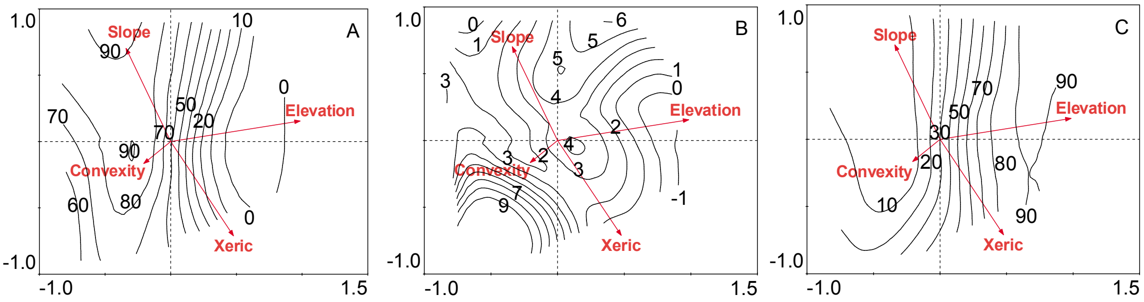

Figure 3. *Regression response surfaces of vegetation layer cover (%) in the context of local indirect environmental gradients. Isolines show predicted cover of: A = tree layer; B = shrub layer; C = herb layer.*

Table 4. *Statistical parameters of the regression models. *P<0.05; ***P<0.001;*

Variable	Distribution	Model	Model significance (*F*)
Mean tree cover per 0.1 ha	Gaussian	GAM	120.55***
Mean shrub cover per 100 m^2	Gaussian	GAM	3.77*
Mean herb cover per 1 m^2	Gaussian	GAM	105.37***

Maximal tree layer cover is predicted for the beech zone, located between 1200-1400 m, which becomes wider towards the more mesic habitats at the same elevation (*Figure 3*). The ecological surface of that variable has Gaussian distribution (*Table 4*). Other forests with high canopy closure occupy the most xeric habitats at about 1300 m. The former case concerns the beech forests from the deep moist ravines at the slope bases

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where the forest canopy closure exceeds 85-90%. The other forests with very high tree layer cover are the coppice beech communities found on flat or convex slopes in the most xeric habitats, where they form very dense sprouting stem pattern and highest canopy closure, reaching over 95% cover. In the xeric habitats, localized at lower altitudes (1200-1000 m), mixed oak forest canopies as well as pine plantation canopies do not reach over 80% cover and continue to fall with the decreasing elevation. On the other hand, with the increase of elevation above 1300 m, tree canopy closure starts to decrease, but this is happening faster in the more xeric places. Above 1500-1600 m on the south slope of Vitosha Mountain, forest vegetation gives way to the subalpine pastures.

Shrub cover correlates negatively with the elevation gradient (*Table 3*). However, there is no clear tendency in the relationship with the moisture gradient. From *Figure 3*, it is evident that shrub cover shows complex response surface, but the statistical test of its form confirmed it to be Gaussian (*Table 4*).

The maximal shrub cover is predicted for the submesic places at about 1200 m as well as for the mesic habitats in the 1400-1600 m elevation belt. The former case is connected with the mixed oak forests and pine plantations at lower altitude, whereas the later one concerns the *Pinus sylvestris* plantations at middle elevation. In the beech forests, shrub cover reaches its minimum.

Herb cover correlates positively with the elevation and habitat moisture, but negatively with the slope inclination. It reaches its maximum in the mesic subalpine habitats, distributed above 1800 m elevation. Herb cover in the forest belt reaches its lowest scores in the beech communities, but increases in the more xeric habitats at middle and lower elevation, where pine plantations and mixed oak forests dominate.

Plant life forms

Plant life form distribution in the context of the local environmental gradients is represented on *Figure 4.* The isolines depict the predicted species number by life form per 0.1 ha. *Table 5* shows the statistical significance of used models as well as the distribution of ecological surfaces and plant life form spectrum. Phanerophytes reach their maximum in the xeric and subxeric habitats at about or below 1000 m (*Figure 4A*). The beech forests and plant communities above the tree line have the lowest number of phanerophytes. With the elevation increase, phanerophyte number decreases much faster in the mesic habitats than in the xeric ones. Chamaephytes show the opposite trend (*Figure 4B*). They have highest species richness in the submesophilic and mesophilic open subalpine communities (mainly species from Ericaceae) and with the elevation decrease, their richness also decreases. Below 1500 m elevation, they are almost lacking.

Hemicryptophytes have complex (bimodal) distribution. The first mode is located in the most xeric habitats of the subalpine belt at elevations between 1800-2000 m. The second one is centered in the xeric places at elevation around and less than 1000 m, where mixed oak forests and mixed pine plantations dominate. A third, slighter mode, appears in the most mesic habitats at about 1800-2000 m. Hemicryptophytes show highly abrupt decrease with the approaching of mesic and submesic beech forests, developed at about 1300-1400 m elevation (*Figure 4C*). With the ascending above 2000 m their number also falls. Cryptophytes have their mode located in the mesophytic beech forests distributed on steeper slopes along moist ravines at about 1200 m (*Figure*

4D). With the ascending towards higher elevation (above 1700-1800), in the most xeric habitats, they disappear. Therophytes have similar distribution to chamaephytes, showing bimodal response surface. The first mode appears at about 1600-1700 m in the most xeric subalpine places, and the second one is located at about and lower than 1000 m elevation, in the subxeric habitats (*Figure 4E*). Their number is lowest in the mesophilic and submesophilic beech forests, distributed at about 1200-1300 m elevation.

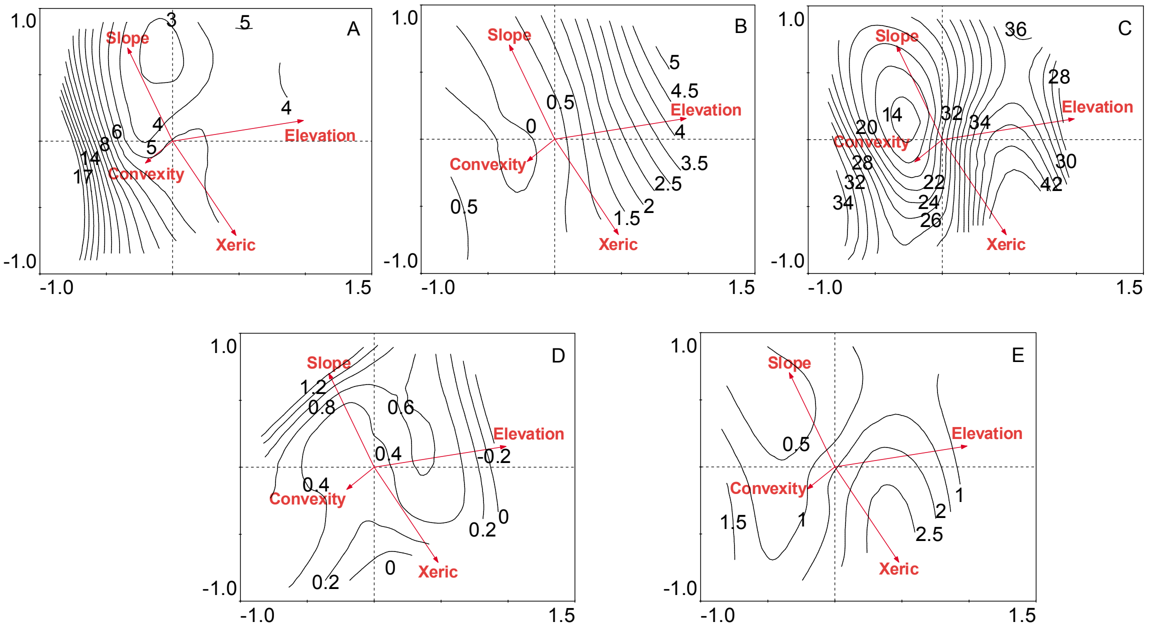

Figure 4. *Regression response surfaces of the plant life forms in the context of local indirect environmental gradients. Isolines show predicted species number per 0.1 ha by plant life form as follows: A = Phanerophytes; B = Chamaephytes; C = Hemicryptophytes; D = Cryptophytes; E = Therophytes.*

Table 5. *Statistical parameters of the regression models. ***P<0.001.*

Variable	Distribution	Model	Model significance	Plant life form spectrum
Phanerophyte species number per 0.1ha	Complex	GAM	29.54***	14.14
Chamaephyte species number per 0.1ha	Gaussian	GAM	61.44***	1.39
Hemicryptophyte species number per 0.1ha	Complex	GAM	17.13***	72.31
Cryptophyte species number per 0.1ha	Gaussian	GAM	10.99***	3.18
Therophyte species number per 0.1ha	Gaussian	GAM	10.41***	8.96

Stand structure

Regression surfaces of stem distribution by diameter classes in the ecological space are represented on *Figure 5. Table 6* can be referred for the model significance and regression surface distributions.

