Chamber series and space-scale analysis of CO_2 gas-exchange in grassland vegetation: A novel approach

Sz. CZÓBEL[*], Sz. FÓTI[*], J. BALOGH[**], Z. NAGY[*], S. BARTHA[***], and Z. TUBA[*,**]

Department of Botany and Plant Physiology, Faculty of Agricultural and Environmental Sciences, Szent István University, H-2103 Gödöllő, Hungary[*]
Plant Ecological Research Group of Hungarian Academy of Sciences, Szent István University, H-2103 Gödöllő, Hungary[**]
Institute of Ecology and Botany, Hungarian Academy of Sciences, H-2163 Vácrátót, Hungary[***]

Abstract

Significant part of our work was developing a new type of CO_2 and H_2O gas exchange chambers fit for measuring stand patches. Ground areas of six chambers (ranged between 0.044–4.531 m^2) constituted a logarithmic series with doubling diameters from 7.5 to 240.0 cm. We demonstrate one of the first results for stand net ecosystem CO_2 exchange (NEE) rates and temporal variability for two characteristic Central European grassland types: loess and sand. The measured mean NEE rates and their ranges in these grasslands were similar to values reported in other studies on temperate grasslands. We also dealt with the spatial scale dependence from ecophysiological point of view. Our chamber-series measurement was performed in a perennial ruderal weed association. The variability of CO_2-assimilation of this weed vegetation showed clear spatial scale-dependence. We found the lowest variability of the vegetation photosynthesis at the small-middle scales. The results of spatial variability suggest the 0.2832 m^2 patch size is the characteristic unit of the investigated weed association and there is a kind of synphysiological minimi-area with characteristic size for each vegetation type.

Additional key words: canopy chamber; grassland; loess; sand; stand CO_2 measurements.

Introduction

Information on the physiology of plant associations (according to our term synphysiology, Tuba *et al*. 1998) comes mainly from two organisation levels of the associations from (*a*) infra-individual (leaf) level and (*b*) ecosystem/community (macro) level that consist of stands laying on several acres of land. Yet we have only limited information on the physiological processes of associations between the infra-individual and landscape levels. The reason of this is mainly the lack of suitable methods for investigating these processes. However, according to the results of synphenetical and vegetation dynamical studies (*e.g*. Bartha *et al.* 1997, Mucina and Bartha 1999) most of the important processes (*e.g*. species exchanges, diversity and coexistence patterns) can be detected in the few dm^2 or m^2 area of the stands—in non-arborescent herbaceous or grassland vegetations.

Adequate techniques for the physiological function of macroscale, such as “eddy correlation” (EC) (Monteith and Unsworth 1995), remote-sensing (GIS, NDVI in *e.g.* Loveland *et al.* 1991, Davis *et al.* 1992), or the so-called aerodynamic (Hall *et al.* 1993) method measure mainly the production. Despite the well-known uncertainties and

Received 22 July 2004, *accepted* 7 March 2005.
Abbrevations: CON – *Convolvulo-Agropyretum*; *E* – transpiration rate; EC – eddy correlation; FES – *Festucetum vaginatae danubiale*; GA – ground area; GIS – geographic information systems; LAI – leaf area index; *n* – number of replications; NDVI – normalised difference vegetation index; NEE – net ecosystem CO_2 exchange; PAR – photosynthetically active radiation; RH – relative humidity; SAL – *Salvio-Festucetum rupicolae*; SD – standard deviation of measurements performed within one-day; SWC – soil water content; T_a – air temperature; T_s – soil temperature.
Acknowledgements: This work was supported by the Hungarian Scientific Research Fund (OTKA-32586), Plant Ecological Research Group of Hungarian Academy of Sciences, Kiskunság National Park and CARBOMONT (EVK2-2002-00549) 5th R & D EU project. This project is also related to CARBOEUROPE-IP (GOCE-CT-2003-50-5572) and GREENGRASS 5th Framework Research Project (EVK-2-CT-2002-00105). We thank Mr. Attila Sóvári for the help in constructing and building the chambers and especially thank him, Mr. János Nagy, and Ms. Zsuzsanna Benkő for their help in the field measurements.
Dedicated to Academician Prof. Gábor Fekete on the occasion of his 75th birthday.

too large spatial scale of the mentioned techniques (see *e.g*. Long and Hällgren 1993) these can not solve some questions (*e.g*. the dependence on the botanical composition or spatial scale of the CO_2-exchange from a few dm^2 to m^2 area in the microscale), and therefore are not suitable between the individual and landscape (macro) levels.

