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Elevated CO_2 affects the content of glomalin related soil protein in xeric temperate loess and temperate semi-desert sand grasslands

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Abstract: Monoliths of temperate loess grassland and temperate semi-desert sand grassland have been exposed to elevated CO_2 (700 µmol mol-1) and present ambient CO_2 concentration in a 6-year open top chamber (OTC) experiment. In loess grassland elevated CO_2 increased both biomass and vegetation cover, whereas there was no similar effect found in semi-desert grassland. The content of glomalin related soil protein (GRSP) increased in both loess and sand grasslands under CO_2 enrichment (early summer aspect). The increase was higher in the case of easily extractable fraction (EEG), representing 14.7 and 22.2% of the chambered control's EEG, for loess and sand grassland respectively. In the case of total glomalin the increase was much lower 7.9% (loess) and 2.6% (sand). On the basis of differences between elevated and ambient CO_2 treatment we could conclude that elevated CO_2 promoted C-deposition in xeric temperate grassland in early summer. Increases of EEG indicate an efficient partitioning of the recently fixed carbon to the soil.

Abbreviations: OTC – open top chamber; GRSP – glomalin related soil protein, BRSP – Bradford reactive soil protein, EEG – easily extractable GRSP, TG – total GRSP, LAI – leaf area index.

1. Introduction

In last years there has been strong scientific effort to understand how ecosystems respond to increases of $[CO_2]_{atm}$. Ecosystems deliver goods and services to mankind that are critical for our survival – food, materials, energy, water etc. Maintaining the flow of these goods and services requires maintaining healthy ecosystems in the face of human caused changes in the global environment. In this respect a carbon cycling is one of key factors participating in the shaping of weather, climate, atmospheric composition and climate change (Norby and Luo 2004). Many studies have been therefore devoted to the carbon sequestration by biological and geological processes. For some important ecosystems such as xeric grasslands, however, the information on responses to elevated $[CO_2]_{atm}$ is still scarce.

With the increase of $[CO_2]_{atm}$ the productivity of grassland ecosystems is predicted to increase. It is suggested that for doubled current atmospheric CO_2 concentration the stimulatory effect will vary between 14-17% (Campbell et al. 2000, Nowak et al. 2004). However, when screening the studies on grassland productivity promotion under elevated CO_2 certain variability can be observed. It seems that production of grasslands is expected to increase in situations where growth limitation by CO_2 is significant when compared to the other main factors such as nutrient availability or temperature (Tuba et al. 2003). When one of the latter is seriously limiting plant growth, increased atmospheric CO_2 concentration is not expected to alleviate this limitation.

In many fumigation experiments the measurements of above-ground production are not paralleled by the measurements below-ground biomass, and unfortunately little is known on the fate of C allocated belowground (Vaccari et al. 2001, Urban et al. 2001). On the basis of existing data it can be estimated that the enhancement of the belowground production is in the similar range to that of the aboveground biomass. Reviewing different non-crop FACE experiments Nowak et al. (2004) reported an 11% increase in belowground productivity for grasslands.

Beside incorporation into biomass, a significant part of the carbon allocated belowground, can be derived to the soil by the process of rhizodeposition (up to 40% of assimilated C; Paterson et al. 1997). It is reported that under elevated CO_2 rhizodeposition will increase (see Pendall et al. 2004a,b) which could enhance carbon sequestration in grasslands. In this process mycorrhizal symbiosis could be of great importance via its effects on root metabolism and exudation (Jones et al. 2004). It is indicated that in the case of arbuscular mycorrhiza a substantial part of the rhizodeposited C is a glycoprotein termed glomalin related soil protein (GRSP, for terminology see Rillig 2004) which could contribute to the pool of recalcitrant C (Wright and Upadhyaya 1996, Rillig et al. 2001, Rillig, 2004). GRSP could be regarded as a part of persistent C and might importantly contribute to carbon sequestration.

In our research the effects of doubled CO_2 concentrations on the temperate loess grassland and temperate semi-desert sand grassland have been studied. These steppe grasslands, differ in the nutritional status and vegetation, have been previously subjected to elevated CO_2 research in which botanical composition (Szerdahelyi et al. 2004), water relations (Szente et al. 1998), photosynthesis (Tuba et al. 1998, Engloner et al. 2003) and morpho- anatomical changes (Engloner et al. 2003) have been studied. In comparison to other types of grasslands, however, the knowledge on response of these two to elevated CO_2 is still scant.

