

1 **Autotrophic component of soil respiration is repressed by**  
2 **drought more than the heterotrophic one in dry grasslands**

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11

1 **Abstract**

2 Summer droughts projected to increase in Central Europe due to climate changes strongly  
3 influence the carbon cycle of ecosystems. Persistent respiration activities during drought  
4 periods are responsible for a significant carbon loss, which may turn the ecosystem from a  
5 sink into a source of carbon. There are still gaps in our knowledge regarding the characteristic  
6 changes taking place in the respiration of the different components of the ecosystem in  
7 response to drought events.

8 In the present study, we combined a physical separation of soil respiration components with  
9 continuous measurements of soil CO<sub>2</sub> efflux and its isotopic (<sup>13</sup>C) signals at a dry grassland  
10 site in Hungary. The physical separation of soil respiration components was performed by  
11 means of inox meshes and tubes inserted into the soil. The root-excluded and root- and  
12 mycorrhiza excluded treatments served to measure the isotopic signals of the rhizospheric,  
13 mycorrhizal fungi and heterotrophic components, respectively.

14 In the dry grassland investigated in the study the three components of the soil CO<sub>2</sub> efflux  
15 decreased at different rates under drought conditions. During drought the contribution made  
16 by the heterotrophic components was the highest (54±8%; mean±SE). Rhizospheric  
17 component was the most sensitive to soil drying with its relative contribution to the total soil  
18 respiration dropping from 66±7% (non-stressed) to 35±17% (mean±SE) under drought  
19 conditions. According to our results the heterotrophic component of soil respiration is the  
20 major contributor to the respiration activities during drought events in the dry grassland  
21 ecosystem studied.

22

# 1 1 Introduction

2 Grassland ecosystems respond forcefully to drought events via substantial reduction of their  
3 primary production (GPP, Hoover et al., 2014; Parton et al., 2012; Reichstein et al., 2013). In  
4 contrast, below-ground respiration is not so strongly affected (van der Molen et al., 2011;  
5 Yang and Zhou, 2013) but tends to be reduced as well under drought (Balogh et al., 2011;  
6 Suseela and Dukes, 2013). Soil respiration is the second largest component of carbon cycling  
7 in grasslands and returns as much as 50–90% of annual GPP back to the atmosphere (Bahn et  
8 al., 2008). Thus, the magnitude of soil respiration can turn the carbon budget from a net sink  
9 into a net source in dry years (Nagy et al., 2007). Here we address the question whether under  
10 drought this is primarily a function of autotrophic respiration declining along with the soil  
11 drying while heterotrophic respiration remains less affected.

12 According to climate change scenarios the frequency of droughts is expected to increase in  
13 Central Europe (Prudhomme et al., 2014) where dry grassland ecosystems represent one of  
14 the major land use types. It is well known that there is a need for better mechanistic models to  
15 address the effects of climatic extremes on carbon fluxes (e.g. Blagodatsky and Smith, 2012).  
16 However, progress has so far been limited due to the high complexity of responses given by  
17 the different ecosystem respiration components to the climatic drivers.

18 Soil organic matter (SOM) and litter derived respiration is considered to belong to the  
19 heterotrophic soil respiration component (Moyano et al., 2009). Their decomposition is  
20 attributed mainly to soil bacteria and fungi and has about 50% share in the total soil  
21 respiration in dry grasslands (Bao et al., 2010; Chen et al., 2009; Gomez-Casanovas et al.,  
22 2012). On the other hand, some of the soil fungi relying on recent photosynthetic assimilates  
23 also contribute to the autotrophic respiration component. Arbuscular mycorrhizal fungi  
24 (AMF) are obligatory symbiont soil fungi, forming intimate mutualistic associations in 70-  
25 90% of the plant species in grasslands (Hiiesalu et al., 2014). About 10-20% of the  
26 assimilated C may be attributed to AMF in exchange for acquiring water and essential  
27 nutrients for plant productivity (van der Heijden et al., 2015). Therefore soil respiration  
28 includes components of an autotrophic-heterotrophic continuum from roots through the root-  
29 associated fungi (rhizospheric and mycorrhizal) to non-root-associated (heterotrophic)  
30 microbial components.

31 Belowground CO<sub>2</sub> production by the autotrophic and heterotrophic components show large  
32 diel and seasonal variability (Fassbinder et al., 2011; Moyes et al., 2010). The drivers behind

1 all this are not fully revealed and the role of soil microbes in the process is still not clear  
2 mainly due to the diversity of soil biota (Bardgett et al., 2008). Moreover, drivers of CO<sub>2</sub>  
3 production frequently interact with each other (Balogh et al., 2015; Vargas et al., 2010),  
4 hampering the partitioning of the total CO<sub>2</sub> efflux into components. Studies found a stronger  
5 effect of photosynthesis than that of temperature on root respiration (Gomez-Casanovas et al.,  
6 2012; Heinemeyer et al., 2012; Hopkins et al., 2013). Both autotrophic and heterotrophic  
7 components were shown to be sensitive to water shortages (Carbone et al., 2011; Moyano et  
8 al., 2013). The autotrophic component was found to be dominant over the heterotrophic one  
9 during drought periods in a Mediterranean woodland ecosystem (Casals et al., 2011) but we  
10 have limited information about grasslands of shallow rooted herb species regarding the  
11 dominant source of carbon during drought periods.

12 The widely used separation techniques (trenching and girdling) are not considered suitable for  
13 grasslands (Epron, 2009), thus the physical separation of the soil CO<sub>2</sub> efflux components via  
14 root exclusion is hardly feasible without seriously disturbing the soil structure and the root  
15 system. A viable option, however, is the use of stable isotopic signatures ( $\delta^{13}\text{C}$ ) of soil  
16 respiration to estimate the relative contributions of the main components (Carbone et al.,  
17 2011; Hopkins et al., 2013). Although diel patterns in  $\delta^{13}\text{C}$  may also be subject to biases in the  
18 measuring methods (Fassbinder et al., 2011; Midwood and Millard, 2011), seasonal changes  
19 are expected to reflect the changes in the contributions of source components rather than the  
20 changes in the isotopic signals of the component itself (Knohl et al., 2005). However, SOM  
21  $\delta^{13}\text{C}$  can also change during the year with fresh plant material being more depleted in  $^{13}\text{C}$  than  
22 the older SOM components (Bowling et al., 2002), therefore fresh litter may contribute to the  
23 decreasing  $\delta^{13}\text{C}$  of the heterotrophic component. Drying of the surface layers can also modify  
24  $\delta^{13}\text{CO}_2$  since heterotrophic respiration could be restricted to the deeper layers of the soil  
25 (Moyes et al., 2010). Drying of the soil can also change the amount of CO<sub>2</sub> produced in the  
26 topsoil layer (Balogh et al., 2015) by allowing greater atmospheric invasion and thereby  
27 enriching soil air in  $^{13}\text{C}$  (Phillips and Nickerson, 2010). The disequilibrium between the  
28 measured isotopic composition and the isotopic composition of the respiratory source could  
29 be significant especially in tracer experiments (Gamnitzer et al., 2011) but it is assumed to be  
30 less pronounced in open chamber measurements due to the steady-state diffusion (Nickerson  
31 et al., 2013).