Closed chamber techniques are suitable tools for studying small-scale spatial variability and dynamics of CO_2 gas-exchange (Angell *et al.* 2001). An evidence for it is based on the comparison between EC and chamber measurement results, which shows an almost 100 % correlation. Chamber methods employing infrared gas-analyzers are simple and fast and can give results similar to those obtained with micrometeorological methods such as eddy covariance and gradient methods (Norman *et al.* 1997). As a consequence we can establish that the chamber measurements with adequate technical background are appropriate for stand CO_2 gas-exchange measurements in spite of some disadvantages (*e.g*. chamber effect, not suitable for arborescent vegetation and non-continuous measurements in the majority of chambers) of this method. There are plenty of CO_2 measuring chambers with a wide range of chamber techniques, from the simple plexi chamber (*e.g*. Zamolodchikov and Karelin 2001) to the more complicated ventilated ones (Dugas *et al.* 1997). For measuring the stand CO_2 gas-exchange on the microscale the only suitable method is a chamber series. The reason of it is the botanical composition, which consists of mosaics of patterns (including vegetation patches with different diameter) in most grassland vegetations. As far as we know our technique with a series of chambers is a pioneer work to investigate the stand CO_2 exchange in the microscale as a function of the measured vegetation.

Our aim was to develop a chamber-series (GA ranged between 0.0044–4.5306 m^2) suitable for studying the CO_2-exchange of non-forested stand under *in situ* circumstances with adequate technical parameters that can be used for fast and reproducible measurements. Beside the description of the detailed technical parameters of our chambers, NEE of three natural temperate grass associations of different species composition, texture, spatial pattern, vegetation dynamic, physiognomy, and architecture were also measured and compared with reference data. The *Festucetum vaginatae danubiale* (FES) sand grassland, the *Salvio-Festucetum rupicolae* (SAL) loess steppe grassland stands, and a fast growing ruderal perennial weed association of *Convolvulo-Agropyretum* (CON) were measured using the medium large chamber (GA 0.2832 m^2).

Our survey also deals with the spatial scale dependence from eco-physiological point of view that was poorly studied until now. We tried to answer the following questions: What is the so-called eco-physiological spatial scale dependence? How is it measurable? How is it estimated? Our approach is based on the spatial variability that informs about the strength of regulation at stand level. Presumably less spatial variability of the physiological parameters indicates higher regulation of the vegetation patches.

The weed vegetation was also chosen as the object on which we performed the spatial scale-dependence of CO_2 assimilation using our newly developed chamber series. These stands together represent well the non-arborescent vegetation of the Carpathian Basin. Investigation of their CO_2-exchange rates is essential in order to estimate the significance of these grasslands in the global carbon cycle and global climate change.

Materials and methods

Vegetations studied: The temperate *Salvio-Festucetum rupicole* climatic zonal grassland vegetation (SAL) has been evolved on loess stone base. It is rich in minerals, the clay minerals ensure a well-structured soil (chernozem). It is a vertically well structured, almost completely closed (up to 90–95 %) steppe community rich in broadleaf dicotyledons and consisting of almost 100 species (Fekete 1992, Zólyomi and Fekete 1994). In many characteristics it is similar to the tallgrass prairie and sagebush grassland of North America. The study site is situated in the Isaszeg loess area (47°34'N, 19°2'E, 230 m a.s.l.) belonging to the Gödöllő Hills. Sample patches showed the sequence of dominant species as follows: *Festuca rupicola* Heuff., *Chrysopogon gryllus* (Torn.) Trin., *Stipa dasyphylla* Czern., *Chamaecytisus austriacus* (L.) Link, and *Carex humilis* Leyss.

The temperate semi-desert FES sand grasslands in the Danube Basin can be found on calcareous and nutrient poor sandy soils of poor water management characteristics showing semi-desert features also due to edaphic causes (Fekete *et al.* 1988, 1995). Dominant perennial grasses, and poikilohydric desiccation tolerant lichens and mosses covering extensive areas are found in large quantities in the association (Verseghy and Kovács-Lang 1971). This vegetation highly resembles other tussock grasslands of the temperate regions. Sampled vegetation patches of *Festucetum vaginatae* association were selected in typical stand at Tece-legelő (47°42'N, 19°15'E, 140 m a.s.l.), near Vácrátót. Few dominant flower species (such as *Festuca vaginata* W. *et* K., *Poa bulbosa* L., *Bromus squarrosus* L., *Stipa borystenica* Klokov, *Fumana procumbens* (Dun) Gren. et Godr.) characterise this stand.