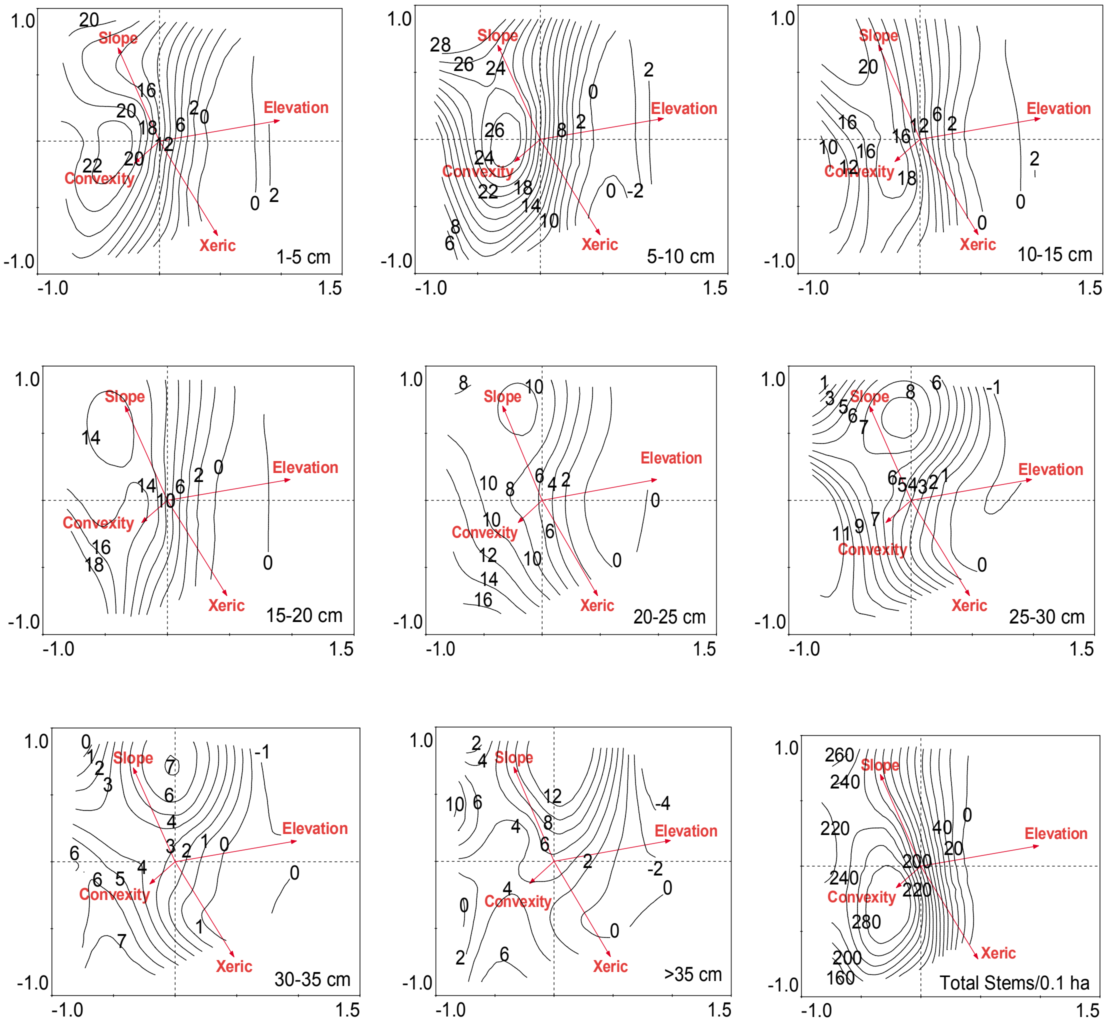

Figure 5. *Regression response surfaces of tree and shrub stem diameter (greater than 1 cm at breast height ≈ 130 cm) in the context of local indirect environmental gradients. Isolines show predicted stem percentage in the stand per 0.1 ha. Stem diameter classes are show in the lower right corner of the graphs. Lower right-corner graph shows total stem number per 0.1 ha.*

All diameter classes have asymmetric or complex distribution. The greatest stem number from 1-5 cm diameter class can be found in the submesic and subxeric habitats at about 1200 m, but also below 1000 m, as well as another slighter mode in the most mesophilic beech forests at lower elevation. The most well expressed mode can be connected to the coppice beech forests at middle elevation, also the mixed oak forests and pine plantations distributed at lower elevation, where the shrub layer is well developed with high species richness. Stem diameter class 5-10 cm shows its mode in the mesic (about and below 1100 m) and submesic habitats at about 1300 m elevation.

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In the former case, it refers to the most mesic ravines along permanent or temporal springs in the mesophilic beech forests. The second mode is related to the coppice beech communities, distributed in the xeric places at middle elevation.

Table 6. *Statistical parameters of the regression models. ***P<0.001.*

Variable	**Distribution**	**Model**	**Model significance *(F)***
% stems 1-5 cm per 0.1ha	Asymmetric	GAM	34.84***
% stems 5-10 cm per 0.1ha	Asymmetric	GAM	57.21***
% stems 10-15 cm per 0.1ha	Complex	GAM	55.66***
% stems 15-20 cm per 0.1ha	Asymmetric	GAM	41.20***
% stems 20-25 cm per 0.1ha	Asymmetric	GAM	38.63***
% stems 25-30 cm per 0.1ha	Asymmetric	GAM	19.45***
% stems 30-35 cm per 0.1ha	Asymmetric	GAM	19.17***
% stems >35 cm per 0.1ha	Asymmetric	GAM	9.43***
Total stems per 0.1 ha	Asymmetric	GAM	44.29***

Each of the middle diameter classes has its specific manner of distribution in the abstract ecological space. For example, stem class 10-15 cm show complex surface form with several modes, centered in habitats with different conditions. These are the most mesic places at about 1200 m and the subxeric habitats at about 1300-1400 m elevation. Third mode can be found in the xeric habitats at about and below 1000 m. The most numerous stems of 15-20 cm class appear in the subxeric and xeric habitats at lower elevation (about and below 1000 m), where mixed oak forests and pine plantations dominate. The stems with diameters 20-25 cm prevail in the same habitats, but with the elevation increase, their numbers slowly decrease. This is happening most slowly in the mesic places and in the mesic beech forests their number almost remains constant in a very broad elevation belt (*Figure 5*).

Stems from thickest classes (25-35 cm) also show peculiar manner of distribution. For example, stems with diameters 25-30 cm have highest density in the submesic habitats at about 1400 m. Second mode appears in the most xeric places at about and below 1000 m. The next group (30-35 cm) has more obscure distribution. As a whole, the trend from previous stem class is repeated, but the stem density here is greatest at the lowest places (about 1000 m) at various habitat moisture conditions. Another slight mode can be found in the most mesic places at about 1400-1500 m. The distribution of the thickest stems (>35 cm) is slightly different. Here can be outlined a well formed mode, centered in the mesic places at about 1400-1500 m elevation. It is obvious that thickest stem distribution is similar to the thinnest stem distribution, which is probably an indication of the “climax” state of these forests and the beginning of regeneration processes. These forests still remain preserved as isolated spots of old beech forests, which should be given special attention in the conservation policies of the management authorities.

The analysis of the total stem distribution shows that the forests with maximum stem number are distributed in the subxeric places on convex slopes at about 1000-1100 m elevation. Second maximum appears in the mesic habitats on steep slopes at elevation about and lower than 1000 m. The first case refers to the coppice beech communities in the xeric places. The second one concerns the beech forests localized at the mesic slope bases along moist ravines. With the increasing elevation stem numbers abruptly falls.

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The same tendency applies when moving away from the centers of maximal stem number towards the low elevation, but the decrease here is much smoother.

Discussion

Gradient relationships

Numerous studies confirm the importance of habitat moisture condition (Whittaker 1960; Whittaker 1965; Whittaker & Niering 1975; Peet & Loucks 1977; Peet 1981; Allen et al. 1991), elevation (Whittaker 1965; Peet 1978; Peet 1981; Allen et al. 1991), geographic gradient, influencing the climate of territories (Whittaker 1960), potential solar radiation (Allen et al. 1991), soil chemical composition (Peet & Christensen 1978; Christensen & Peet 1984; Peet & Christensen 1988), soil physical structure (Peet & Loucks 1977), the bedrock type (Whittaker 1960), successional gradient (Peet & Loucks 1977), and geographic latitudinal position (Peet 1978) for the vegetation structure and composition. The importance of habitat moisture and elevation is confirmed in this study too. Since the scale of our study is rather local, i.e. do not embraces territories with different climate regime, we cannot support or reject the importance of climatic gradient. The same is true for the bedrock type or soil composition because we did not used these variables in the current investigation.

The compensating effect against the moisture gradient, where the mesophilic vegetation types, ascending at greater elevation, are frequently found at more xeric conditions and vice versa, has been recognized Whittaker (1960). However, slope topography and elevation are not direct environmental gradient, but rather, a surrogate for complex interlocking environmental variables, which determine plant distribution (Whittaker 1956, 1960; Austin et al.1984; Austin 1985). If a particular biological meaning of the explained data variation is demanded, more appropriate would be a greater number of direct environmental gradients to be used in the gradient analyses (Austin 1985; Austin 2005). Unfortunately, measurement of direct environmental gradient is expensive and laborious task. On the other hand, indirect variables are easy, fast and cheap to be obtained.

On a regional scale, other studies (Bergmeier & Dimopoulos 2001; Tsiripidis *et al.* 2007a), similar to the current one, which we can juxtapose to ours, have been done. The DCA ordination of Bergmeier & Dimopoulos (2001) describes the moisture gradient effect on the mesic and relatively xeric habitats in Greece, which also applies to the subalpine and semi mountainous habitats. Significant correlations between the two DCA axes and the elevation have been found and interpreted as the gradients of temperature and rainfall. The significant correlation between the elevation and xeric habitat condition in our study can be explained with the presence of rain shadow on the south slope of Vitosha Mountain. Thus, this phenomenon leads to more xeric conditions across the subalpine habitats at about 1500-1600 m during the summer, especially over the south and southwest slopes, which inevitably influences vegetation structure and composition. Despite the scale difference in the two studies, their results confirm the primary importance of the elevation and habitat moisture for the forest and subalpine vegetation.

The environmental gradients are recognized as primary determinants for beech forest differentiation in Northern Greece (Tsiripidis *et al.* 2007). The elevation is qualified as the most important environmental gradient as well as the soil mineral content (Tsiripidis *et al.* 2007). With the secondary importance was the substrate type, which was in a

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direct dependence of the elevation and soil nutrient composition. It predetermines the physical texture and the chemical characteristics (calcium concentration and pH) of the soil. These speculations cannot be confirmed or rejected in our study because such soil parameters were not analyzed here. The fine differentiation within the different ecological groups were ascribed to the microclimate and soil quality of the habitat (Tsiripidis *et al.* 2007), dependent on the exposition, relief and slope inclination, which were also confirmed in the current study.

Despite the primary importance of the complex environmental gradients, profound influences also have the natural disturbances such as forest fires (Whittaker 1960), landslides, windthrows, biotic disturbances etc. (White 1979), as well as the anthropogenic disturbance gradients like livestock grazing (Naveh & Whittaker 1979), logging and others, which can influence to greater extent the vegetation composition and structure. However, frequent fires with low intensity in the past could only decrease stand density without causing substantial changes in their characteristics (Whittaker 1960).