32 Uncertainties in estimating the contributions of soil respiration components could be reduced  
33 by a combination of different methodologies (Risk et al., 2012). The question we are asking

1 is: which of the investigated soil respiration components (autotrophic - including rhizospheric  
2 and mycorrhizal fungi - and heterotrophic components) of the dry grasslands dominates  
3 during drought? Our hypothesis was that autotrophic respiration would be reduced linearly  
4 with photosynthesis, whereas heterotrophic respiration might not be affected as strongly,  
5 resulting in a net loss of C from the soil carbon reservoir. In order to achieve our goals we  
6 used an experimental approach based on the physical separation of soil respiration  
7 components combined with measurements of soil CO<sub>2</sub> efflux and its isotopic (<sup>13</sup>C) signal.

8

## 9 **2 Methods**

### 10 **2.1 Site description**

11 The vegetation at the Bugac site (46.69° N, 19.6° E, 114 m above sea level) is a dry sandy  
12 grassland dominated by *Festuca pseudovina*, *Carex stenophylla* and *Cynodon dactylon* and it  
13 was under extensive grazing for 20 years prior to our study. Ten-year mean annual  
14 precipitation (2004-2013) was 575 mm and the mean annual temperature reached 10.4 °C.  
15 The soil is a chernozem type sandy soil with high organic carbon content (Balogh et al.,  
16 2015).

### 17 **2.2 Spatial separation of soil CO<sub>2</sub> efflux components**

18 In 2010 ten soil cores (160 mm in diameter and 800 mm in depth, one of them 600 mm in  
19 diameter) were excavated. The roots were removed and the root-free soil was packed back -  
20 layer by layer - into PVC tubes with the same dimensions. Four tubes were used to exclude  
21 both roots and mycorrhiza. Walls of another 6 tubes were partially removed and replaced by  
22 inox mesh (40 μm mesh size) to exclude roots while ensuring that the mycorrhiza filaments  
23 can grow into the tubes (Moyano et al., 2007). These root-free and root- and mycorrhiza-free  
24 soil cores were settled at a distance of 6 m from the eddy covariance tower to the south  
25 direction (Fig. S1.). The distance between the soil cores/tubes was 50 cm.

26 Soil CO<sub>2</sub> efflux and its isotopic signal were measured in plots:

- 27 - with undisturbed soil (various positions, 36 positions in total within a ~4 m<sup>2</sup> plot): total soil  
28 respiration,  $R_{\text{soil}}$ ,  $\delta^{13}\text{C}_{R_{\text{soil}}}$ ,
- 29 - without roots and arbuscular mycorrhizal fungi (4 spatial replications) = heterotrophic  
30 component only,  $R_{\text{rme}}$ ,  $\delta^{13}\text{C}_{R_{\text{rme}}}$ ,

1 - with root-excluded soil (6 spatial replications) = without roots, but with arbuscular  
2 mycorrhizal fungi,  $R_{re}$ ,  $\delta^{13}C_{Rre}$ .

### 3 **2.3 Gas exchange measuring systems**

4 Three different gas exchange systems were used in our study: eddy-covariance system (EC),  
5 automated soil respiration measuring system (SRS) connected to an isotopic CO<sub>2</sub> analyser  
6 (cavity ring-down spectroscopy system, CRDS). The experimental area was in the EC  
7 footprint (Supplementary material Fig. S1) but the size of the EC flux footprint area was  
8 larger by several orders of magnitude than the area covered by the SRS. Care was taken  
9 during the establishment of the experiment to select a plot with the same average soil  
10 characteristics and vegetation cover as found in the EC footprint area. Hence, the net  
11 ecosystem exchange (NEE) and evapotranspiration (ET) estimates obtained in this way can be  
12 considered representative also for the small-scale SRS and isotope measurements.

13 Data from 15<sup>th</sup> May 2013 to 12<sup>th</sup> November 2013 (182 days) were analysed in the present  
14 study.

#### 15 **2.3.1 Eddy covariance setup**

16 The EC system at the Bugac site measured the CO<sub>2</sub> and H<sub>2</sub>O fluxes continuously from 2002.  
17 In dry years the grassland can turn into a net carbon source (Nagy et al., 2007) but the longer-  
18 term annual sums of NEE showed it to be a net sink, ranging from -171 to +106 g C m<sup>-2</sup> yr<sup>-1</sup>  
19 (Pintér et al., 2010) with a -100 g C m<sup>-2</sup> yr<sup>-1</sup> average.

20 The EC system consists of a CSAT3 sonic anemometer (Campbell Scientific, USA) and a Li-  
21 7500 (Licor Inc, USA) open-path infra-red gas analyser (IRGA), both connected to a CR5000  
22 data logger (Campbell Scientific, USA) via an SDM (synchronous device for measurement)  
23 interface. Additional measurements used in this study were: air temperature and relative  
24 humidity (HMP35AC, Vaisala, Finland), precipitation (ARG 100 rain gauge, Campbell, UK),  
25 global radiation (dual pyranometer, Schenk, Austria), incoming and reflected  
26 photosynthetically active radiation (SKP215, Campbell, UK), volumetric soil moisture  
27 content (CS616, Campbell, UK) and soil temperature (105T, Campbell, UK). These  
28 measurements were performed as described by Nagy et al. (2007) and Pintér et al. (2010).  
29 Fluxes of sensible and latent heat and CO<sub>2</sub> were processed using an IDL program after Barcza  
30 et al. (2003) adopting the CarboEurope IP methodology. For a detailed description of data  
31 processing and gap-filling see Nagy et al. (2007) and Farkas et al. (2011).

### 1 **2.3.2 Soil respiration system**

2 The 10 chamber automated soil respiration system was set up in July 2011. The system is an  
3 open dynamic one, consisting of an SBA-4 infrared gas analyser (PPSystems, UK), pumps,  
4 flow meters (D6F-01A1-110, Omron Co., Japan), electro-magnetic valves, and PVC/metal  
5 soil chambers (Fig. S3). The chambers were 10.4 cm high with a diameter of 5 cm, covering a  
6 soil surface area of 19.6 cm<sup>2</sup>. The flow rate through the chambers was 300 ml min<sup>-1</sup>, replacing  
7 the air in the chamber in 40 seconds. The PVC chambers were enclosed in a white metal  
8 cylinder with 2 mm airspace in between to stabilize the chamber and to prevent warming by  
9 direct radiation. Four vent holes with a total area of 0.95 cm<sup>2</sup> were drilled on the top of the  
10 chambers. Vent holes also served to allow precipitation to drip into the chambers. Chamber  
11 walls exceeded the chamber top by 3 mm directing precipitation to the vent holes. The system  
12 caused minor disturbances in the soil structure and the spatial structure of the vegetation. It  
13 was applied without cutting the leaves/shoots of the plants, so it did not disturb transport  
14 processes taking place inside the plant stems and roots. It was suitable for continuous, long-  
15 term unattended measurements of soil CO<sub>2</sub> efflux and was also used in previous experiments  
16 (Balogh et al., 2015; Nagy et al., 2011). The soil respiration chambers contained no standing  
17 aboveground plant material. Before the study the system was tested on a calibration tank  
18 (CzechGlobe, Brno, Czech Republic) against known fluxes ( $F_{\text{SRS}} = 0.98 \times F_{\text{calibration\_tank}}$ ,  
19  $R^2 = 0.92$ ,  $n = 86$ ) and it was also compared to a LI-6400 system at the study site ( $F_{\text{SRS}} =$   
20  $0.92 \times F_{\text{LI6400}}$ ,  $R^2 = 0.92$ ,  $n = 36$ ).

21 Other studies (Nickerson et al., 2013; Risk et al., 2011) also used this chamber size, arguing  
22 that these chambers could be placed between the plants in grasslands, while larger chambers  
23 might create a non-representative surface due to the cutting necessary for placing the  
24 chambers on the ground (Risk et al., 2011).