The main objective of the study was to assess what is the response of GRSP pool to 6-years CO_2 exposure in temperate loess and temperate semi-desert sand grasslands. We hypothetized that two grasslands would respond to elevated CO_2 not only by changing productivity but also by changes in GRSP production. Two fractions of glomalin-related soil protein, easy extractable (EEG) and total one (TG) were analyzed. The LAI, vegetation cover and above ground biomass were also followed.

2. Materials and methods

2.1. Site and vegetation description

Temperate loess grassland. The studied vegetation is a xeric temperate loess steppe (*Salvio-Festucetum rupicolae pannonicum*) (Zólyomi and Fekete 1994) situated at the border of the Hungarian Great Plain (Albertirsa, Monor-Irsa hills, 48 km south east of Budapest) at 160 m altitude a.s.l. The parent rock is sandy loess and loess with thick humus-and nutrient-rich A layer (humus layer: 100 cm, humus content: 6.1 % and available nitrogen: 14.2 g m^{-2}). The original grassland is made up of more than 90 species. The climate is temperate continental with hot dry summers; mean annual precipitation 500 mm or less; annual mean temperature of 11 ^{o}C; and large annual amplitude of temperature changes (22 ^{o}C).

Temperate semidesert sand grassland. The semidesert sand grassland can be found near Vácrátót, Hungary (latitude 47^{o}42'N, longitude 19^{o}15'E, 140 m a.s.l.). The climate of the site is of temperate continental type. Total annual precipitation is about 500 mm, the mean annual air temperature is 10.5 ^{o}C. The temperature of the sand surface regularly reaches 60 ^{o}C at noon in summer. The vegetation is an open semi-arid grassland (*Festucetum vaginatae danubiale*), which is distributed in the Carpathian Basin, evolved on sandy soils and has semi-desert characteristics due to edaphic causes (Fekete et al. 1988). This community is dominated by *Festuca vaginata* W. et K. and *Stipa capillata* Klokov. The average cover by vascular plants is 30-40% and mosses (mainly *Tortula ruralis* (Hedw). Gaertn.) contribute 18-20% to the total cover.

2.2. Experimental CO_2 exposure.

The CO_2 exposure was carried out in the Global Climate Change and Plants Long-term Experimental Ecological Research Station (Gödöllő, 28 km east of Budapest) for 6 years using open top chambers with a total surface area of 1.2 m^2 (Ashenden et al. 1992, Tuba et al. 1996). From the temperate semi-desert sand grassland and loess grassland 50 cm x 50 cm x 30 cm monoliths were extracted and transplanted into the open top chambers (Tuba et al. 1996) filling the profile which was exposed when original soil was removed from the chamber. Four weeks after the transplantation of the monoliths the grass was cut. Following a two-month adaptation period, the monoliths were exposed gradually, over a 4-weeks period, to 700 μmol mol^{-1} CO_2. The elevated CO_2 treatments were maintained at 700 μmol mol^{-1} and the present CO_2 treatments at 350 μmol mol^{-1}. The air CO_2 concentrations in the elevated and present CO_2 chambers were maintained as described earlier (Tuba et al. 1996). The monoliths were not watered.

During the whole six years of the experiment three different treatments were applied as follows: Control in chambers at ambient (present-day) CO_2 concentration (approximately 350 μmol mol^{-1}), elevated CO_2 concentration (approximately 700 μmol mol^{-1}) and absolute control plots without chambers at ambient (present-day) CO_2 concentration. Two replicates were applied for each treatment.

Aboveground biomass measurements, canopy cover and leaf area index determinations. Aboveground biomass was harvested in July 2003 and dry mass was measured after oven drying.

Canopy cover was estimated in May and September according to Braun-Blanquet (1964). Leaf area index (LAI) was calculated by the averaged values of Agricultural Camera (ADC, Dycam Inc., Hills, CA, USA). The LAI values calculations used to generate each index are based on amount of red (the R pixel value) and infrared (the G pixel value) at each point in the image.

The analysis of glomalin related soil protein. Soil samples for glomalin analysis were taken two times, in the middle of June and November 2003, by drilling with 2 cm ∅ borer to the depth of 10 cm.