Vegetation cover and plant life forms

Studying vegetation of Santa Catalina Mountains, Whittaker and Niering (1975) claimed for the presence of some trends, which we also try to verify in the current study. According to the authors, there was no correlation between the species number and productivity within the tree layer. This statement is clearly supported by our results, since the beech forests, where the canopy closure is highest, had lowest species richness, often presented only by the common beech itself. The second claim was, that the productivity and the diversity of perennial herbs should have bimodal distribution and relaxed competition. We obtained Gaussian distribution of herb cover, but our regressions incorporate four indirect gradients – not only one, as was the case in Whittaker and Niering (1975). However, we found strong correlation between the herb cover and diversity (*Table 3*). It is also stated that therophytes are concentrated in the open xeric habitats with increasing diversity and productivity from forests towards the open communities, which is supported by our results too (*Figure 3; Figure 4*).

Positive relationship between habitat moisture and vegetation cover (Whittaker 1956), dry/live leaf mass (Whittaker 1966), and productivity (Whittaker and Niering 1975) of tree layer have been found in other studies. Decrease of productivity and aboveground biomass of the mature “climax” forests with the advance towards the xeric places and higher elevation has also been found (Whittaker 1966), as well as positive correlation between the xeric gradient and shrub cover (Whittaker 1956), and productivity (Whittaker 1966; Whittaker & Niering 1975). According to Whittaker (1956), along the moisture gradient shrub cover increases conversely to herb cover, i.e. they show negative relationship. Negative relationship has also been found between tree and shrub cover in forest communities (Whittaker, 1956; Whittaker and Woodwell, 1969). We have found similar trend along the elevation gradient. Herb cover increased and the shrub cover had complex relationship with the elevation (*Figure 3; Table 3*).

Despite that the herb productivity distribution along moisture gradient had bimodal form (Whittaker and Niering 1975) – with two maxima, first one in the subxeric habitats, and second one in the mesic ones – the results confirm its positive relationship with the more xeric habitats. Whittaker (1966) reported similar results, where productivity of herb layer was maximal in the marginal (extreme) moisture gradients parts, but the herb biomass increased along the elevation and reached its maximum in the mountainous fir forests.

Shrub productivity and diversity were weakly correlated when all species were taken together, not separated by plant life forms. Within the shrub layer, the different life forms had different centers of productivity and diversity (Whittaker and Niering 1975). However, we obtained strong positive correlation between shrub cover and diversity of phanerophytes, thereby rejecting the first hypothesis. Probable reason for this discrepancy can be found in the fact that we use as a surrogate for productivity the vegetation layer cover, which for the shrubs is higher in the poorer and dryer places as well as in the open subalpine habitats, where the species diversity is higher too. The second claim is supported by our results because it is obvious that phanerophytes and chamaephytes have different diversity centers (*Figure 4*).

In the primary vegetation layer there was no correlation between the diversity and productivity in habitats where the corresponding life form dominates (Whittaker and Niering 1975). This hypothesis is entirely supported since we have not found significant correlations between the cover of primary vegetation layer with species diversity of the dominant life form in neither of the analyzed plant communities.

In the temperate climate zone, the tree layer as a whole is poorer on species than the shrub or herb layers. The herb and shrub layers are at average poorer on species in highly closed forests than in more open forests and the herb communities (Whittaker and Niering 1975). Both statements are confirmed by us. According to Whittaker (1953), structural-functional character of the vegetation is determined by the relationship between plant life forms and their partial or complete turnover along the environmental gradient change. We have found obvious trend in the species turnover of some life forms and absence of tendency in others. For example, *Figure 4* and *Table 3* show that phanerophyte and chamaephyte distribution changes considerably with elevation change. Decreasing of phanerophytes and increasing of chamaephytes and hemicryptophytes with elevation increase have been found by Whittaker (1960), and Whittaker and Niering (1965) too. Chamaephytes seem to be influenced by the moisture gradient too, since species number in submesic subalpine habitats was higher. The relative continuity in the change of broadleaf forests (mainly common beech forests, *Fagus sylvatica*) into coniferous ones (mainly pine plantations, *Pinus sylvestris* and *Pinus nigra*), likewise the situation on the south slope of Vitosha Mountain, might not be always evident. Abrupt transition between community types can be seen most often when one or few tree species dominate in the forest vegetation (Whittaker 1956). In our case, abrupt change can only be observed in the transition between forests and open grasslands in the forest belt or between forests and subalpine meadows and pastures at higher elevation. Most probable reason for this phenomenon are the natural fires, human caused disturbances (fires, livestock grazing, mowing, agriculture) or abrupt change in environmental conditions of the habitat (Whittaker 1956).

Hemicryptophytes have not showed clear tendency, but as a whole, they were more numerous in the open subxeric subalpine habitats, as well as at lower elevation in variable moisture conditions. Probable limiting factor for their distribution is the dominant tree species competition since their numbers are significantly lower in the highly closed beech forests. According to Whittaker (1956), the contrast between different plant life form dominance, for example, between pastures and forests, can be explained in terms of the environmental conditions, giving advantage of one life form, which in its turn prevents the establishment of individuals from other life forms. Due to this phenomenon, both dominant life forms can sometimes replace each other abruptly instead of forming continuum along the environmental gradients.

Cryptophytes are most numerous in the beech forests and with the increasing elevation and xerophytisation of habitats they become less important. This can be explained with the ephemeral life cycle of most cryptophytes, which they complete before the emergence of tree leafs in the spring.

Therophytes reach its maximum in the most elevated and most xeric habitats, as well as in the lowland, highly influenced by human activity, territories. It is not surprising, given their ability to survive during extremely unfavorable periods, resulting from natural or anthropogenic factors. Positive relationship between therophyte richness and elevation has also been found by Whittaker (1960), which was explained with the relatively drier climate in the studied territory. However, negative relationships were also reported (Whittaker & Niering 1968). In our case, we confirm the results of the former study, because, as already mentioned, during the summer months, rain “shadow” on the south and southwest slopes causes extreme drought, which inevitably induces species compositional changes in favor of therophyte number and decreasing of phanerophyte and cryptophyte numbers.

Scale

Studying understory vegetation of North Carolina at different scales, Reed et al. (1993) attributed correlation decrease with the decreasing scale of measurement to the interaction between the plant individuals, which mitigated the influence of environment at the smaller scales, where plant individuals compete directly for the same resources. This is mainly applicable to the open herb communities, but unfeasible to forest vegetation, where the competition between the individuals cannot be easily proved. The weaker correlation between the environment and vegetation at the smaller scales is due probably to unavoidable error in sampling. In other words, in the smaller sampling plots are found smaller number of plants, influenced to greater extent by chance (Reed et al. 1993). This contributes to the greater heterogeneity in the database and weaker correlations. For example, the total inertia in the DCA analysis at the 1 m^2 scale is much greater, hence the explained variation in the data is lesser (*Table 1*).

Numerous previous studies assumed that the environmental gradients, correlated most strongly with species composition would have to be similar at the different scales of measurement (Reed et al. 1993). We have confirmed this hypothesis, but this, however, does not mean that the vegetation pattern at the smaller scale is a miniature version of that on the coarser scales. Consequently, extrapolation between the different scales should be avoided at any cost (Palmer, 1990).

In contrast to Reed et al. (1993), we confirmed the hypothesis that with the increase of sampling area, covered by the sampling plots, the correlation between the environment and vegetation variables also increases. According to the authors, the reason for the weaker correlation found in their study was the data heterogeneity and the large number of interacting variables when sampling extensive territories. Like Wiser et al. (1996), however, we maintain that, despite the great variation in vegetation composition, the continuity of indirect gradient importance is confirmed at both scales as well as across different scales.

In conclusion, it can be summarized, that the predictive power of the indirect gradients is valid for the habitat scale (0.1 ha), as well as at the microhabitat scale (1m^2). Nevertheless, for future environmental gradient-vegetation studies it should be kept in mind that the use of different sampling scales is of crucial importance for the correct understanding of the vegetation pattern and processes (Reed et al. 1993).

Stand structure

Positive relationship between xeric gradient and the total stem number have been previously reported (Whittaker 1956), where along the xeric environmental gradient stand height decreased. According to the author, the increasing stem number expresses the stand low stature and higher density, where the numerous thin stems in the xeric habitats belong to species with low habitus. However, in our case, it refers to the management practices of the beech forests. The higher stem number is the outcome from the coppice nature of the beech forests in these habitats, rather than high species number. Detected tendency in Whittaker (1956), at some extent, applies to the oak forests and pine plantations in the area, where, indeed, the greater stem number is due to higher species richness.

Three general types of stand development have been suggested (Peet 1981). They were considered as three referent points in the cenocline continuum varying accordingly to the elevation and habitat moisture, which are applicable to the analyzed by us stands. In the favorable sites at middle elevation, tree species establish immediately after the disturbance event. This is followed by rapid growth, canopy closure and competitive displacement of the undergrowth. This results in bell-shaped diameter distributional curves. Meanwhile, the diversity and productivity decrease, while the biomass remains constant (Peet 1981). After decades or centuries of stagnation, following large tree collapse, the forest canopy eventually opens, allowing regeneration to be restarted. During this period, the biomass, diversity and productivity undergo dramatic changes, responding to the forest structural reorganization. This dynamic model is applicable to the *Fagus-Hepatica, Fagus-Galium, Fagus-Luzula* and *Fagus-Festuca* type communities in the studied area. The regeneration in these forests is periodical and expresses the variability of seedling germination in the favorable for growth places. The competition between tree species in these places is relatively unimportant and the diameter distribution shows irregular peaks, resulting from periodic regeneration.