25  $R_{\text{soil}}$  was measured by 6 SRS chambers, while  $R_{\text{rme}}$  and  $R_{\text{re}}$  were measured by 2-2 SRS  
26 chambers, respectively.

### 27 **2.3.3 Isotopic (<sup>13</sup>CO<sub>2</sub>) measurements**

28 A Picarro G1101-i gas analyser (CRDS, Picarro Inc., CA, USA) was attached to the soil  
29 respiration system from May to November in 2013. This CRDS system measured the isotopic  
30 composition inside the chambers and in the reference air. Reference air was sampled 10 cm  
31 above the surface in the foliage of plants. The SRS sequentially measured each of the 10  
32 chambers for 3 minutes. Every second chamber was additionally probed for isotopic signature

1 measurements by the CRDS (3 minutes), followed by reference air measurements for another  
2 3 minutes. Thus, the isotopic measurements of 5 chambers took 30 minutes in a single cycle.  
3 The CRDS integration time was set at 10 seconds, thus the CRDS provided 18 measurement  
4 points per chamber per cycle. Although the system response of the CRDS was clearly slower  
5 than the response of the SRS, the 3-minute duration was long enough to obtain robust results.  
6 Since CRDS followed the 3-minute intervals of SRS measurements no additional grace time  
7 had to be considered for the isotopic measurements.

8 Although this sampling scheme provides very good temporal coverage (replication in time), it  
9 is not perfectly addressing spatial variability and hence the position of each of the chambers  
10 was moved 11 times to randomly selected locations during the study period (i.e., every 2–3  
11 weeks) to obtain sequential spatial replications for each plot type (undisturbed, root-excluded,  
12 root- and mycorrhizal fungi excluded; see Supplementary material Figs S1 and S2). More  
13 precisely,  $\delta^{13}\text{C}_{\text{Rsoil}}$  was measured by 3 chambers at 36 (3 chambers x 12 positions) randomly  
14 selected positions within the experimental area (undisturbed soil, Supplementary material Fig.  
15 S1).  $\delta^{13}\text{C}_{\text{Rre}}$  was measured by 1 chamber which was moved to positions 1, 3, 5, 6, 8, 9 during  
16 the study period (Supplementary material Fig. S2).  $\delta^{13}\text{C}_{\text{Rrme}}$  was measured by 1 chamber  
17 which was moved to positions 2, 4, 7, 10 during the study period (Supplementary material  
18 Fig. S2).

19 Since contributions by the different soil CO<sub>2</sub> efflux components were estimated for five  
20 different periods within the study period distinguished by NEE, SWC values and isotopic  
21 signals (see Results), data for each estimation originated from 2-3 spatial replications.

## 22 **2.4 Data processing and modelling**

23 Data processing and statistical analysis were done in R (R Core Team 2014). Before  
24 calculating daily averages of  $\delta^{13}\text{C}$  values a filtering method was applied to each dataset. Out  
25 of each 180-second-long measurement on a certain chamber, the first 70 s (to measure a  
26 steady state signal) and the last 20 s were cut and the remaining values were used for further  
27 calculations. As reference and chamber air were measured sequentially reference values  
28 during chamber measurements were estimated by linear interpolation between the  
29 neighbouring reference sequences.

30 After the interpolation,  $\delta^{13}\text{C}$  values of the soil CO<sub>2</sub> efflux were calculated using the isotopic  
31 mass balance approach in each plot:

$$32 \quad \delta^{13}\text{C}_R = \frac{\delta^{13}\text{C}_{out} \times c_{out} - \delta^{13}\text{C}_{in} \times c_{in}}{c_{out} - c_{in}} \quad (1)$$



1 where  $\delta^{13}\text{C}_{\text{out}}$  and  $\delta^{13}\text{C}_{\text{in}}$  are the isotopic signature of the outgoing and incoming air of the  
2 chamber and  $c_{\text{out}}$  and  $c_{\text{in}}$  are the  $\text{CO}_2$  concentrations of the outgoing and incoming air of the  
3 chamber, respectively.

$$4 \quad \delta^{13}\text{C} = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \quad (2)$$

5 and  $R$  stands for the  $^{13}\text{C}:^{12}\text{C}$  isotope ratio of the sample and the international VPDB standard  
6 (0.011182), respectively.

7 Individual measurements were filtered out by using a moving-window procedure if the  
8 investigated value (at the window center) was outside the range of the mean  $\pm$  median  
9 absolute deviation of the values in a 10 day moving window. This filtering procedure left an  
10 overall data availability of 68-70%. Daily averages were calculated using the remaining data.

11 To determine the isotopic signature of the ecosystem respiration ( $R_{\text{eco}}$ ), Keeling plots were  
12 constructed by plotting the night-time  $\delta^{13}\text{C}$  values measured 10 cm over the surface against  
13 the inverse of the  $\text{CO}_2$  concentration. The extrapolated y-intercept of the linear regression was  
14 used as  $\delta^{13}\text{C}_{R_{\text{eco}}}$  values.

15 Total soil  $\text{CO}_2$  efflux was separated isotopically into its components. We defined the  
16 components following the terminology presented by Moyano et al. (2009):

17 Heterotrophic respiration= microbial respiration from litter and SOM decomposition.

18 Autotrophic respiration= mycorrhizospheric respiration including rhizospheric and  
19 mycorrhizal fungi components.

20 Rhizospheric respiration= respiration of roots and root-associated microorganisms in the  
21 rhizosphere, not including mycorrhizal fungi.

22 Two-source mixing models were used to estimate the fraction ( $a$ ) of the rhizospheric and ( $b$ )  
23 mycorrhizospheric components based on the measured isotopic signals:

$$24 \quad \delta^{13}\text{C}_{R_{\text{soil}}} = a \times \delta^{13}\text{C}_{R_{\text{rhizo}}} + (1 - a) \times \delta^{13}\text{C}_{R_{\text{re}}} \quad (3)$$

$$25 \quad \delta^{13}\text{C}_{R_{\text{soil}}} = b \times \delta^{13}\text{C}_{R_{\text{mycrhiz}}} + (1 - b) \times \delta^{13}\text{C}_{R_{\text{rme}}} \quad (4)$$

26 where  $\delta^{13}\text{C}_{R_{\text{soil}}}$  is the  $\delta^{13}\text{C}$  of the total soil  $\text{CO}_2$  efflux,  $\delta^{13}\text{C}_{R_{\text{re}}}$  is the  $\delta^{13}\text{C}$  of the root-excluded  
27 soil,  $\delta^{13}\text{C}_{R_{\text{rme}}}$  is the  $\delta^{13}\text{C}$  of the root- and mycorrhiza excluded soil (heterotrophic respiration),  
28  $a$  is the fraction of the rhizospheric component ( $R_{\text{rhizo}}$ ) and  $b$  is the fraction of the  
29 mycorrhizospheric component ( $R_{\text{mycrhiz}}$ ) to the total soil efflux. According to these equations  
30  $1-b$  represents the ratio of heterotrophic respiration component to the total soil efflux and  $b-a$   
31 represents the ratio of mycorrhizal fungi component.