GRSP, operationally defined as Bradford-reactive soil protein (Rillig 2004), was extracted from soil subsamples (4) as easily extractable glomalin (EEG) and as total glomalin (TG) as described by Wright and Upadhyaya (1998). EEG was extracted from 1 g of ground dry-sieved soil with 8 ml of 20 mM citrate, pH 7.0 at 121 °C for 30 min. TG was obtained by repeated extraction from 1 g of ground dry-sieved soil with 8 ml of 50 mM citrate, pH 8.0 at 121 °C for 60 min. After each autoclaving cycle supernatant was removed by centrifugation at 5000 rpm for 20 min and stored. TG extraction cycles were repeated till the glomalin content of supernatant was under the method detection limit (ca. 2 μg/ml). Extracts from each cycle were pooled, centrifuged at 10,000 rpm for 10 min to remove soil particles and then analyzed.

Table 1. Growth response of xeric temperate loess steppe (*Salvio-Festucetum rupicolae pannonicum*) and temperate semidesert sand grassland (*Festucetum vaginatae danubiale*) to elevated [CO_2]. Parameters were measured at the end of six years experiment. Plants were fumigated with elevated CO_2 (ca.700 µmol mol^{-1}) in OTCs or exposed to present-day ambient CO_2 concentration (ca. 350 µmol mol^{-1}, chambered and un-chambered treatment included). Means ± standard deviations are presented. Different letters within the rows indicate significant difference between the means at p < 0.05 (*t*-test).

	ambient [CO_2] – chamber free	ambient [CO_2] – OTC	elevated [CO_2] - OTC
Loess grassland			
vegetation cover [%]	140.5 ± 26.4 ab	121.3 ± 33.2 a	150.4 ± 29.6 b
leaf area index	1.74 ± 0.66	1.57 ± 0.33	1.74 ± 0.54
above ground biomass [g DW m^{-2}]	994.1 ± 58.8 ab	811.9 ± 39.6 a	1656.3 ± 33.2 b
Sandy grassland			
vegetation cover [%]	97.1 ± 51.3	96.9 ± 46.9	91.7 ± 42.6
leaf area index	1.27 ± 1.31	0.89 ± 0.49	1.21 ± 1.08
above ground biomass [g DW m^{-2}]	510.8 ± 96.2	499.8 ± 23.3	471.5 ± 78.2

Protein content in the supernatant was determined by Bradford assay (Sigma–Aldrich Inc.) with bovine serum albumin as the standard. Concentration of GRSP was extrapolated to mg g^{-1} by correcting for the dry weight of coarse fragments included in the extraction of soil.

Statistics

At least three repetitions, i.e., samples per chamber have been done at all treatments for each measurement. Data were analysed by Student *t*-test or ANOVA ($p < 0.05$).

3. Results

Aboveground plant biomass, canopy cover and leaf area index

Above ground biomass was much higher in loess grassland than in sandy grassland (Table 1). Under elevated CO_2 concentration aboveground biomass of loess grassland was substantially increased. The same trend was found for total canopy cover but not for leaf area index. In sandy grassland there was no effect of doubled CO_2 atmospheric concentration on growth.

GRSP content

In June the concentrations of easily extractable GRSP (EEG) were in the same range for both types of grasslands (round 1 mg g^{-1} dry soil) reaching the highest average values in elevated CO_2 treatment (Figure 1). In agreement with this, when data for chambered monoliths were analysed by two-way ANOVA (factors: soil type and CO_2 concentration), no significant effect of the soil was found but analysis revealed significant difference between CO_2 treatments. No effect of fumigation on EEG was found at the end of the season (Figure 2) the concentrations were, in general, similar to those found in early summer.

The concentration of total GRSP in single samples reached maximal 8 mg g^{-1} (loess). The average values were significantly higher in loess (3.9-6.5 mg g^{-1}), than in temperate semi-desert sand grassland (1.6-2 mg g^{-1}). For mid-June