At high or middle elevation, in the xeric and poor habitats, seedling establishment after disturbances is unusually slow (Peet 1981). These habitats on the south slope of Vitosha are dominated by *Pinus-Fragaria, Fagus-Corylus-Brachypodium, Quercus-Cornus* and *Pinus-Crataegus* communities. The forests in such places slowly reach the biomass, diversity and productivity from the period before the disturbance, while the regeneration rate is constant. It is typical for the beech forests in the area, where the regeneration is exceptionally of sprouting origin and seedling regeneration is almost lacking. Similar situation is found in the pine plantations of *Pinus sylvestris* and *Pinus nigra* in the xeric habitats at middle and low elevation, where seedling regeneration is also lacking. The common beech there can be found in multiply separated sprouting spots, where canopy openings allow undergrowth to occur.

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COMPARISON OF DIFFERENT SPACE INDEXING METHODS FOR ECOLOGICAL EVALUATION OF URBAN OPEN SPACES

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Abstract. Nowadays we meet many different evaluation methods regarding the ecological performance of green surfaces and parks. All these methods are extremely valuable in determining how well a green surface performs from ecological aspect and to what extent the environment were damaged if these sites would be built or would be developed any other way causing reduction of green surfaces. The goal of the article is to clarify the differences between two evaluation methods (GSI – Green Space Intensity, BARC – Biological Activity Rate Calculation) suitable for urban green infrastructure analysis and to see if any significant difference can be observed evaluating the same site by these methods. Our research sites are in Budapest and their sizes vary between 2,5-8 acres. The most important aspects of site analysis are the following: size and boundaries of the park, existence or lack of water features, the characteristics of their surfaces and the complexity of vegetation. We summarize the data of the site analysis in tables, make a summarizing diagram for visual representation and draw conclusions from the results. As a final step, we evaluate how these two evaluation systems relate to urban open space developments.

Keywords: *remote sensing, urban vegetation, vegetation index, biological activity, green surface*

Introduction

Nowadays we meet many different evaluation methods regarding the ecological performance of green surfaces and parks. Some of them measure the ecological or biological assessment of a park by using questionnaires. The answers are converted into numbers referring to the ecological value of the measured site. There are also methods which evaluate infrared photo and conclude the biological performance of the site from certain data gained from the photo.

All these methods are extremely valuable in determining how well a park or green surface performs from an ecological aspect and to what extent the environment were damaged if these site would be built or would be developed any other way causing reduction of green surfaces and loss of recreational function of the site.

In our time we have more and more opportunities to evaluate any specific site by different methods. Sometimes even professionals get lost among the many different methods. Are all of them usable for the same purposes, or should we prefer certain methods in certain cases? Learning more about the different evaluation methods and comparing them will help in getting a clearer picture.

The goal of the article is to clarify the differences between two evaluation methods [GSI – Green Space Intensity *(*according to Jombach, 2012: "… the GSI database contains values from 0 to 100 percent, representing a special mixture of green coverage and quality, called green space intensity.") and BARC – Biological Activity Rate Calculation (ÖTM, 9/2007.)] suitable for urban green infrastructure analysis to see if any significant variance can be observed evaluating the same site by these methods. We

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used the data emerged from Normalized Difference Vegetation Index (NDVI) evaluation method to determine GSI value of each sample plot.

Review of Literature

While there is only Hungarian literature about the description and calculation method of Biological Activity (BA), the satellite-based green surface mapping processes – NDVI calculations as well – have been being used for decades in international practice. One of the very first comment and description of this method is in a periodical (1973 March – Sept.) report of Remote Sensing Center at Texas A&M University. The documentation discusses the calculation procedure in detail, which was called Vegetation Index (VI) calculation. The scientific name NDVI (Normalized Vegetation Index) came in use only few years later (Rouse et al., 1973).

The evaluation method has become more and more popular in vegetation monitoring procedures since the middle of 1990s. In 1994 Myneni and Williams investigated the influence of pixel heterogeneity, background, atmospheric and bidirectional effects on the relationship between fraction of photosynthetically active radiation absorbed by the photosynthesizing tissue in a canopy (FAPAR) and Normalized Difference Vegetation Index (NDVI) by using a three-dimensional model of radiation transfer (Myneni, 1994).

Three years later, Carlson and Riziley used a simple rudiative transfer model with vegetation, soil, and atmospheric components in their study to illustrate how the NDVl, Leaf Area Index (LAI), and fractional vegetation cover are dependent on each other (Carlson, 1997).

In 2005 a study was made, which reviewed the rapidly increasing use of the NDVI in recent ecological studies and outlined its possible key role in latter research of environmental change in an ecosystem context (Pettorelli et al., 2005).

In 2012 a detailed research was made in the United States, which dealt with the exploration of the applicability of Biophysical Composition Index (BCI) in various remotely sensed images at different spatial resolutions. The results indicate that, BCI has a closer relationship with impervious surface abundance than those of Normalized Difference Vegetation Index (NDVI), Normalized Difference Built-up Index (NDBI) and Normalized Difference Impervious Surface Index (NDISI). The study compared several recent evaluation methods in same aspect, which constitutes the value of it (Deng, 2012).

In Hungary, evaluation methods based on NDVI came into use in the last decade. As a result a supporting study was made in 2006 to create the Development Concept and Program of Green Space System in Budapest (Pro Verde!). According to the workgroup, to justify the recommendations of the program a complex green surface analysis is necessary, which shows processes and trends related to green surfaces in the last 15 years by objective Geographical Information System (GIS) methods. They made the analysis by comparing the changes in NDVI indices on several land-use units in Budapest between 1990 and 2005 (Gábor et al., 2006). It is important to be mentioned, that the study uses the expression 'BA index' for NDVI values.

In 2007 a regulation regarding the calculation method of BA on different surfaces has been introduced by the Hungarian Ministry of Governance and Urban Planning. The main goal of this regulation is to set up a control mechanism to control the modification of urban development plans regarding new developments. From that time on the domestic practice uses 'BA rate' expression in the legal regulation (ÖTM, 9/2007.).

In 2008 a detailed study was made regarding the analysis of green surface-covering in Budapest as a result of the research started in 2006. The base of the research was also the evaluation of satellite photos taken in 1990 and 2005 (Ongjerth, 2008).

In the last few years, the opportunities offered by NDVI evaluation method are more and more popular in the domestic scientific life. The introduction of BA evaluation method into the legal regulation offers a possibility to control to certain degree the urban development processes from ecological aspect.

Materials and Methods

The biological activity is the conditioning effect of vegetation-covered surfaces on the environment through different physical, physiological and biological processes. The Biological Activity Rate (BAR) represents the level of biological intensity (Jámbor-Szilágyi, 2006).

According to the BA regulation aforementioned, every kind of surface could be characterized by a BAR, which has to be calculated from the area's size and a pre-determined BA rate/acre relating to the features of the area (ÖTM, 9/2007.). The regulation has two tables in its annex. The first includes the determined BA rates of several land-use units and the second shows the values to be used for BA rate calculations on different kind of surfaces *(Table 1.)*. Each biologically active surface has significant effect on its environment. Closed and multi-level vegetation has significantly higher effect on its close environment than a paved surface, therefore it also has higher BA rate. From this point of view, this formula can be used well in valuating any kind of surface in ecological and/or environmental aspects.

Table 1. *Pre-determined Biological Activity Rates of several surfaces (2. annex in ÖTM, 9/2007)*

1.	**(A)** **Surface-quality**	**(B)** **index BAR/acre**
2.	Built-up area, or impervious pavement	0
3.	Surface without any kind of pavement or with pervious pavement	1
4.	Road with allée on one side (trees with large canopies)	1,5
5.	Road with allée on both sides (trees with large canopies)	3
6.	Motorway, highway and main road with green stripe wider than 1/3 of road width	1,2
7.	Secondary roads, local main road, service road, bicycle or pedestrian streets and railway line with a green stripe wider than 1/3 of road width	1,4
8.	Groves, bushy areas	6
9.	Protective tree-plantation less than 20 m wide	6
10.	**Green surface on the ground**	
11.	Green surface with one-level vegetation	5
12.	Green surface with two-level vegetation (grass and 40 bushes/150 m^2, or grass and 1 tree with large canopy/150 m^2)	6
13.	Green surface with three-level vegetation (grass and 40 bushes/150 m^2 and 1 tree with large canopy/150 m^2)	7

14.	**Green roof on built structure**	
15.	Green roof with one-level vegetation, extensive green roof	2
16.	Green roof with one-level vegetation, semi-intensive green roof	2,5
17.	Green roof with two-level vegetation (grass and 40 bushes/150 m^2, or grass and 1 tree with large canopy/150 m^2), intensive green roof	3
18.	Green roof with three-level vegetation (grass and 40 bushes/150 m^2 and 1 tree with large canopy/150 m^2), intensive green roof	4
19.	Plough land	3,2
20.	Grassland, pasture	6
21.	Orchard, vineyard, garden	5
22.	Forest	9
23.	Reed surface	8
24.	Water surface	6
25.	Open pit, quarry, regularly disturbed area, waste deposit	0,2

The NDVI is a simple numerical indicator, which has been being used by natural resource researches, forestry and agricultural for decades (Jombach, 2012). Its value is determined by the quotient of the difference and the amount of radiation intensity reflected by plants in Near-Infrared (NIR) ($\lambda \sim 0{,}8$ μm) and Visible Red (VIR) ($\lambda \sim 0{,}6$ μm) regions (Carlson, 1997).

$$NDVI = (NIR - VIR) / (NIR + VIR) \qquad \text{(Eq.1)}$$

Chlorophyll in plants is responsible for the light absorption and reflection in the visible and near-infrared regions (Eredics, 2007). Therefore the quantity and quality of chlorophyll production determines the Normalized Difference Vegetation Index, whose value is largely influenced by the healthiness of the vegetation and by the size of tree canopy. Therefore it is a suitable indicator for evaluating the physical condition of the vegetation in a certain area. The rate of the chlorophyll coverage can be estimated on satellite photos which by and large matches the vegetation (green) coverage of the area, thus irrespective of its thickness (Ongjerth, 2008).

NDVI takes on values between -1.0 and +1.0. The vegetation is very poor near the 0.0 values, between -1.0 and 0.0 there is no plant life (only impervious surfaces: buildings, pavement or naked surface). Above 0.5 the vegetation is perfect (complex, prolific and rich) (Bakay, 2012).