32  $\delta^{13}\text{C}_{R_{\text{rhizo}}}$  value was estimated by plotting  $\delta^{13}\text{C}_{R_{\text{soil}}}$  values against the  $R_{\text{re}}/R_{\text{soil}}$  ratio  
33 (Supplementary material Fig. S3b). Since  $R_{\text{re}}/R_{\text{soil}}$  is hypothetically zero when only

1 rhizospheric respiration is present, y-intercept of the linear regression was assumed as  
 2  $\delta^{13}\text{C}_{\text{Rrhizo}}$ .  $\delta^{13}\text{C}_{\text{Rmycrhiz}}$  was estimated using the same approach (Supplementary material Fig.  
 3 S3a),  $\delta^{13}\text{C}_{\text{Rsoil}}$  values were plotted against the  $R_{\text{rme}}/R_{\text{soil}}$  ratio and y-intercept of the linear  
 4 regression was assumed as  $\delta^{13}\text{C}_{\text{Rmycrhiz}}$ . Similarly,  $\delta^{13}\text{C}_{\text{Rre}}$  values were plotted against the  
 5  $R_{\text{rme}}/R_{\text{re}}$  ratio and y-intercept of the linear regression was assumed as  $\delta^{13}\text{C}_{\text{Rmyc}}$  (Supplementary  
 6 material Fig. S4c) but this value was not used in further calculations.

7 Contributions of rhizospheric, mycorrhizal fungi and heterotrophic respirations to total soil  
 8 respiration were calculated by the mixing models applied on subsets (periods) of the dataset  
 9 of the total study period. Estimated values of rhizospheric ( $R_{\text{rhizo}}$ ), mycorrhizal fungi ( $R_{\text{myc}}$ )  
 10 and heterotrophic respiration ( $R_{\text{het}}$ ) were calculated by multiplying the measured  $R_{\text{soil}}$  rates  
 11 (total soil respiration) with the estimated fractional contributions (F) of each component as  
 12 follows:

$$13 \quad R_{\text{het}} = R_{\text{soil}} \times F_{\text{het}} \quad (5)$$

$$14 \quad R_{\text{rhizo}} = R_{\text{soil}} \times F_{\text{rhizo}} \quad (6)$$

$$15 \quad R_{\text{myc}} = R_{\text{soil}} \times F_{\text{myc}} \quad (7)$$

16 where,  $F_{\text{het}}$ ,  $F_{\text{rhizo}}$  and  $F_{\text{myc}}$  are the fraction of the heterotrophic, rhizospheric and mycorrhizal  
 17 respiration in total soil respiration, respectively.

## 18 **2.5 Microbial investigations**

19 Soil samples for the microbial investigations were taken after the gas exchange measurements  
 20 in May 2014 to avoid the disturbance of the measurements by sampling the soil. Sampling  
 21 date was chosen considering the maximum of the carbon sequestration capacity of the  
 22 investigated grassland (Nagy et al., 2007). 5-5 samples were taken from 5 soil layers (0-10  
 23 cm, 10-20 cm, 20-30 cm, 30-40 cm and 40-50 cm) in each plot.

24 Determination of AM fungal hyphal length in the soil was based on the methods of Bååth and  
 25 Söderström (1979) using separation by wet-sieving and centrifugation. The separated fungal  
 26 hyphae were stained using agar solution (0.75%) containing trypan blue (0.05%) then dried  
 27 for 24 h at 70°C. The hyphal length was measured in the dried agar film by the intersection  
 28 method (Tennant, 1975) under a binocular microscope.

29 The fluorescein diacetate (FDA) hydrolysis assay was used to estimate the total microbial  
 30 activity in soil samples and expressed as mg fluorescein released  $\text{kg}^{-1}$  dry soil (Adam and  
 31 Duncan, 2001).

## 1 **2.6 Uncertainty assessment**

2 Isotopic signals of soil respired CO<sub>2</sub> were studied extensively but several uncertainties related  
3 to the different methods were also revealed. Steady-state methods were found to provide more  
4 robust estimates than static chambers but still charged with biases (e.g. diffusive fractionation,  
5 Nickerson and Risk 2009). Open systems have the advantage of unattended automatic  
6 measurement collecting large amount of data but are less sensitive to small isotopic  
7 differences (Midwood and Millard, 2011).

8 In our study  $\delta^{13}\text{C}_{\text{Reco}}$  estimates were independent of chamber related biases, using night-time  
9  $\delta^{13}\text{CO}_2$  and CO<sub>2</sub> concentration data of the free air over the surface for the calculation  
10 (Keeling-plot approach). This approach gave similar results to the chamber-based  
11 measurements, providing also partial verification of the latter ones. Moreover, isotopic  
12 measurements were independent on soil CO<sub>2</sub> efflux measurements, since IRGA and CRDS  
13 systems took different air samples from the same soil chambers. Isotopic data together with  
14 CO<sub>2</sub> efflux rates were collected during 1980 measurement cycles on 182 days in order to have  
15 robust estimates of isotopic signals.

16 A C4 grass (*Cynodon dactylon*) was also present in the study site potentially modifying the  
17  $\delta^{13}\text{C}$  of the respired CO<sub>2</sub>. Its cover was about 10% in the pasture (Koncz et al., 2014) but it  
18 was less frequent (i.e. less than 5%) in the experimental area. Calculated uncertainties of the  
19 relative contributions of each components (rhizospheric, mycorrhizal fungi and heterotrophic)  
20 contain the uncertainty due to a possible 5% contribution by the C4 grass. The isotopic signal  
21 of CO<sub>2</sub> efflux by the C4 plant was supposed to be -14‰.

22 In order to estimate the uncertainty of the measurements and estimated contributions by the  
23 different components to the total soil respiration random errors of each factor (CO<sub>2</sub>  
24 concentrations, isotopic compositions, model fit errors and possible C4 contribution) were  
25 propagated by Gaussian error propagation (Lo, 2005).

## 26 **3 Results**

### 27 **3.1 Meteorological conditions, NEE, ET, soil CO<sub>2</sub> efflux, $\delta^{13}\text{C}$ of CO<sub>2</sub> efflux**

28 The end of May and the beginning of June was the most productive period in the year due to  
29 ample water availability with the lowest NEE (strongest carbon sink activity) and highest  
30 evapotranspiration (ET) values being measured in this period (Fig. 1a). It rained only a few  
31 times from the end of June to 19<sup>th</sup> August (total precip: 10 mm) and the accompanying high  
32 temperature resulted in drought. Daily minimum NEE was around zero at the end of July and

1 in August. Rain events after the drought period had significant effects on soil CO<sub>2</sub> effluxes  
2 (Fig. 1c). There was a second active period following autumn rains but CO<sub>2</sub> uptake and ET  
3 were smaller than in May or June.

4 R<sub>soil</sub> was the highest among the soil CO<sub>2</sub> effluxes, while R<sub>rme</sub> was the lowest, the average CO<sub>2</sub>  
5 effluxes in the whole study period were 5.0±2.1, 3.8±1.6 and 2.6±1.2 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>  
6 (mean±SD) in R<sub>soil</sub>, R<sub>re</sub> and R<sub>rme</sub>, respectively (Table 2). R<sub>re</sub> was sometimes higher than R<sub>soil</sub>,  
7 especially shortly after rain events. The lowest daily average total soil CO<sub>2</sub> efflux was  
8 measured in 15<sup>th</sup> August (2.22 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), while the lowest daily average R<sub>re</sub> and R<sub>rme</sub>  
9 values were observed in 2<sup>nd</sup> October (1.25 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) and 2<sup>nd</sup> November (1.04 μmol  
10 CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), respectively. The highest values of soil CO<sub>2</sub> effluxes were measured in May in  
11 all treatments (R<sub>soil</sub>, R<sub>re</sub> and R<sub>rme</sub>). Sudden increases in R<sub>re</sub> and R<sub>rme</sub> were observed shortly  
12 after rain events but R<sub>soil</sub> showed slower (but more persistent) response to precipitation (Fig.  
13 1c).