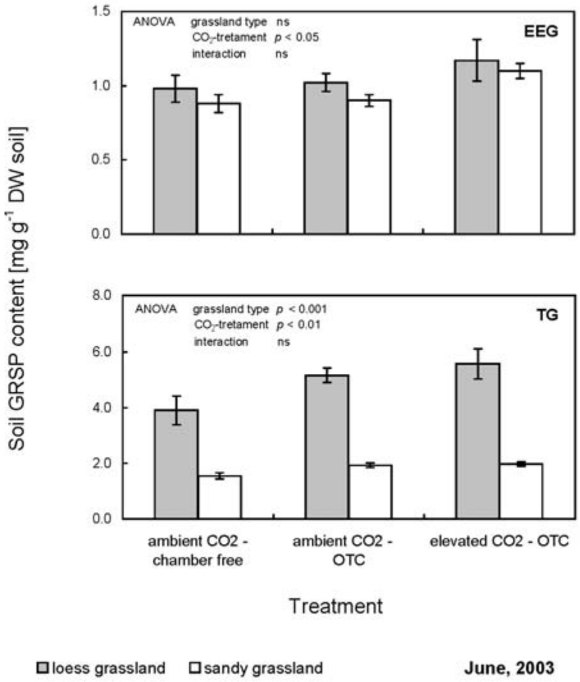

Figure 1. Soil glomalin related soil protein content (EEG - easy extractable fraction, upper panel and TG – total GRSP, lower panel) in xeric temperate loess steppe (*Salvio-Festucetum rupicolae pannonicum*) and temperate semidesert sand grassland (*Festucetum vaginatae danubiale*) – June, 2003. Monoliths of both grasslands have been fumigated in OTC's for 6 years with elevated CO_2 (ca.700 µmol mol^{-1}) or exposed to present-day ambient CO_2 concentration (ca. 350 µmol mol^{-1}, chambered and un-chambered treatment included). Means ± standard errors are shown.

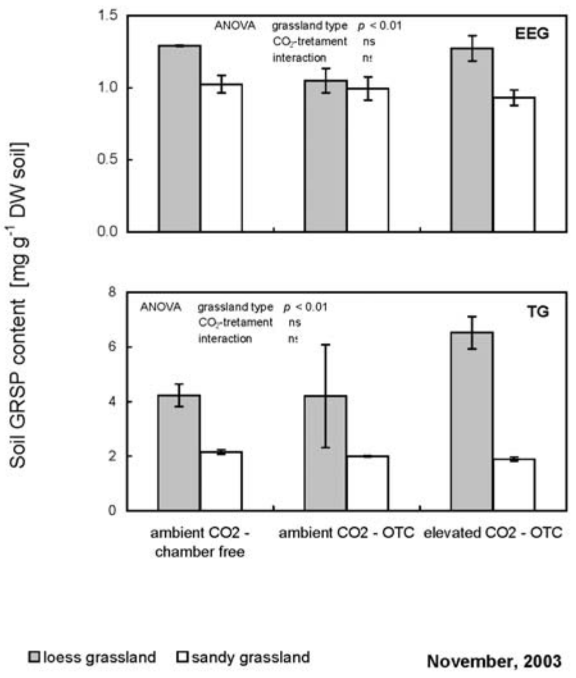

Figure 2. Soil glomalin related soil protein content (EEG - easy extractable fraction, upper panel and TG - total, lower panel) in xeric temperate loess steppe (*Salvio-Festucetum rupicolae pannonicum*) and temperate semidesert sand grassland (*Festucetum vaginatae danubiale*) – November, 2003. Monoliths of both grasslands have been fumigated in OTC's for 6 years with elevated CO_2 (ca.700 µmol mol^{-1}) or exposed to present-day ambient CO_2 concentration (ca. 350 µmol mol^{-1}, chambered and un-chambered treatment included). Means ± standard errors are shown.

the two way ANOVA revealed significant difference between the soils and, similar to EEG, the significant influence of CO_2 concentration on TG content. In the late autumn the content of TG was fairly even for all three treatments in sandy grassland. In loess, the highest average TG was reached in elevated CO_2 treatment, due to high variability of ambient CO_2-OTC data, however, the effect of fumigation could not be supported by ANOVA.

4. Discussion

The content of the total glomalin related soil protein (determined as Bradford reactive protein - BRSP) in both types of the soil was in the range known from other studies. Wright and Upadhyaya (1998) and Wright et al. (1999) report a high abundance of GRSP for both native and agricultural soil (2-14 mg g^{-1} and 2-5 mg g^{-1} soil, respectively). In our case much higher GRSP concentration was found in loess soil that in the sandy soil, which correlates well with higher (4-5 fold) organic matter content in loess than in sand (ca. 20 % and 4-5 % respectively). Beside the environmental conditions influencing organic matter turnover, differences in glomalin content in both soils can be directly related to different species composition, both of plants and soil microorganisms, and to different mycorrhizal dependency of plant species forming two types of grasslands.