The spatial scale-dependant measurements were carried out in the CON perennial ruderal weed association located in the suburb of Gödöllő (47°36'N, 19°24'E, 210 m a.s.l) that has developed on a year long abandoned area dominated by three typical weed species: *Elymus* *repens* (L.) Gould., *Artemisia vulgaris* L., and *Daucus carota* L. Its soil is semi-wet and neutral, rich in nutrients. The measured ruderal vegetation is less structured and has simpler physiognomical characteristics than the SAL association.

Canopy chambers and system used: Ground areas of the six stand measuring chambers (ranged between 0.0440–4.5306 m^2) constituted a logarithmic series with diameter doubling from 7.5 to 240.0 cm (Fig. 1). The height of each chamber was 70 cm. The cylinder-jacket of the chambers was arched from ultraviolet-B resistant water clean plexi sheets. The thickness of these was 5–10 mm depending on the chamber type (the larger the thicker) (Table 1). Outer fans supplied air motion within the four smaller chambers and the blowing vent was 10 cm above the ground level fitted on the cylinder jacket, while the outlet was fitted through the cover. A 150-mm diameter tube creating a closed system connected the two vents. As concerns air ventilation the two largest chambers were exceptions where the ventilation systems were within the chamber, but these two chambers were also ones of a closed system (Table 1). All the chambers could be fitted to a 5 cm wide metal soil collar.

Before the *in situ* vegetation measurements the chambers were tested on different (biologically inert) surfaces (*e.g.* gravel, asphalt) which are inactive from biological point of view and on one species dominating homogeneous grass patches.

NEE measurements: The chamber was allowed to set back in the ground and after a 30-s mixing interval, the measurement period commenced. CO_2 concentration was measured using a portable CO_2 measurement system (*LI-COR-6200*; *Li-Cor*, Lincoln, NE, USA) for up to 90 s (30 s in average) depending upon the flux rate. Within each measurement period a set of NEE measurements

Fig. 1. The view of Gödöllő chamber series arranged in descending ground area (4.5306—0.0044 m^2; diameter from 240.0–7.5 cm) order (*left to right*).

Table 1. Basic parameters of the chambers and fans. *Includes the volume of the ventilating course if any, **mean of 10 measured points. Standard height of chambers was 0.7 m, voltage of fans was always 230 V.

Ground area [m^2]	0.0044	0.0177	0.0708	0.2832	1.1327	4.5306
Diameter [m]	0.075	0.15	0.3	0.6	1.2	2.4
Chamber volume [m^3]	0.003092	0.012370	0.049479	0.197915	0.791658	3.166632
Total volume [m^3]*	0.010632	0.022575	0.063050	0.219381	0.791658	3.166632
Diameter of the ventilating course [m]	0.08	0.10	0.12	0.145	-	-
Length of the ventilating course [m]	1.5	1.3	1.2	1.3	-	-
Localization of fans	outer	outer	outer	outer	inner	inner
No. of fans	1	1	1	1	1	2
Power supply of fans [W]	20	40	76	76	200	400 (2×200)
Nominal air supply [$m^3\ s^{-1}$]	0.0236		0.139	0.139	0.83	1.66
Measured air supply [$m^3\ s^{-1}$]	0.015		0.043	0.080		
Calculat. time for total ventilation [s]	0.7	1.0	1.5	2.7	0.7	1.1
Highest air velocity [$m\ s^{-1}$]	3.0		3.8	4.5	3.0	3.9
Lowest air velocity [$m\ s^{-1}$]			1.9	1.1		1.1
Average air velocity [$m\ s^{-1}$]**			2.5	1.4	1.9	1.8

(average minimum of 15 and 30) was used, along with soil [soil water content (SWC) and soil temperature (T_s)] and micrometeorological [transpiration rate (*E*), photosynthetically active radiation (PAR), relative humidity (RH), and air temperature (T_a)] parameters. These were completed with botanical (species list, percentage cover of each species) features in order to characterise the investigated vegetation patches and sites. Ambient conditions at the beginning of each measurement were re-established by lifting the chambers while the fans were running. Measurement periods were brief to minimize chamber effects. NEE values were averaged in each of the sampled sites and chamber sizes (if relevant). Standard deviation (SD) numbers were calculated from these mean data.