These numbers are being utilized and processed by the Green Space Intensity (GSI) method. This method was born to could the existence and quality of regional and urban green spaces be revealed simply. It is based on the numbers of NDVI. The GSI percentages could be directly got from the NDVI indices by multiplying them by 200. Every negative value has 0 % GSI and every value between 0,5 and 1 get 100 % GSI (Jombach, 2012). The distribution of the intermediate GSI percentages is demonstrated by the *Table 2*.

An area's GSI is determined by averaging the GSI percentages of each pixel covering its whole surface.

Table 2. *The correlation between GSI percentages and NDVI indices*

NDVI	GSI (%)
(-1) - 0	0
0 - 0,1	0,01 - 19,99
0,1 - 0,2	20 - 39,99
0,2 - 0,3	40 - 59,99
0,3 - 0,4	60 - 79,99
0,4 - 0,5	80 - 99,99
0,5 - 1	100

We also used these two evaluation methods to quantify the urban ecological significance of several areas in Budapest in this study. As mentioned before, both evaluation methods provide good opportunity to evaluate certain sites in ecological aspect, but their evaluation procedures are considerably different.

The GSI valuation is based on the physical features of the vegetation in contrast with the biological activity valuation, which can be determined by site analysis and by grouping the different surfaces into categories. We made a detailed analysis in order to compare the GSI and BA evaluation methods of eight sample plots with different characteristics. The main aims of this study are to analyze the applicability of these methods in urban ecological aspects, to reveal their strengths and weaknesses by comparing them and to determine their potential role in recent urban planning processes.

We chose eight urban parks as research sites. Significant attention was paid on their surface characteristics, so that the differences and similarities between GSI and BA evaluations could be analyzed and determined more easily. We could use satellite photos taken by Landsat 8 satellite in 2013 of the research areas to determine their NDVI and GSI value. Each pixel of the photos covers 30 x 30 m area. We made site analysis on each site and categorized the different surfaces - according to the second table in the official regulation of BA calculation. We had vector-graphical and pixel-based site plans as well as geodesic surveys about each area. Besides, we checked all the site plans in case of all areas and if it was necessary, we actualized and refined the data by complemented the survey plans manually during site visits in July 2014.

The GSI maps we used during the research are fitted onto vector graphic surveys. The plot boundaries of each area are also represented on these vector graphic maps and they could be clearly observed on the *Fig. 2.* as well. We made the BA analyzes inside these boundaries and created the site maps by using the same vector graphic system.

Although the satellite photos used for NDVI analysis were taken in spring-summer 2013, there was no significant change on the areas which could affect the success of the analysis. Therefore we consider the influence effect of the occurring deviations negligible in point of this analysis. Due to continental climate in Hungary, the vegetation has the most active period in spring and summertime. Therefore the analyzed satellite photos were taken in these seasons.

Sample plots in Budapest

All of the researched areas are in Budapest, the capital city of Hungary. Their sizes vary from 2-3 to 6-8 acres. Four of the sites (Millenáris Park, Gellért Hill water reservoir, Germanus Gyula Park and Lake Feneketlen) are located in Buda, the other four (Erzsébet Square, Golgota Square, Haller Park and Szent István Park) are on the Pest side of the Danube river *(Fig. 1.)*.

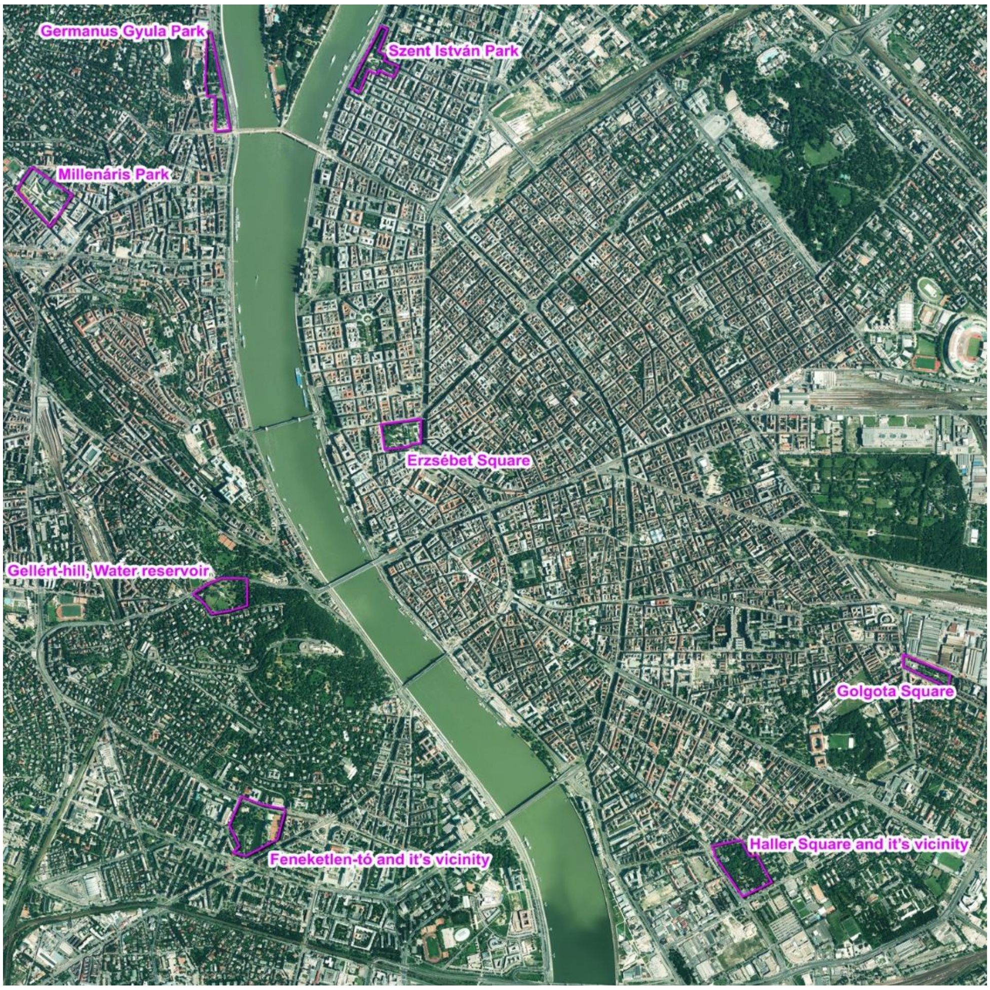

Figure 1. *Location of analyzed and reference parks on the orthophoto of Budapest (2010)*

The most important aspects of site analysis are the following: size and boundaries of the park, existence or lack of water features, the characteristics of their surfaces and the complexity of vegetation. All kind of surfaces had to be categorized according to the 2nd table in the appendix of the decree about the biological activity calculation (ÖTM, 9/2007.). The values and rates of different ecological evaluation methods referring to each plot are summarized in tables.

According to the regulation of BA calculation method, beside the built-in surfaces two kinds of pavement quality was differentiated: the solid pavements with waterproof surface (e.g. asphalt, rubber) having 0 BAR/acre and the pervious pavements with pervious and diffuse surface (e.g. rubble pavement, sand) having 1 BAR/acre. The green surfaces which are directly connected to the ground could be divided into several categories depending on the structure of their vegetation. The one-level vegetation has 5, the two-level has 6 and the three-level vegetation which consists of trees, shrubs and grass has 7 BAR/acre. There is one more category for groves having the same BAR/acre value (6) as the two-level vegetation (ÖTM, 9/2007.).

In case of green roofs, the following BAR/acre values are determined depending on the number of levels the vegetation has: one-level has 2,5, two-level has 3 and the three-level vegetation on green roofs has 4 BAR/acre (ÖTM, 9/2007.).

Although water surfaces are disregarded by NDVI, they obviously have positive effect on the environment. Therefore they are being considered with significant 6 BAR/acre in the biological activity calculations (ÖTM, 9/2007.).

By using the data got from the vector graphic maps, we created tables for each site which include the sizes of the surveyed surfaces. We calculated the BA rates of each surface and determined the Total Biological Activity Rate (TBAR) of each sample area, by summarizing the BA rate of each surface. We determined the Total Area (TA) of each sample plot by measuring the regions inside their boundaries. The tables are also representing the area which was got by summarizing the pixels of NDVI maps on each plot. In addition we represented the Green Space Index (GSIN) which shows the percentage of the green surfaces related to the TA, and the GSI.