14 Isotopic signature of R<sub>eco</sub> was the lowest in May and June, increased in July and August and  
15 decreased again in October and November (Fig. 1e). δ<sup>13</sup>C<sub>Reco</sub> showed clear responses to  
16 precipitation pulses with sudden declines being observed during the rain events. Chamber-  
17 based δ<sup>13</sup>C<sub>Rsoil</sub> showed similar changes during the study period. δ<sup>13</sup>C<sub>Rrme</sub> and δ<sup>13</sup>C<sub>Rre</sub> showed  
18 large scatter during the whole study period with no clear and detectable trends (Fig. 1d).  
19 Differences between δ<sup>13</sup>C<sub>Rsoil</sub> and δ<sup>13</sup>C<sub>Rrme</sub> were the largest in the active period and the  
20 smallest under drought conditions.

21 According to the NEE, SWC values and isotopic signals we distinguished 5 periods within the  
22 study period: an active period from 15<sup>th</sup> May to 20<sup>th</sup> June, a drying (stress development)  
23 period from 21<sup>st</sup> June to 22<sup>nd</sup> July, a drought period from 23<sup>rd</sup> July to 19<sup>th</sup> August, a wetting  
24 (stress release) period from 20<sup>th</sup> August to 16<sup>th</sup> September and a re-greening (recovery) period  
25 from 17<sup>th</sup> September to the end of the study period (11<sup>th</sup> November) (Fig. 1).

### 26 **3.2 δ<sup>13</sup>C of the respiration components**

27 Fig 2. shows the measured and estimated δ<sup>13</sup>C values of the different soil CO<sub>2</sub> efflux  
28 components. δ<sup>13</sup>C<sub>Rrme</sub> was the highest, while δ<sup>13</sup>C<sub>Rsoil</sub> was the lowest, suggesting that it was  
29 the rhizospheric respiration that was the most substantially depleted, while heterotrophic  
30 respiration was the least depleted in <sup>13</sup>C. Mean values of δ<sup>13</sup>C<sub>Reco</sub>, δ<sup>13</sup>C<sub>Rsoil</sub>, δ<sup>13</sup>C<sub>Rre</sub> and  
31 δ<sup>13</sup>C<sub>Rrme</sub> were -27.9±0.5‰, -26.8±1.3‰, -26.4±1.8‰ and -25.7±2‰ (mean±SE),  
32 respectively. The estimated isotopic signals of the respiration of mycorrhizospheric

1 ( $\delta^{13}\text{C}_{\text{Rmycrhiz}}$ ), rhizospheric ( $\delta^{13}\text{C}_{\text{Rrhizo}}$ ) and mycorrhizal fungi components ( $\delta^{13}\text{C}_{\text{Rmyc}}$ ) were -  
2  $28.6\pm 1.6\text{‰}$ ,  $-28.9\pm 1.7\text{‰}$  and  $-27.2\pm 2.3\text{‰}$  (estimate $\pm$ SE), respectively (Fig. 2).

3 36% of the variation in  $\delta^{13}\text{C}_{\text{Rsoil}}$  was explained by SWC ( $\delta^{13}\text{C}_{\text{Rsoil}} = -0.1267 \times \text{SWC} - 25.537$ ,  
4  $R^2=0.36$ ,  $P<0.0001$ ), while only 3% of the variation of  $\delta^{13}\text{C}_{\text{Rrme}}$  was explained by SWC and  
5 there was no correlation between  $\delta^{13}\text{C}_{\text{Rre}}$  and SWC. Similar results were obtained between  $T_s$   
6 and the isotopic signals but the correlation was weaker ( $\delta^{13}\text{C}_{\text{Rsoil}} = 0.1056 \times T_s - 28.588$ ,  
7  $R^2=0.11$ ,  $P<0.0001$ ). Daily minimum NEE (NEE<sub>min</sub>, Fig. 1b) explained 29% of the variation  
8 in  $\delta^{13}\text{C}_{\text{Rsoil}}$  ( $\delta^{13}\text{C}_{\text{Rsoil}} = 0.0941 \times \text{NEE}_{\text{min}} - 26.245$ ,  $R^2=0.29$ ,  $P<0.0001$ ) but no correlation was  
9 found between NEE<sub>min</sub> and  $\delta^{13}\text{C}_{\text{Rrme}}$  and between NEE<sub>min</sub> and  $\delta^{13}\text{C}_{\text{Rre}}$ .

### 10 **3.3 Fraction of the different components in total soil respiration during the** 11 **vegetation period**

12 Two end-member mixing models (eq. 3 and 4) were used to estimate the relative contributions  
13 of rhizospheric, mycorrhizal fungi and heterotrophic components to total soil respiration  
14 during the study period. The estimated contributions by the different components were  
15  $50\pm 6\%$ ,  $13\pm 8\%$  and  $37\pm 6\%$  (mean $\pm$ SE) for the rhizospheric, mycorrhizal fungi and  
16 heterotrophic components, respectively. The autotrophic component (mycorrhizospheric  
17 component) of soil respiration showed significant decrease during the drying and drought  
18 periods. Rhizospheric component was the most sensitive to drying and drought. Average  
19 contributions by the rhizospheric component to total soil CO<sub>2</sub> efflux decreased from  $66\pm 7\%$   
20 (mean $\pm$ SE) in the active period to  $35\pm 13\%$  during the drought period (Fig. 3). After drought  
21 rhizospheric contributions increased again and become dominant during the re-greening  
22 period in autumn  $63\pm 7\%$  (mean $\pm$ SE). During the transient (drying and wetting) periods the  
23 rhizospheric contributions to the total soil CO<sub>2</sub> efflux were  $38\pm 11\%$  and  $46\pm 8\%$ , respectively.  
24 Relative mycorrhizal contributions were between 8-21% during the whole study period, with  
25 the highest contribution ( $21\pm 11\%$ ; mean $\pm$ SE) during the wetting period. Heterotrophic  
26 contributions to soil respiration were the lowest in the active period ( $21\pm 7\%$ ) and the highest  
27 under drought ( $54\pm 13\%$ ) (Fig. 3).

28 Changes in soil CO<sub>2</sub> effluxes showed similar responses to drying and drought conditions as  
29 isotopic signals. Average  $R_{\text{soil}}$  decreased by 60% (referenced to the average during the active  
30 period) as a response to drought, while  $R_{\text{re}}$  and  $R_{\text{rme}}$  showed declines of 56 and 52%  
31 respectively, suggesting that declines in root respiration were substantially larger than those in  
32  $R_{\text{soil}}$  (60%).

1 The estimated rates of rhizospheric, mycorrhizal fungi and heterotrophic components (eq. 5-7)  
2 are shown in Table 2. Pearson correlation coefficients pairing the estimated respiration rates  
3 and their possible driving variables (NEE, Ts, SWC) showed significant negative correlation  
4 between  $R_{rhiz}$  and NEE ( $R = -0.94$ ,  $p < 0.05$ ) and a significant positive correlation between  $R_{rhiz}$   
5 and SWC ( $R = 0.82$ ,  $p < 0.05$ ).  $R_{het}$  changed with Ts but the correlation was not significant.

### 6 **3.4 Microbial biomass and activity**

7 Hyphal length (on dry soil weight basis) was significantly lower in the upper layers of root-  
8 and mycorrhiza excluded soil than in undisturbed soil, while it was significantly higher in  
9 root-excluded plots at 10-20 cm depth. Hyphal length in the root-excluded soil was similar to  
10 undisturbed soil in the other soil layers. Fluorescein values were significantly lower in all soil  
11 layers in the root- and mycorrhiza excluded plots than in the undisturbed soil. Fluorescein  
12 values in the root-excluded plots were also lower than in undisturbed soil but this difference  
13 was not significant (Fig. 4).