Measuring circumstances: All measurements were carried out on a clear day with perpendicular incidence of sunlight and under very similar micrometeorological conditions (PAR, RH, T_a). CO_2-exchange, *E*, T_a, RH, and vapour pressure were measured and stomatal conductance was calculated using a portable closed-loop IRGA gas-exchange system (*LI-COR-6200*; *Li-Cor*, Lincoln, NE, USA) sampling the air in plexi-chambers of different diameters. For temporal (time-to-time) variation of photosynthesis close vegetation patches were selected from the same area of the investigated sand and loess associations and a set of measurements was made with one chamber (GA 0.2832 m^2) at each sampling date. Then the values were averaged. PAR values were recorded using sunfleck ceptometers (*Decagon Devices*, Pullman, WA, USA). Leaf area index (LAI) was calculated from the sunfleck/PAR-trasmittance values, using software written in *Excel (Visual/Basic)* macro based on algorithms by Campbell (1986). Canopy-surface and soil temperature were measured with an infrared thermometer (*Raynger MX4*; *Raytek Co.*, Santa Cruz, CA, USA). SWC was determined by a *TDR* reflectometer (*ML2*, *Delta-T Devices Co.*, Cambridge, UK) in three replications at 5, 10, and 20 cm soil depths.

Physiognomically uniform stands of each association were selected. We measured the NEE of different vegetation patches (GA 0.2832 m^2) of two completely different non-arborescent vegetation types, the open sand grassland (FES), and the loess grassland (SAL). The measurements were carried out during severe drought stress SWC [m^3 m^{-3}] of 3.1–3.8 at sand and 6.5–6.9 at loess grassland, and in more favourable “non-stressed” SWC [m^3 m^{-3}] of 5.6–6.0 at sand and 10.7–15.6 at loess grassland phases between 2000 and 2004.

To analyse the stand photosynthetic activity in different spatial scale we measured 30 vegetation patches in the *Elymus* dominated sub-association of the ruderal weed association in each of the six scales. Our scale-dependent coenological and synphysiological studies were carried out on 17^{th} October, 2001. At this time the CON was over the intense growth period fully developed and apparently not suffered from any kind of stress variability. Similar micrometeorological conditions allowed to focus on the spatial (area to area) variation within the photosynthesizing vegetation at the different measurement scales as well as to exclude the temporal (time to time) variation in photosynthesis.

Results and discussion

The essential part of our work was developing new type of CO_2 and H_2O exchange measuring chambers fit for measuring stand patches. Many factors were taken into account during the design procedure of the canopy chambers suitable for any particular study. For quantitative studies on photosynthetic rates, it will generally be necessary to comply with the critical conditions of such systems (Šesták *et al.* 1971). These criteria were met by the chambers, independent from their volumes, despite of the well-known fact: the higher the chamber the more problems one gets. The technical parameters also reinforced the suitability of these portable instruments.

Our study demonstrated one of the first results for stand Net Ecosystem Exchange (NEE) rates for two characteristic Central European non-arborescent vegetation types, sand and loess grasslands. The measured mean NEE rates showed similar values (Table 2) as other studies related to grassland CO_2 flux measurements both in FES sand (Frank *et al.* 2002, Hunt *et al.* 2002) and SAL loess (Angell *et al.* 2001, Suyker and Vermal 2001) grass-dominated associations. The SD numbers of the measured grassland sites were of a similar range (Table 2) as in other *in situ* case studies based on other steppes with similar physiognomical characteristics (see *e.g.* Suyker and Vermal 2001, Frank *et al.* 2002, Hunt *et al.* 2002). The similarity of NEE values and its SD provide further evidence for the reliability of this technique. The physiological activity of the CON weed vegetation showed lower mean and SD data than the loess grassland on the same (0.2832 m^2) scale (Fig. 2).