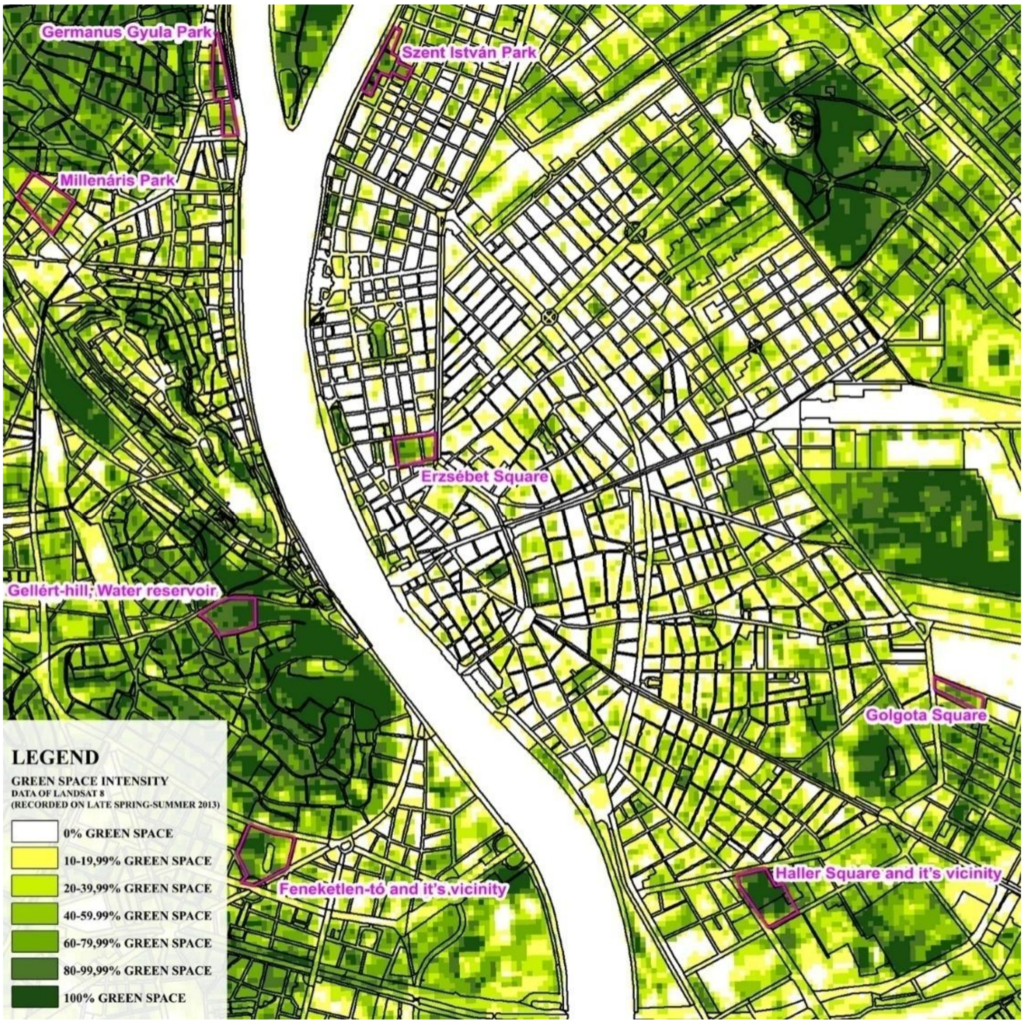

Figure 2. *Intensity of green surface in Budapest (late spring-summer 2013)*

Erzsébet Square and Park

Erzsébet Square is located in the heart of the city at a downtown location. The eastern part of the 3,4 acres size park was a former bus terminal; the western part of it was a formal park earlier with a large decorative fountain in the middle. The two parts are separated by the building of a previous bus terminal, now a historical monument used as an exhibition area. The eastern part of the site is roof garden with 2 m soil cover and with a glass-bottom pool in the center. In this part of the park there is maximum two-level vegetation, while on the other side of the park mostly two- and three-level vegetation can be found. Besides, the trees on the western part are much older (mostly middle-aged) and taller than on the east, they also have larger canopies whereby they have positive effect on the park's climate.

The duality of the park could be obviously noticed during the site visits. The eastern part of the park has got large scale of paved and built-up surfaces which could not be reduced by the existing green roof with one- and two-level vegetation even in visually aspect. Due to its central location, the park is extremely popular, which was noticeable even in the western part. To serve the needs of the visitors lots of pavements, built structures and equipments could be found here *(Table 3.)*.

Table 3. *Biological activity and green surface calculation of Erzsébet Square and Park, Budapest*

Total area calculated from NDVI maps:	34 200 m^2
Total Area (TA) determined by plot boundaries:	33 500 m^2

Surface	**Area**	**Surface rate** (based on the TA)	**BAR**	BAR /acre
Built-in area	1 235,1 m^2	3,69 %	0,00	0
Solid pavement	15 010,4 m^2	44,81 %	0,00	0
Pervious pavement	2 722,2 m^2	8,13 %	0,27	1
Water surface	630,3 m^2	1,88 %	0,38	6
Green surface with one-level vegetation	3 113,0 m^2	9,29 %	1,56	5
Green surface with two-level vegetation	6 516,2 m^2	19,45 %	3,91	6
Green surface with three-level vegetation	712,2 m^2	2,13 %	0,50	7
Half-intensive green roof with one-level vegetation	2 005,8 m^2	5,99 %	0,50	2,5
Intensive green roof with two-level vegetation	1 554,8 m^2	4,64 %	0,47	3

Total Biological Activity Rate (TBAR):	**7,58**
Green Surface Index (GSIN):	**41,50 %**
Green Space Intensity (GSI):	**57,45 %**

Lake Feneketlen and its vicinity

Lake Feneketlen ("Bottomless Lake") and its vicinity can be found in the 11th district in a residential area. The 7,7-acre site is around a former clay pit, which had to be closed down quite urgently, due to subsurface waters which broke in rather unexpectedly in the 1930s. In the place of the former pit a 1 acre size natural lake was created, which is approx. 5 m deep. The most significant built structure in the park is an open-air theater, which is out of use recently. There is a big playground in the northwestern corner of the site. The park has never been renewed, but many trees have been planted here since the 1960s. The vegetation is rather overgrown today. About one third of the park's area is covered by solid and pervious pavement and one fourth is covered by two-level vegetation (mostly by trees and grass under it) *(Table 4.).*

Table 4. *Biological activity and green surface calculation of Lake Feneketlen and its vicinity, Budapest*

Total area calculated from NDVI maps:	77 400 m^2
Total Area (TA) determined by plot boundaries:	76 908 m^2

Surface	**Area**	**Surface rate** (based on the TA)	**BAR**	BAR /acre
Built-in area	4 217,1 m^2	5,48 %	0,00	0
Solid pavement	15 867,3 m^2	20,63 %	0,00	0
Pervious pavement	13 513,4 m^2	17,57 %	1,35	1
Water surface	9 819,3 m^2	12,77 %	5,89	6
Green surface with one-level vegetation	5 135,2 m^2	6,68 %	2,57	5
Green surface with two-level vegetation	19 282,4 m^2	25,07 %	11,57	6
Green surface with three-level vegetation	9 073,0 m^2	11,80 %	6,35	7

Total Biological Activity Rate (TBAR):	**27,73**
Green Surface Index (GSIN):	**43,55 %**
Green Space Intensity (GSI):	**68,58 %**

The site has an important role in the green surface provision of the district due to the large percentage of pervious pavements and to old and multi-level vegetation surfaces. There is a huge water surface in the middle of the site which is not only popular among the citizens but influences significantly the climatic features of the park by its conditioning effect (Oláh, 2012). This surface was not considered by GSI analysis in absence of chlorophyll.

Water reservoir on Gellért Hill

The 4,7-acre park on the northern slopes of Gellért Hill was built around 1975 as a compromise. Due to the huge housing estate developments in the southern part of Buda at that time, the need for potable water grew tremendously, which could be fulfilled only by building a new water reservoir. From technical point it had an ideal location at the top of Gellért Hill. On the other hand it is visually an extremely sensitive location in the city, and a great tourist attraction with a fantastic view. As a compromise an underground water reservoir was built, very unusual at that time with a public park on the top of it. The soil cover above the tanks (each 40 000 m^3) varies between 0,6 to 1 m. Unique retaining wall system made of concrete, reminiscent of old fortification and steep slopes span the elevation difference. In the eastern part of the site, where no big scale earth work was done, some old honey-locust (*Robinia sp.*) trees from the time before the construction can be still found. Most of the woody vegetation was planted at the time of the construction and the plants are located to strengthen the grading concept, so more or less along contour lines *(Fig. 3.)*.

Figure 3. *Site map for biological activity calculation (water reservoir on Gellért Hill, Budapest)*

Large part of the area is covered by vegetation. The paved walkways take up about 12% of the total area which is one of the lowest percentages among our sample areas. It is important to be mentioned, that green surfaces above the water tanks must be classified in the green roof categories, therefore their BAR values are lower than the other green surfaces *(Table 5.)*.

Germanus Gyula Park

Germanus Gyula Park (approx. 4,1 acres) is located close to the Danube just north from Margaret Bridge on the Buda side. It is a narrow site with main traffic artillery on the eastern border. The strange feature about the park is that even if the Danube is very close, there is no visual or mental connection to the river due to the extremely intensive traffic line dividing them. On the other side the park is bordered by a hospital building and by an indoor spa. The park is used mainly by dog owners, though there is also a small playground. Huge sycamore (*Platanus sp.*) trees make the park really shady. It has not been renewed since 1958 except the area in front of a hospital, which was designed in 1980 with a small water feature, which does not function any more.

Table 5. *Biological activity and green surface calculation of water reservoir on Gellért Hill, Budapest*

Total area calculated from NDVI maps: 47 700 m^2

Total Area (TA) determined by plot boundaries: 47 050 m^2

Surface	**Area**	**Surface rate** (based on the TA)	**BAR**	BAR /acre
Built-in area	119,8 m^2	0,25 %	0,00	0
Solid pavement	5 094,9 m^2	10,83 %	0,00	0
Pervious pavement	424,1 m^2	0,90 %	0,04	1
Green surface with one-level vegetation	9 238,4 m^2	19,64 %	4,62	5
Green surface with two-level vegetation	14 654,6 m^2	31,15 %	8,79	6
Green surface with three-level vegetation	6 937,3 m^2	14,74 %	4,86	7
Half-intensive green roof with one-level vegetation	6 836,7 m^2	14,53 %	1,71	2,5
Intensive green roof with two-level vegetation	3 140,9 m^2	6,68 %	0,94	3
Intensive green roof eith three-level vegetation	603,3 m^2	1,28 %	0,24	4

Total Biological Activity Rate (TBAR): **21,20**

Green Surface Index (GSIN): **88,02 %**

Green Space Intensity (GSI): **92,55 %**

About 46% of the park is covered by pavement which includes walkways of several length and width, traffic roads and leisure surfaces *(Table 6.)*. Therefore the green surfaces with mostly two-level vegetation are extremely fragmented, with old and tall trees having huge canopy.

Golgota Square

Golgota Square Park is a 2,3-acre local park in the southeastern side of the 8th district. In 1966 a playground and a football field were built on the western part of it. In 1999 a gas station was placed in the corner of the park reducing its size and causing serious environmental damages. Most of the solid (impervious) pavement can be found around this gas station, only small size solid paved areas are next to the playground and football fields.

In the park there are green surfaces mostly with two-level vegetation (approx. 15 000 m^2) except in the vicinity of the petrol station. These surfaces are fragmented by several walkways with pervious pavements. Tall trees with huge canopy stand around the leisure part of the park but unfortunately lots of them are in very poor condition *(Table 7.)*.