In early summer the content of glomalin increased in both loess and sand grassland soils under elevated CO_2 treatment. For easily extractable fraction (EEG) the increase represented 14.7 and 22.2 % of the chambered control's EEG for loess and sandy grassland, respectively. In the case of TG the increase was 7.9 % (loess) and 2.6 % (sand). Similar results were observed in sorghum (*Sorghum bicolor*) FACE experiment (Rillig et al. 2001) where CO_2 enrichment (ambient + 200 µmol mol^{-1}) increased EEG but had no significant effect on total one. In our case, furthermore, no clear effect of fumigation was found on the level organic matter as a whole.

The two fractions of EEG and TG are distinguished on the basis of operationally defined ease of extraction in citrate with heat, and the reaction with a monoclonal antibody against glomalin (Wright and Upadhyaya, 1996). EEG is currently interpreted to be mostly recently deposited material. It is therefore to presume that also in our case the recently fixed carbon was efficiently partitioned to the soil. On the basis of differences between elevated and ambient CO_2 treatment we could conclude that elevated CO_2 promoted mycorrhizal deposition in xeric temperate grassland in early summer while in the late autumn no difference in EEG soil content among the three treatments was found. The TG results for November could, on the other hand, indicate a cumulative effect of CO_2 fumigation in loess grassland (Figure 2).

The observed differences in glomalin summer deposition can not be directly paralleled to the enhancement of plant biomass under elevated CO_2 concentration. Elevated CO_2 increased both biomass and vegetation cover in loess- but not in sand grassland. At last partly the lack of any effect in this grassland type could be related to very high variability of biomass in chambers with elevated CO_2 treatment.

Other CO_2 fumigation experiments indicate (Rogers et al. 1994) that the elevated CO_2-promoted rhizodeposition could result from stimulation of root biomass production. Indeed, in recent studies by Pendall and co-workers (Pendall et al. 2004a,b) a significant increase of root biomass (23 %) was related to the increase of rhizodeposition (roughly doubled in elevated compared with ambient). The same relation could not be proved in our experiment where no enhancement of root biomass by twice ambient CO_2 over 6 years has been found (data not shown). The lack of the effect could also result from the high variability of bellow-ground biomass data, which can be partly related to the difficulties inherent to root biomass determination. On the other hand, previous work in intact native grassland systems has revealed that significant stimulation of the size of root systems (biomass, length density or root number) is not a universal response to elevated CO_2.

In general, our results could be regarded also as a certain disproportion between the plant growth response and the response of GRSP pool to elevated CO_2. The 20 % increase in

EEG fraction found in sandy soil, was not accompanied by the significant promotions of plant production. On the other hand the increase of EEG was lower in loess soil, but there an increase of aboveground biomass can be detected. It could be speculated that two plant-soil systems differed in carbon allocation patterns. These could be directly or indirectly influenced by elevated CO_2 treatment on the level of soil and plant processes (e.g., changed plant-soil water relations under elevated CO_2; Tuba et al. 1996)

By applying isotopic techniques it is possible to study allocation patterns and also to study the fate of carbon allocated belowground (Jones and Donnelly, 2004). Pendall and coworkers (2004a,b) reported that increased turnover rates of older soluble organic matter negated the gain of new, rhizodeposited C, resulting in no difference in net ecosystem production of semiarid grassland between ambient and elevated treatments. The importance of extra deposited glomalin related soil protein for C sequestration in xeric temperate loess steppe would therefore depend also on the decomposability and on the turnover of different GRSP fractions. At present, however, little is known about these processes (Rillig 2004).

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References

Ashenden, T.W., R. Baxter and C.R. Rafarel. 1992. An inexpensive system for exposing plants in the field to elevated concentrations of CO_2. *Plant Cell Environ.* 15: 365-372.

Braun-Blanquet, J. 1964. *Pflanzensoziologie.* Wien, New-York.

Campbell, B.D., D.M. Stafford-Smith, A.J. Ash, J. Fuhrer, R.M. Gifford, P. Hiernaux, S.M. Howden, M.B. Jones, J.A. Ludwig, R. Manderscheid, J.A. Morgan, P.C.D. Newton, J. Nösberger, C.E. Owensby, J.F. Soussana, Z. Tuba and C. ZouZhon. 2000. A synthesis of recent global change research on pasture and rangeland production: reduced uncertainties and their management implications. *Agric. Ecosyst. Environ.* 82: 39-55.

Engloner, A.I., D. Kovács, J. Balogh and Z. Tuba. 2003. Anatomical and eco-physiological changes in leaves of couch-grass (*Elymus repens* L.), a temperate loess grassland species, after 7 years growth under elevated CO_2 concentration. *Photosynthetica* 41, 185-189.