The different NEE values within the two associations (Table 2) showed the importance of the actual SWC for the physiological processes. The means of NEE were significantly different (p<0.05) under well-watered (non-stressed) and non-optimally watered (water-shortage or stress) circumstances in both investigated stands. The stressed values decreased in harmony with SWC and negative carbon-balance characterising this stage, such as winter stress when only respiration dominated in the C-balance. But inter-annual climatic differences also played an important role in the actually measured NEE values of these grasslands as CO_2-exchange was subjected to a large interaction with climate. Even the variability of NEE could be significantly different among years both in

Table 2. Inter-annual variability of the net ecosystem CO_2 exchange (NEE) of naturally induced drought stressed and non-stressed temperate sand and loess grasslands. Measured in different vegetation patches in a portable chamber (ground area = 0.2832 m^2). Means of minimum 15 and 30 measurements.

	Sand grassland				Loess grassland			
	Stressed		Non-stressed		Stressed		Non-stressed	
Date of measurements	06/07/2000	01/06/2001	22/06/2000	14/09/2000	22/06/2000	14/09/2000	15/06/2001	18/05/2004
NEE [μmol(CO_2) m^{-2} s^{-1}]	1.426	0.188	-0.745	-1.615	-3.939	-3.240	-9.013	-11.183
Standard deviation (SD)	0.727	0.573	0.411	0.916	1.881	1.827	3.818	7.790

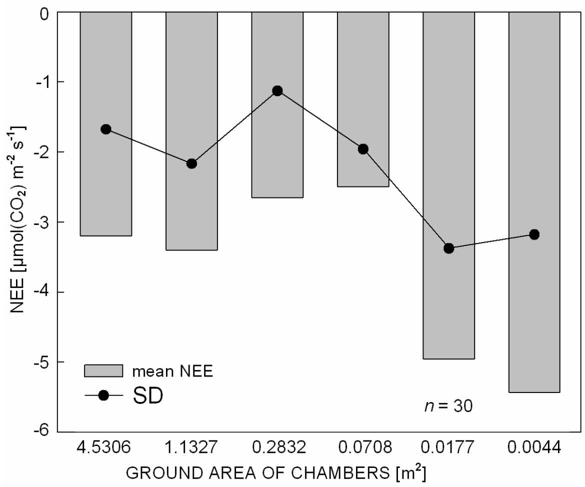

Fig. 2. Spatial scale-dependence of net ecosystem exchange (NEE) in the ruderal weed vegetation, measured on 17 October 2001, Gödöllő, Hungary. Measurements were performed with six different chambers (ground area ranged between 4.5306—0.0044 m^2). Different symbols represent NEE (*columns*) and their SD (*black dots*) from different chamber sizes, based on 30 replications (*n*) in each scale.

the stressed and non-stressed sand vegetation.

In ecology the decreased variability indicates the more regulated state. Presumably the spatial scale with the lowest variability represents the supra-individually most regulated physiological (CO_2-exchange) units of the grassland. Concerning the chamber-series measurement, the variability of CO_2-assimilation of the weed vegetation showed clear spatial scale-dependence as a sign of regulation (Fig. 2). The lowest variability of the vegetation photosynthesis was at the small-middle scale (GA 0.2832 m^2). The highest variability was observed at the smallest scale according to the heterogeneity of vegetation patterns, which was inversely proportional to the chamber periphery/surface ratio. Therefore the higher the chamber the more predictable data we can expect. The variability in absolute value of the spatial scale-dependence of the stand photosynthesis was the highest for the second smallest chamber (GA 0.0177 m^2). It seems that the absolute value of the stand photosynthesis linking to the spatial scale was not a determining factor from the variability point of view. The results suggest that the 0.2832-m^2 patch area is the characteristic unit of the investigated weed association and there is a kind of syn-physiological minimi-area with characteristic size for each vegetation type.

References

Angell, R.F., Svejcar, T., Bates, J., Saliendra, N.Z., Johnson, D.A.: Bowen ratio and closed chamber carbon dioxide flux measurements over sagebrush steppe vegetation. – Agr. Forest Meteorol. **108**: 153-161, 2001.

Bartha, S., Czárán, T., Scheuring, I.: Spatiotemporal scales of non-equilibrium community dynamics: a methodological challenge. – New Zeal. J. Ecol. **21**: 199-206, 1997.

Campbell, G.S.: Extinction coefficients for radiation in plant canopies calculated using an ellipsoidal inclination angle distribution. – Agr. Forest Meteorol. **36**: 317-321, 1986.

Davis, F.W., Schimel, D.S., Friedl, M.A., Michaelsen, J.C., Kittel, T.G.F., Dubayah, R., Dozier, J.: Covariance of biophysical data with digital topographic and land use maps over the FIFE site. – J. geophys. Res. **97**: 19,009-19,021, 1992.