14

## 15 **4 Discussion**

16 Our approach combined the root- and root- and mycorrhiza exclusion treatments with isotopic  
17 measurements. The aim of this combination was to assess the contributions made by the  
18 heterotrophic and autotrophic components in soil  $CO_2$  efflux of the undisturbed soil. Although  
19 the root- and mycorrhiza exclusion caused large disturbances in soil structure by inserting the  
20 tubes into the soil, we used these treatments only for identifying the isotopic signals of the  
21 investigated components. All of the estimated contributions to soil  $CO_2$  efflux by  
22 rhizospheric, mycorrhizal fungi and heterotrophic components were applied for the  
23 undisturbed soil.

### 24 **4.1 Estimated contributions made by the different components to the total** 25 **soil $CO_2$ efflux and effect of drought on $CO_2$ effluxes and $\delta^{13}C$ values**

26 While the percentages of the autotrophic component in the total soil  $CO_2$  efflux were  $63 \pm 6\%$   
27 on average (rhizospheric and mycorrhizal fungi components,  $50 \pm 6\%$  and  $13 \pm 8\%$ ,  
28 respectively) being much higher than the average percentage of the heterotrophic ( $37 \pm 6\%$ )  
29 component, the contributions by the different components showed significant changes during  
30 the growing season. In other studies conducted in grassland ecosystems the estimated yearly

1 average ratio of the autotrophic component was found to be lower accounting for 38-52% of  
2 the total soil respiration (Bao et al., 2010; Heinemeyer et al., 2012), while reaching 74%  
3 during the growing season in a prairie grassland (Gomez-Casanovas et al., 2012) and 60-74%  
4 in an arid perennial grassland (Carbone et al., 2008). Our study was conducted from May to  
5 the beginning of November, therefore we can assume considering the lower vegetation  
6 activity in the dormant season (Nagy et al., 2007) that the contribution of the autotrophic  
7 component could be lower while that of the heterotrophic component higher for the whole  
8 year than the estimations for the growing season.

9 Soil CO<sub>2</sub> effluxes decreased in all treatments ( $R_{\text{soil}}$ ,  $R_{\text{re}}$ ,  $R_{\text{rme}}$ ) under dry conditions, the largest  
10 decline being observed in total soil respiration ( $R_{\text{soil}}$ ), therefore a strong response of the  
11 autotrophic component to drought could be assumed. The measured isotopic signals also  
12 showed decreasing autotrophic contributions to CO<sub>2</sub> efflux during soil drying.  $\delta^{13}\text{C}_{\text{Rsoil}}$   
13 showed negative responses to SWC and was more enriched when SWC was low, while  $\delta^{13}\text{C}$   
14 of the root- and mycorrhiza-excluded respiration ( $R_{\text{rme}}$ ) showed no response. Since  $\delta^{13}\text{C}_{\text{Reco}}$   
15 was the lowest of the measured isotopic signals it can be assumed that the isotopic signals of  
16 the above-ground respiration could be the most depleted  $\delta^{13}\text{C}$ . Therefore, the observed  
17 increase in  $\delta^{13}\text{C}_{\text{Reco}}$  and  $\delta^{13}\text{C}_{\text{Rsoil}}$  values during the drying period and during the drought also  
18 showed the decline of both the above- and below-ground autotrophic components. The same  
19 phenomenon was shown by the modelling results with the smallest contribution made by the  
20 rhizospheric component estimated for the drought period (35±13%; mean±SE), while the  
21 highest for the active period (66±7%; mean±SE). Fractions of the heterotrophic respiration  
22 were the highest during drought (54±13% mean±SE) and the mycorrhizal fungi respiration  
23 showed only a small decrease during drought compared to the active period (from 13±10%;  
24 to 11±18%;), suggesting that the non root-associated microbes and mycorrhizal filaments  
25 were less sensitive to water shortages than the rhizosphere. Soil aggregates are expected to  
26 provide micro-habitats for soil organisms that should be moist enough for those organisms to  
27 thrive even under drought (Davidson et al., 2012). Since there was an absence in plant  
28 photosynthetic supply during drought period, mycorrhizal fungi component is expected to use  
29 stored carbon for respiration (van der Heijden et al., 2008).

30 Low  $\delta^{13}\text{C}_{\text{Rsoil}}$  and  $\delta^{13}\text{C}_{\text{Reco}}$  values were measured in the wetting and re-greening periods due to  
31 the drought-induced fall of the fresh litter to the surface as fresh plant material could be more  
32 depleted than the old litter (Bowling et al., 2002). The declines in  $\delta^{13}\text{C}_{\text{Rsoil}}$  and  $\delta^{13}\text{C}_{\text{Reco}}$   
33 immediately after the rain events during drying and drought periods could also be explained

1 by the wetting of the litter layer, exposing relatively fresh substrate to degradation for short  
2 periods. This phenomenon could also cause an overestimation in contributions made by the  
3 depleted components (rhizospheric) during rain events. Since the rhizospheric contribution  
4 estimated for the re-greening period was high it is assumed that this result was obtained partly  
5 due to the increased amount of fresh litter. Similar results were obtained in a tallgrass prairie  
6 by Gomez-Casanovas et al. (2012), where the autotrophic components were more sensitive to  
7 soil drying than the heterotrophic ones. In contrast, Carbone et al. (2008) found more sensitive  
8 response by the heterotrophic component in an arid (<150 mm annual precipitation) perennial  
9 grassland. Fractions of autotrophic components were reported to increase in response to  
10 drought in a woodland ecosystem, supposing that the signature of the recent photosynthetic  
11 supply became enriched during drought and that could also explain the increase in the soil  
12 respired CO<sub>2</sub> (Casals et al., 2011). A drought induced increase in  $\delta^{13}\text{C}$  of root respiration of  
13 trees was also assumed in a recent study (Risk et al., 2012), suggesting that the isotopic  
14 signals of the assimilates, thereby the signals of the autotrophic component might also  
15 increase. In our study,  $R_{\text{rme}}/R_{\text{soil}}$  showed significant positive correlation with  $\delta^{13}\text{C}_{R_{\text{soil}}}$  (the  
16 regression was used to estimate  $\delta^{13}\text{C}_{R_{\text{mycrhiz}}}$ , Supplementary material Fig S4), so  $\delta^{13}\text{C}_{R_{\text{soil}}}$  was  
17 high if the fraction of heterotrophic CO<sub>2</sub> efflux to the total soil CO<sub>2</sub> efflux was found to be  
18 high. Moreover, NEE<sub>min</sub> values were close to zero during drought (average daily minimum  
19 NEE was  $-0.91 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) showing the lack of the photosynthetic supply in this  
20 period. Photosynthetic CO<sub>2</sub> uptake of this vegetation was found to be sensitive to drought  
21 conditions (Nagy et al., 2007) and it can act as a driver of the soil CO<sub>2</sub> production and efflux  
22 (Balogh et al., 2015). The observed strong correlation between the estimated rhizospheric  
23 respiration and NEE can also be explained by the interacting effects of drought and  
24 photosynthetic supply of respiration. These findings support that in the grasslands under study  
25 the autotrophic respiration component was more sensitive to soil drying and its activity  
26 determined the isotopic signals of the total soil respiration during the study period.