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

Jones, M.B. and A. Donnelly. 2004. Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO_2. *New Phytol.* 164: 423-439.

Jones, D.L., A. Hodge and Y. Kuzyakov. 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytol.* 163: 459-480.

Norby, R.J. and Y. Luo. 2004. Evaluating ecosystem responses to rising atmospheric CO_2 and global warming in a multi-factor world. *New Phytol.* 162: 281-293.

Nowak, R.S., D.S. Ellsworth and S.D. Smith. 2004. Functional responses of plants to elevated atmospheric CO_2 - do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytol.* 162: 253-280.

Paterson, E., J.M. Hall, E.A.S. Rattray, B.S. Griffiths, K. Ritz and K. Killham. 1997. Effect of elevated CO_2 on rhizosphere carbon flow and soil microbial processes. *Global Change Biol.* 3: 363-377.

Pendall, E., A.R. Mosier and J.A. Morgan. 2004a. Rhizodeposition stimulated by elevated CO_2 in a semiarid grassland. *New Phytol.* 162: 447-458.

Pendall, E., S. Bridgham, P.J. Hanson, B. Hungate, D.W. Kicklighter, D.W. Johnson, B.E. Law, Y.Q. Luo, M. Megonigal-Olsrud, M.G. Ryan and S.Q. Wan. 2004b. Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. *New Phytol.* 162: 311-322.

Rillig, M.C., S.F. Wright, B.A. Kimball, P.J. Pinter, G.W. Wall, M.J. Ottmanand and S.W. Leavitt. 2001. Elevated carbon dioxide and irrigation effects on water stable aggregates in a *Sorghum* field: a possible role for arbuscular mycorrhizal fungi. *Global Change Biol.* 7: 333-337.

Rillig, M.C. 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Can. J. Soil Sci.* 84: 355-363.

Rogers, H.H., B.G. Runion and S.V. Krupa.1994. Plant-responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environ. Pollut.* 83:155-189.

Szerdahelyi, T., J. Nagy, Sz. Fóti, Sz. Czóbel, J. Balogh and Z. Tuba. 2004. Botanical composition and some CO_2 exchange characteristics of temperate semi-desert sand grassland in Hungary under present-day and elevated air CO_2 concentracions. *Ekologia (Bratisl.)* 22: 124-163.

Szente, K., Z. Nagy and Z. Tuba. .... Enhanced water use efficiency in dry loess grassland species grown at elevated air CO_2 concentration. *Photosynthetica*, 35: 637-640.

Tuba, Z., K. Szente, Z. Nagy, Z. Csintalan and J. Koch. 1996. Responses of CO_2 assimilation, transpiration and water use efficiency to long-term elevated CO_2 in perennial C_3 xeric loess steppe species. *J. Plant Physiol.* 148: 356-361.

Tuba, Z., M.B. Jones, K. Szente, Z. Nagy, L. Garvey and R. Baxter. 1998. Some ecophysiological and production responses of grasslands to long-term elevated CO_2 under continental and atlantic climates. *Ann. New York Acad. Sci.* 851: 241-250.

Tuba, Z., A. Raschi, G.M. Lanini, Z. Nagy, L. Helyes, D. Vodnik and L. Sanità di Toppi. 2003. Plant and ecosystem response to elevated carbon dioxide, in: Sanità di Toppi L, Pawlik-Skowrońska B (Eds.) *Abiotic Stresses in Plants.* Kluwer Academic Publishers, Dordrecht. pp*:*157-203.

Urban,O., D. Janouš, R. Pokorný, I. Marková, M. Pavelka, Z. Fojtík, M. Šprtová, J. Kalina and M.V. Marek. 2001. Glass domes with adjustable windows: A novel technique for exposing juvenile forest stands to elevated CO_2 concentration. *Photosynthetica* 39: 395-401.

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

Vaccari, F.P., I. Bettarini, A. Giuntoli, F. Miglietta, A. Raschi. 2001. Mediterranean grassland community under elevated CO_2: observations from a CO_2 spring. *Journal of Mediterranean Ecology* 2: 41-50.

Wright, S.F. and A. Upadhyaya. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Sci.* 161: 575-586.

Wright, S.F. and A. Upadhyaya. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil* 198: 97-107.

Wright, S.F., J.L. Starr and I.C. Paltineanu. 1999. Changes in aggregate stability and concentration of glomalin during tillage management transition. *Soil Sci. Soc. Am. J.* 63: 1825-1829.

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