Dugas, W.A., Reicosky, D.C., Kiniry, J.R.: Chamber and micrometeorological measurements of CO_2 and H_2O fluxes for three C_4 grasses. – Agr. Forest Meteorol. **83**: 113-133, 1997.

Fekete, G.: The holistic view of succession reconsidered. – Coenoses **7**: 21-29, 1992.

Fekete, G., Tuba, Z., Melkó, E.: Background processes at the population level during succession in grasslands on sand. – Vegetatio **77**: 33-41, 1988.

Fekete, G., Tuba, Z., Précsényi, I.: Application of three approaches to evaluate abundance and rarity in a sand grassland community. – Coenoses **10**: 29-38, 1995.

Frank, A.B., Liebig, M.A., Hanson, J.D.: Soil carbon dioxide fluxes in northern semiarid grasslands. – Soil Biol. Biochem. **34**: 1235-1241, 2002.

Hall, D.O., Scurlock, J.M.O., Bolhàr-Nordenkampf, H.R., Leegood, R.C., Long, S.P.: Photosynthesis and Production in a Changing Environment. A Field and Laboratory Manual. – Chapman & Hall, London – Glasgow – New York – Tokyo – Melbourne – Madras 1993.

Hunt, J.E., Kelliher, F.M., McSeveny, T.M., Byers, J.M.: Evaporation and carbon dioxide exchange between the atmosphere and a tussock grassland during a summer drought. – Agr. Forest Meteorol. **111**: 65-82, 2002.

Long, S.P., Hällgren, J.-E.: Measurement of CO_2 assimilation by plants in the field and the laboratory. – In: Hall, D.O., Scurlock, J.M.O., Bolhàr-Nordenkampf, H.R., Leegood, R.C., Long, S.P. (ed.). Photosynthesis and Production in a Changing Environment. A Field and Laboratory Manual. Pp. 129-167. Chapman & Hall, London – Glasgow – New York – Tokyo – Melbourne – Madras 1993.

Loveland, T.R., Merchant, J.W., Ohlen, D.O., Brown, J.F.: Development of a land-cover characteristics database for the conterminous U. S. – Photogrammetric Eng. remote Sensing **57**: 1453-1463, 1991.

Monteith, J.L., Unsworth, M.H.: Principles of Environmental Physics. – Edward Arnold, London – New York – Melbourne – Auckland 1995.

Mucina, L., Bartha, S.: Variance in species richness and guild proportionality in two contrasting dry grassland community. – Biológia (Bratislava) **54**: 67-75, 1999.

Norman, J.M., Kucharik, C.J., Gower, S.T., Baldocchi, D.D., Crill, P.M., Rayment, M., Savage, K., Striegl, R.G.: A comparison of six methods for measuring soil-surface carbon dioxide fluxes. – J. geophys. Res. **28**: 771-777, 1997.

Šesták, Z., Čatský, J., Jarvis, P.G. (ed.): Plant Photosynthetic Production, Manual of Methods. – Dr W. Junk Publ., The Hague 1971.

Suyker, A.E., Vermal, S.B.: Year-round observations of the net ecosystem exchange of carbon dioxide in a native tallgrass prairie. – Global Change Biol. **7**: 279-289, 2001.

Tuba, Z., Csintalan, Zs., Nagy, Z., Szente, K., Kemény, G., Takács, Z., Koch, J., Badacsonyi, A., Murakeözy, P., Palicz, G., Kóbor, Sz., Ötvös, E., Bartha, S.: [Synphysiology: basic theories and explorative investigations for an emerging field of plant ecology.] – In: Fekete, G. (ed.) Front Lines of the Community Ecology. Pp. 171-196. Scientia Publisher, Budapest 1998. [In Hung.]

Verseghy, K., Kovács-Láng, E.: Investigations on production of grassland communities of sandy soil in the IBP area near Csévharaszt (Hungary) I. Production of lichens. – Acta biol. Acad. Sci. hung. **22**: 393-411, 1971.

Zamolodchikov, D.G., Karelin, D.V.: An empirical model of carbon fluxes in Russian tundra. – Global Change Biol. **7**: 147-161, 2001.

Zólyomi, B., Fekete, G.: The Pannonian loess steppe: Differentiation in space and time. – Abstr. Bot. **18**: 29-41, 1994.