27 According to these studies and to our results we can assume that the different vegetation types  
28 may respond differently to drought: woodlands may increase their autotrophic contribution  
29 while grasslands may decrease it (Casals et al., 2011; Gomez-Casanovas et al., 2012; Risk et  
30 al., 2012). Plants with different rooting habits have different water availability during dry  
31 periods (van der Molen et al., 2011), which could explain the differences between the  
32 different ecosystems in their response to drought.



## 1 **4.2 Measured and estimated isotopic signals of the soil respiration** 2 **components**

3 Measured and calculated  $\delta^{13}\text{C}$  values of the different respiration components showed  
4 differences similar to the ones reviewed by Bowling et al. (2008).  $\delta^{13}\text{C}_{\text{Reco}}$  (containing also  
5 the signal from above ground green biomass) was the most depleted, while  $\delta^{13}\text{C}_{\text{Rrme}}$   
6 (heterotrophic components only) was the least depleted.  $\delta^{13}\text{C}$  of the root- and mycorrhiza  
7 excluded respiration was similar to SOM  $\delta^{13}\text{C}$  measured in a previous study (Denef et al.,  
8 2013): -25‰ and -26‰ in the topsoil layers (without the litter layer).  $\text{CO}_2$  effluxes from  
9 mycorrhizal fungi were expected to be more enriched in  $^{13}\text{C}$  relative to the total soil  
10 respiration (about +3‰, Bowling et al. 2008). Estimated  $\delta^{13}\text{C}$  of mycorrhizal fungi  
11 component was  $-27.2 \pm 2.3\text{‰}$  (estimate  $\pm$  SE), which is 1.7‰ higher than the rhizospheric  
12 component ( $-28.9 \pm 1.7\text{‰}$ ; estimate  $\pm$  SE).

13 In our study neither  $\delta^{13}\text{C}_{\text{Rrme}}$  values (heterotrophic respiration), nor  $\delta^{13}\text{C}_{\text{Rre}}$  values  
14 (heterotrophic+mycorrhizal fungi respiration) showed correlation with SWC but  $\delta^{13}\text{C}_{\text{Rsoil}}$   
15 (total soil respiration) showed significant negative correlation with SWC. We can assume that  
16  $\delta^{13}\text{C}$  of heterotrophic respiration was not influenced by SWC changes during the growing  
17 season as it was found also by other studies (Phillips and Nickerson, 2010; Risk et al., 2012).  
18 Furthermore, the lack of correlation with the present study also suggests that soil moisture  
19 induced changes in diffusivity (disequilibrium effect due to changing soil moisture) were not  
20 large enough to affect the measured  $\delta^{13}\text{C}$  values.

## 21 **4.3 Microbial investigations**

22 High hyphal density was maintained in  $\text{R}_{\text{re}}$  plots and low but still significant microbial  
23 activities (SOM decomposition) were detected in  $\text{R}_{\text{rme}}$  plots, therefore the measured  $\delta^{13}\text{C}$   
24 values characterized the sources of the root-free ( $\delta^{13}\text{C}_{\text{Rre}}$ ) and root- and mycorrhiza-free  
25 ( $\delta^{13}\text{C}_{\text{Rrme}}$ ) soils. The fact that very high amounts of hyphae were found in the root-excluded  
26 soil in the 10-20 cm layer proved that mycorrhizal fungi filaments were able to penetrate  
27 through the inox mesh and supported significant microbial activity. Grasses have extensive  
28 fibrous root systems with moderate to high levels of mycorrhizal colonization (van der  
29 Heijden et al., 2015). The range of AM hyphal lengths found in this study ( $1.9\text{--}8.8 \text{ m g}^{-1}$  soil)  
30 were similar to those reported in the literature (e.g. Mummey and Rillig 2008). The higher  
31 hyphal densities found in root-free soil might have been related to the higher availability of  
32 SOM-derived nutrients and to more space without the roots (i.e. lack of competition).

1 According to our results significant amount of CO<sub>2</sub> was respired from mycorrhizal filaments  
2 in the undisturbed soil, having a 12-31% share in the respiration carried out by the autotrophic  
3 component.

4 Values of fluorescein in root-excluded plots were similar to those measured in the undisturbed  
5 soil probably because hyphae of AM fungi provide an increased area for interaction with  
6 other microorganisms (hyphosphere, Andrade et al. 1997), but were much lower in root- and  
7 mycorrhiza excluded soil. These results support the component estimations showing the  
8 significant activities of root-associated microorganisms.

## 9 **5 Conclusions**

10 In the dry grasslands investigated in our study all three components of the soil CO<sub>2</sub> effluxes  
11 decreased, following different dynamics under drought conditions. Both the measured CO<sub>2</sub>  
12 effluxes and the isotopic signals showed similar results regarding the component responses.  
13 The strongest decrease in response to drought was seen in rhizospheric respiration (relative  
14 contribution to the total respiration decreased from 66±7% to 35±13%; mean±SE), while the  
15 relative contribution to the total soil respiration by the heterotrophic components increased  
16 during soil drying. During drought the contribution of the heterotrophic component was found  
17 to be the highest (54±8%; mean±SE). Mycorrhizal fungi respiration had its highest share in  
18 soil respiration (21±11%; mean±SE) in the wetting period after drought. According to these  
19 results the autotrophic component of the soil respiration is more sensitive to drought than the  
20 heterotrophic one in the dry grassland ecosystem studied. Thus, carbon source activities  
21 during drought periods identified by NEE measurements originated from carbon sources  
22 already stored, thereby decreasing the carbon content of the soil.

23 Drought events are expected to be more frequent in Central Europe in the future, and it is  
24 expected that the productivity of grassland ecosystems may strongly respond to projected  
25 dryness, influencing the carbon cycle of the ecosystems. Since potential productivity is  
26 generally linked to soil carbon content a pronounced decrease in soil organic matter due to the  
27 enhanced activity of the heterotrophic component under drought may directly affect the long  
28 term productivity of grasslands.

29

## 30 **Author contributions**

1 J. Balogh, M. Papp, K. Pintér and Z. Nagy conceived and designed the experiment, M. Papp,  
2 K. Pintér and K. Posta performed the experiment, J. Balogh, Sz. Fóti, W. Eugster and Z. Nagy  
3 analyzed the data and wrote the paper, but all co-authors contributed to writing.

4

## 5 **Acknowledgements**

6 The authors are grateful to the editor and to the three anonymous reviewers who provided  
7 constructive comments and suggestions that improved the paper. The authors gratefully  
8 acknowledge the financial support of the projects OTKA-PD 100575, OTKA-PD 105944,  
9 Research Centre of Excellence (11476-3/2016/FEKUT) and KTIA\_AIK\_12-1-2012-0012.  
10 János Balogh and Szilvia Fóti acknowledge the support of the János Bolyai Research  
11 Scholarship of the Hungarian Academy of Sciences. Isotopic measurements were supported  
12 by Gábor Baross Programme (REG-KM-09-1-2009-0029).