Table 6. *Biological activity and green surface calculation of Germanus Gyula Park, Budapest*

Total area calculated from NDVI maps:	41 400 m^2
Total Area (TA) determined by plot boundaries:	41 425 m^2

Surface	**Area**	**Surface rate** (based on the TA)	**BAR**	BAR /acre
Built-in area	138,6 m^2	0,33 %	0,00	0
Solid pavement	17 639,3 m^2	42,58 %	0,00	0
Pervious pavement	1 617,6 m^2	3,90 %	0,16	1
Water surface	143,4 m^2	0,35 %	0,09	6
Green surface with one-level vegetation	7 172,3 m^2	17,31 %	3,59	5
Green surface with two-level vegetation	10 143,0 m^2	24,49 %	6,09	6
Green surface with three-level vegetation	4 570,8 m^2	11,03 %	3,20	7

Total Biological Activity Rate (TBAR):	**13,12**
Green Surface Index (GSIN):	**52,83 %**
Green Space Intensity (GSI):	**55,02 %**

Table 7. *Biological activity and green surface calculation of Golgota Square, Budapest*

Total area calculated from NDVI maps:	22 500 m^2
Total Area (TA) determined by plot boundaries:	22 950 m^2

Surface	**Area**	**Surface rate** (based on the TA)	**BAR**	BAR /acre
Built-in area	263,5 m^2	1,15 %	0,00	0
Solid pavement	2 209,0 m^2	9,63 %	0,00	0
Pervious pavement	3 637,3 m^2	15,85 %	0,36	1
Green surface with one-level vegetation	1 444,5 m^2	6,29 %	0,72	5
Green surface with two-level vegetation	15 395,7 m^2	67,08 %	9,24	6

Total Biological Activity Rate (TBAR):	**10,32**
Green Surface Index (GSIN):	**73,38 %**
Green Space Intensity (GSI):	**59,20 %**

Haller Square and its vicinity

The 6,5-acre Haller Square in the outer part of the 9th district actually did not exist till the beginning of the 1960s. At the time a typical district park was created here. In the southern part of the park there are some residential buildings, belonging to a housing estate built also in the 1960s. Nowadays different sport fields, a playground, a dog runner and some elements for roller skaters can be found in the site. There is well developed vegetation with large shrubs and significant size of trees.

Just by looking at the data it is obvious, that the park has got large scale of green surface. (approx. 70%) The pervious pavements with good water absorbing capacity and the groves including tall trees with closed canopy are very important features regarding total biological activity rate (TBAR) of the site *(Table 8.).*

Table 8. *Biological activity and green surface calculation of Haller Square and its vicinity, Budapest*

Total area calculated from NDVI maps:	67 500 m^2
Total Area (TA) determined by plot boundaries:	65 000 m^2

Surface	**Area**	**Surface rate** (based on the TA)	**BAR**	BAR /acre
Built-in area	4 057,0 m^2	6,24 %	0,00	0
Solid pavement	9 772,1 m^2	15,03 %	0,00	0
Pervious pavement	5 269,8 m^2	8,11 %	0,53	1
Water surface	0,0 m^2	0,00 %	0,00	6
Green surface with one-level vegetation	6 362,9 m^2	9,79 %	3,18	5
Green surface with two-level vegetation	28 257,7 m^2	43,47 %	16,95	6
Green surface with three-level vegetation	4 268,1 m^2	6,57 %	2,99	7
Grove, copse	7 012,4 m^2	10,79 %	4,21	6

Total Biological Activity Rate (TBAR):	**27,86**
Green Surface Index (GSIN):	**70,62 %**
Green Space Intensity (GSI):	**88,69 %**

Millenáris Park

Millenáris Park is a typical brown-field development in the heart of Buda. The 5,4-acre site used to be an electrical machine factory, causing a severe soil and groundwater contamination. Before the construction works of a cultural and entertainment center started in 2000, the soil had to be removed 1-1,5 m deep. This made it possible to create extended water surfaces in the park. Some of the old factory building were turned into exhibition halls or were used for other cultural purposes. There is a popular playground in one corner of the site. Recently a community garden was placed in the park. Due to the industrial past and the extended earthwork all the trees were planted at the same time of the constructions, so they are approx. 15 years old by now.

The park as a popular place and with multi-functional buildings has small scale (only 34%) of green surface coverage. The water surface in the middle is giving an extra 5 % to the conditioning surfaces. The vegetation of the park is relatively young; therefore it has not created yet a closed, multi-level green surface. The TBAR is being decreased by the underground garages serving the two office buildings in the southwestern part *(Table 9.).*

***Table 9.** Biological activity and green surface calculation of Millenáris Park, Budapest*

Total area calculated from NDVI maps: 55 800 m^2

Total Area (TA) determined by plot boundaries: 53 930 m^2

Surface	**Area**	**Surface rate** (based on the TA)	**BAR**	BAR /acre
Built-in area	18 172,1 m^2	33,70 %	0,00	0
Solid pavement	10 776,4 m^2	19,98 %	0,00	0
Pervious pavement	4 007,0 m^2	7,43 %	0,40	1
Water surface	2 594,6 m^2	4,81 %	1,56	6
Green surface with one-level vegetation	5 443,3 m^2	10,09 %	2,72	5
Green surface with two-level vegetation	7 222,6 m^2	13,39 %	4,33	6
Green surface with three-level vegetation	1 672,7 m^2	3,10 %	1,17	7
Half-intensive green roof with one-level vegetation	2 879,6 m^2	5,34 %	0,72	2,5
Intensive green roof with two-level vegetation	1 161,7 m^2	2,15 %	0,35	3

Total Biological Activity Rate (TBAR): **11,25**

Green Surface Index (GSIN): **34,08 %**

Green Space Intensity (GSI): **40,61 %**

Szent István Park

Szent István Park is located next to the Danube on the Pest side opposite to Margaret Island. This 3,4-acre park was built in the 1930s, as part of an extensive residential development in the neighborhood. The 'T' shaped site determines the unique form of the park with two symmetrical green surfaces at both sides and a bigger paved area in the middle. The green „wings" are mostly for strolling or jogging, most of the activities are concentrated in the middle, like a small sport field, pergolas, a 2000 m^2 pool, fountains. The playground is separated from the rest of the park by a street. There are impressive alleys in the park; some of them are even protected.

Beside the low amount of built-up areas, large scale (approx. 73%) of the paved surfaces are pervious pavement, only the pedestrian, the traffic areas and the sport field have solid (e.g. asphalt and stone) pavements. The green surfaces are covered mostly by two-level vegetation, only a small part of the area near the paved leisure surface in the middle has three-level structure with approx. 600 m^2. *(Fig. 4., Table 10.)*

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***Figure 4.** Site map for biological activity calculation of Szent István Park, Budapest*

***Table 10.** Biological activity and green surface calculation of Szent István Park, Budapest*

Total area calculated from NDVI maps:	33 300	m^2
Total Area (TA) determined by plot boundaries:	34 025	m^2

Surface	**Area**	**Surface rate** (based on the TA)	**BAR**	BAR /acre
Built-in area	34,6 m^2	0,10 %	0,00	0
Solid pavement	4 162,8 m^2	12,23 %	0,00	0
Pervious pavement	11 513,2 m^2	33,84 %	1,15	1
Water surface	585,0 m^2	1,72 %	0,35	6
Green surface with one-level vegetation	5 571,8 m^2	16,38 %	2,79	5
Green surface with two-level vegetation	11 548,9 m^2	33,94 %	6,93	6
Green surface with three-level vegetation	608,7 m^2	1,79 %	0,43	7

Total Biological Activity Rate (TBAR):	**11,64**	
Green Surface Index (GSIN):	**52,11**	**%**
Green Space Intensity (GSI):	**69,08**	**%**

Results

We summarized the data of the site analysis in a summary table and made a summary diagram for the visual representation. The GSI and the TBAR indicators cannot be compared with each other directly because of their different units. The GSI applies to the ratio of green surfaces based on the whole size of the plot and the TBAR is the sum

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of the BA rates of each surface of the area. (GSI index is a ratio, while TBAR index is just a value depending on the size of the analyzed surface.) A new index had to be introduced in order to make the GSI and TBAR values comparable. This is Maximum Biological Activity Rate (MBAR) which shows the activity rate that can be reached in the case of maximum (100%) GSI. We supposed that the maximum GSI can be reached in case of three-level vegetation which situation also corresponds with the maximum TBAR affordable on these urban located areas. The analysis and comparison of the graphic maps, based on site visits and the satellite pictures (taken by the Landsat 8 satellite) verified our supposal *(Fig. 5.)*.

BA map of Feneketlen-Lake and its vicinity

Joint short-wave infrared and ortho map of Feneketlen-Lake and its vicinity

NDVI map of Feneketlen-Lake and its vicinity

BA map of Millenáris Park

Joint short-wave infrared and ortho map of Millenáris Park

NDVI map of Millenáris Park

BA map of Haller Square

Joint short-wave infrared and ortho map of Haller Square

NDVI map of Haller Square

Figure 5. *Comparison of Biological Activity, short-wave infrared and NDVI maps*

Where the pixels on the NDVI pictures are dark green, the vegetation structure is mostly two- or three-level on each plot. Therefore it is proposed to determine the

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MBAR on each sites supposed that there are green surfaces with three-level vegetation. We used the following formula:

$$\text{MBAR} = 7 * \text{TA} \qquad \text{(Eq.2)}$$

where MBAR means the Maximum Biological Activity Rate, no. 7 is the Biological Activity Rate (BAR) of green surface with three-level vegetation and TA means the Total Area of the site. Finally we determined the Total Biological Activity Index (TBAI) of each areas by using the following formula:

$$\text{TBAI} = \text{TBAR} / \text{MBAR} * 100 \qquad \text{(Eq.3)}$$

[Total Biological Activity Rate (TBAR) of each plot is calculated, by summarizing the BA rates of each surface.]

In *Table 11.* TBAI and GSI are based on common benchmark, thus they can be compared with each other. We calculated the Green Surface Index (GSIN) of each site as well as their rank (R) among the sample plots according to different rates (TBAR, TBAI, GSI, GSIN) for further analyses.

Table 11. *Summary table of TBAR, TBAI, GSI and GSIN rates*

Sample-area	**TBAR (rate)**	**TBAI* (%)**	**P**	**GSI (%)**	**P**	**GSIN (%)**	**P**
Erzsébet Square and	**7,58**	**32,34**	7	**57,45**	6	**41,50**	7
Feneketlen-Lake and its vicinity	**27,73**	**51,51**	4	**68,58**	4	**43,55**	6
water reservoir on Gellért-Hill	**21,20**	**64,38**	1	**92,55**	1	**88,02**	1
Germanus Gyula Park	**13,12**	**45,24**	6	**55,02**	7	**52,83**	4
Golgota Square	**10,32**	**64,26**	2	**59,20**	5	**73,38**	2
Haller Square	**27,86**	**61,23**	3	**88,69**	2	**70,62**	3
Millenáris Park	**11,25**	**29,81**	8	**40,61**	8	**34,08**	8
Szent István Park	**11,64**	**48,89**	5	**69,08**	3	**52,11**	5

* According to Eq.3

As it can be noticed from the summary diagram, there are significant differences between the TBAI and GSI values in cases, like at water reservoir on Gellért Hill *(Fig. 6.)*. The main reason of it is particularly the difference between the evaluation processes. Based on the comparison of data in the summarizing table we can make certain statements regarding the different evaluation methods.

The calculations of the two methods using different evaluation processes and the rates associated to them as reference points gave similar results regarding the hierarchy (position) of the analyzed plots.

The water reservoir on Gellért Hill is a significant area, which reached the best rank (R = 1) according to all three methods. The reason of this is obvious just by looking at the survey map: This area has the lowest built-up and paved surface-rate (11,98%). The Millenáris Park reached the worst rank (R = 8) according to every aspects. The reason of this – in contrast to the water reservoir on Gellért Hill – the high built-up (33,7%) and impervious pavement (27,51%) surface-rate.

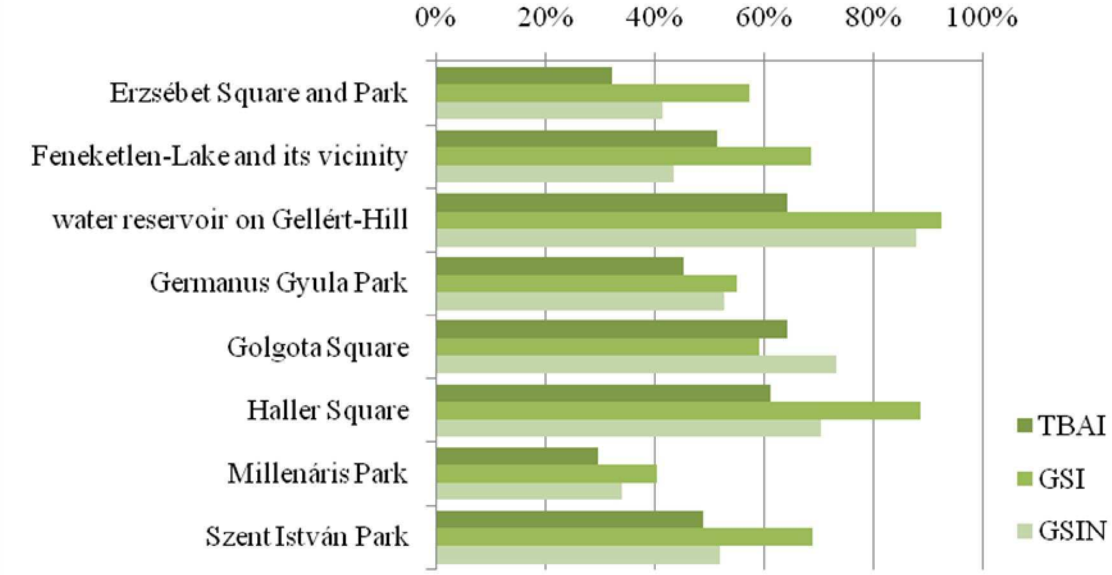

Figure 6. *Summary diagram of TBAI, GSI and GSIN rates*

In the case of Golgota Square, the TBAI (64,26%) is much higher than the GSI (59,20%). A reason could be that the structure of the vegetation is more significant for the BA calculation, then its condition. In contrast to this, in the case of GSI rate the condition of vegetation is concluded from the chlorophyll amount. Another reason of the difference comes from the resolution of the satellite photos. The photos which are available for us, are not detailed enough to get exact view of the GSI data of each areas. This is true especially in the case of long and narrow sites, where 80-90% of a pixel is on the edge of the inner and outer part of the area. In this case the chlorophyll index of the pixel is determined by rate of the inner and outer areas' chlorophyll indices like on Golgota Square.

The GSI (92,55%) and GSIN (88,02%) values of the water reservoir on Gellért Hill are very close to the 100% and to each other. In contrast to this, the TBAI (64,38%) is pretty low, which could be explained by the high green-roof rate (22,49%). These green surfaces cannot participate in to the water circulation of the natural areas in the city; therefore they have lower BAR value, than the green surfaces on the ground.

We have to highlight the Lake Feneketlen and its vicinity, because only this is the only site having higher TBAI (51,51%) than GSIN (43,55%). The reason is, that the site has a huge water surface (approx. 9800 m^2 – 12,77%) in the middle, which is noted with very high BAR/acre value (6) during the calculations. This case is a good example for showing the defect of the BA calculation process: The formula gives a BAR/acre value for water surfaces, but does not deal with the way these elements were created, whether they are natural lakes or just ornamental pools, which make a huge difference in urban ecology.

In five out of the eight analyzed sites, there is a significant difference (14,18% - 25,03%) between the GSI and the GSIN. Based on these differences it can be stated that NDVI does not give real picture about the green surface rates of any areas.

Summary

This study deals with the comparison of the GSI and BA evaluation methods in urban ecology aspects. The comparison process was based on the analysis of eight sample plots in Budapest, whereby we could enlighten the strengths and weaknesses of the two methods.

The GSI evaluation method is based on high resolution satellite photos and analyzes the physical features of the vegetation, which helps to evaluate the quality of the vegetation quite precisely:

- It can judge the condition of the vegetation through the quantity of chlorophyll.
- Although the GSI value is determined by the chlorophyll quantity, it is suitable to estimate the vegetation structure (one-, two- or three-level) as well.

Based on our analysis it is evident, that many factors influence its accuracy, and the following mistakes cannot be eliminated:

- The tree-canopies covering paved surfaces deform the result.
- Except vegetation it cannot differentiate the ecological efficiency of different surfaces.
- In some cases it gives an extreme (usually extremely high) green surface rate of the sites.

The BA evaluation method is based on pre-determined values, and it can demonstrate the ecological effects of several surfaces. But at the same time there are also many factors which cannot be eliminated by the method:

- Although the method considers the absorbing ability of the pavements, it does not calculate its capacity.
- Although water surfaces as biologically active surfaces are taken into account, but the method does not deal with the character of the water feature (natural or man-made) so important from urban ecological point.
- It is based on flat areas: It disregards the overlapping surfaces, for example the size of overlapping tree canopies.
- The evaluation procedure is based on pre-determined rates. These rates are fixed; therefore they do not refer to the present biological state of vegetation.

According to the results it can be stated that neither GSI nor BA evaluation methods could be used solely for making a detailed ecological evaluation on urban areas. Both methods have strengths as well as weaknesses.

The BA evaluation method is dealing not only with the vegetation and its structure but other surfaces (e.g. water surfaces and pervious pavements) which have ecological effect on urban environment as well. The GSI method, due to NDVI indices is more sensitive for the actual physical condition of surfaces. None of existing empiric evaluation method could match its accuracy.

The recent technology provides opportunity to make several different evaluations related to urban ecological aspect. The Leaf Area Index (LAI) mentioned in Review chapter, is the total one-sided area of leaf tissue per unit ground surface area. It is a key parameter in ecophysiology, especially for scaling up the gas exchange from leaf to canopy level. It characterizes the canopy-atmosphere interface, where most of the energy fluxes exchange (J. J. Bréda, 2003). The evapotranspiration ability of different plant surfaces and their effect on microclimate could be measured by LAI.

This study, as well as the review chapter reveal, that ecological evaluation of urban areas is getting more and more important especially in recent urban development processes. The growing popularity of BCI and NDISI evaluation methods used in the United States is good example to show this tendencies. It is high time to overview the domestic BA evaluation method, to which the detailed analyzes performed in the study on different sample sites with different characteristics could be a potential base. Due to the differences of urban surfaces it is neccessary to know and create methodologies for evaluations, which are sensitive for their physical and biological features. By summarizing the strengths of these procedures, a more advanced evaluation method can be worked out, which gives a more detailed picture about the ecological value of different urban areas.

Besides the methods which measure the ecological value of a site by using remote sensing we should not underestimate the importance of field study. The database provided by orthophotos or satellite images cannot give a comprehensive view of the ecological diversity of a certain site. However the diversity of the flora and the fauna cannot be "measured" by the above mentioned methods, they can be useful indicating those areas which are likely to be ecologically complex and rich enough, to be studied more detailed on the field.

The „green city password" as synonym of livable and pleasant urban environment pops-up frequently in urban development programs all over Europe. The landscape, the green and open spaces play a significant role in these urban development or urban rehabilitation projects (Pro Verde!, 2006). One of the most important aspects in recent urban ecological design processes is the evaluation of existing green surfaces, parks, squares and urban neighborhoods in environmental aspects. Due to this demand, more and more evaluation methods are coming up (e.g. Leadership in Energy and Environmental Design – LEED which is a green-building certification program and based on an ecological/green index calculated during the evaluation process of any kind of project; or the Green City Idea which primarily focuses on open space projects and helps cities restoring and use their potential and opportunities instead of taking further territories away from nature and agriculture); some of them are based on quantity, others on quality aspects. The complexity of the growing cities' structures and the continuous development of information technology strongly require the common usage of both aspects in any evaluation methods.

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