## 13 **References**

- 14 Adam, G. and Duncan, H.: Development of a sensitive and rapid method for the measurement  
15 of total microbial activity using fluorescein diacetate (FDA) in a range of soils, *Soil Biol.*  
16 *Biochem.*, 33(7), 943–951, 2001.
- 17 Andrade, G., Mihara, K. L., Linderman, R. G. and Bethlenfalvay, G. J.: Bacteria from  
18 rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi, *Plant Soil*,  
19 192(1), 71–79, doi:10.1023/A:1004249629643, 1997.
- 20 Bååth, E. and Söderström, B.: The significance of hyphal diameter in calculation of fungal  
21 biovolume, *Oikos*, 33, 11–14, 1979.
- 22 Bahn, M., Rodeghiero, M., Anderson-Dunn, M., Dore, S., Gimeno, C., Drösler, M., Williams,  
23 M., Ammann, C., Berninger, F., Flechard, C., Jones, S., Balzarolo, M., Kumar, S., Newesely,  
24 C., Priwitzer, T., Raschi, A., Siegwolf, R., Susiluoto, S., Tenhunen, J., Wohlfahrt, G. and  
25 Cernusca, A.: Soil Respiration in European Grasslands in Relation to Climate and Assimilate  
26 Supply., *Ecosystems*, 11(8), 1352–1367, doi:10.1007/s10021-008-9198-0, 2008.
- 27 Balogh, J., Pintér, K., Fóti, S., Cserhalmi, D., Papp, M. and Nagy, Z.: Dependence of soil  
28 respiration on soil moisture, clay content, soil organic matter, and CO<sub>2</sub> uptake in dry  
29 grasslands, *Soil Biol. Biochem.*, 43(5), 1006–1013, doi:10.1016/j.soilbio.2011.01.017, 2011.
- 30 Balogh, J., Fóti, S., Pintér, K., Burri, S., Eugster, W., Papp, M. and Nagy, Z.: Soil CO<sub>2</sub> efflux  
31 and production rates as influenced by evapotranspiration in a dry grassland, *Plant Soil*, 388(1-  
32 2), 157–173, doi:10.1007/s11104-014-2314-3, 2015.
- 33 Bao, F., Zhou, G., Wang, F. and Sui, X.: Partitioning soil respiration in a temperate desert  
34 steppe in Inner Mongolia using exponential regression method, *Soil Biol. Biochem.*, 42(12),  
35 2339–2341, doi:10.1016/j.soilbio.2010.08.033, 2010.
- 36 Barcza, Z., Haszpra, L. and Kondo, H.: Carbon exchange of grass in Hungary, *Tellus B*, 187–  
37 196 doi: 10.1034/j.1600-0889.2003.00014.x/full, 2003.
- 38 Bardgett, R. D., Freeman, C. and Ostle, N. J.: Microbial contributions to climate change  
39 through carbon cycle feedbacks., *ISME J.*, 2(8), 805–814, doi:10.1038/ismej.2008.58, 2008.

- 1 Blagodatsky, S. and Smith, P.: Soil physics meets soil biology: Towards better mechanistic  
2 prediction of greenhouse gas emissions from soil, *Soil Biol. Biochem.*, 47, 78–92,  
3 doi:10.1016/j.soilbio.2011.12.015, 2012.
- 4 Bowling, D., McDowell, N., Bond, B., Law, B. and Ehleringer, J.:  $^{13}\text{C}$  content of ecosystem  
5 respiration is linked to precipitation and vapor pressure deficit, *Oecologia*, 131(1), 113–124,  
6 doi:10.1007/s00442-001-0851-y, 2002.
- 7 Bowling, D. R., Pataki, D. E. and Randerson, J. T.: Carbon isotopes in terrestrial ecosystem  
8 pools and  $\text{CO}_2$  fluxes., *New Phytol.*, 178(1), 24–40, doi:10.1111/j.1469-8137.2007.02342.x,  
9 2008.
- 10 Carbone, M. S., Winston, G. C. and Trumbore, S. E.: Soil respiration in perennial grass and  
11 shrub ecosystems: Linking environmental controls with plant and microbial sources on  
12 seasonal and diel timescales, *J. Geophys. Res.*, 113(G2), G02022,  
13 doi:10.1029/2007JG000611, 2008.
- 14 Carbone, M. S., Still, C. J., Ambrose, A. R., Dawson, T. E., Williams, A. P., Boot, C. M.,  
15 Schaeffer, S. M. and Schimel, J. P.: Seasonal and episodic moisture controls on plant and  
16 microbial contributions to soil respiration., *Oecologia*, 167(1), 265–78, doi:10.1007/s00442-  
17 011-1975-3, 2011.
- 18 Casals, P., Lopez-Sangil, L., Carrara, A., Gimeno, C. and Nogués, S.: Autotrophic and  
19 heterotrophic contributions to short-term soil  $\text{CO}_2$  efflux following simulated summer  
20 precipitation pulses in a Mediterranean dehesa, *Global Biogeochem. Cycles*, 25(3), GB3012,  
21 doi:10.1029/2010GB003973, 2011.
- 22 Chen, S., Lin, G., Huang, J. and Jenerette, G. D.: Dependence of carbon sequestration on the  
23 differential responses of ecosystem photosynthesis and respiration to rain pulses in a semiarid  
24 steppe, *Glob. Chang. Biol.*, 15(10), 2450–2461, doi:10.1111/j.1365-2486.2009.01879.x, 2009.
- 25 Davidson, E. A., Samanta, S., Caramori, S. S. and Savage, K.: The Dual Arrhenius and  
26 Michaelis-Menten kinetics model for decomposition of soil organic matter at hourly to  
27 seasonal time scales, *Glob. Chang. Biol.*, 18(1), 371–384, doi:10.1111/j.1365-  
28 2486.2011.02546.x, 2012.
- 29 Deneff, K., Galdo, I., Venturi, A. and Cotrufo, M.: Assessment of Soil C and N Stocks and  
30 Fractions across 11 European Soils under Varying Land Uses., *Open J. Soil Sci.*, 3, 297–313,  
31 2013.
- 32 Epron, D.: Separating autotrophic and heterotrophic components of soil respiration: lessons  
33 learned from trenching and related root exclusion experiments, in *Soil Carbon Dynamics: An  
34 Integrated Methodology*, edited by M. Bahn, A. Heinemeyer, and W. Kutsch, pp. 289–310,  
35 Cambridge University Press., 2009.
- 36 Farkas, C., Alberti, G., Balogh, J., Barcza, Z., Birkas, M., Czobel, S., Davis, K. J., Fuehrer,  
37 E., Gelybo, G., Grosz, B., Kljun, N., Koos, S., Machon, A., Marjanovic, H., Nagy, Z.,  
38 Peressotti, A., Pinter, K., Toth, E. and Horvath, L.: Methodologies, in *Atmospheric  
39 Greenhouse Gases: The Hungarian Perspective.*, edited by L. Haszpra, pp. 65–90, Springer,  
40 New York., 2011.
- 41 Fassbinder, J. J., Griffis, T. J. and Baker, J. M.: Interannual, seasonal, and diel variability in  
42 the carbon isotope composition of respiration in a  $\text{C}_3/\text{C}_4$  agricultural ecosystem, *Agric. For.  
43 Meteorol.*, 153, 144–153, doi:10.1016/j.agrformet.2011.09.018, 2011.
- 44 Gannitzer, U., Moyes, a. B., Bowling, D. R. and Schnyder, H.: Measuring and modelling the  
45 isotopic composition of soil respiration: insights from a grassland tracer experiment,  
46 *Biogeosciences*, 8(5), 1333–1350, doi:10.5194/bg-8-1333-2011, 2011.

1 Gomez-Casanovas, N., Matamala, R., Cook, D. R. and Gonzalez-Meler, M. a.: Net ecosystem  
2 exchange modifies the relationship between the autotrophic and heterotrophic components of  
3 soil respiration with abiotic factors in prairie grasslands, *Glob. Chang. Biol.*, 18, 2532–2545,  
4 doi:10.1111/j.1365-2486.2012.02721.x, 2012.

5 van der Heijden, M. G. A., Bardgett, R. D. and van Straalen, N. M.: The unseen majority: soil  
6 microbes as drivers of plant diversity and productivity in terrestrial ecosystems., *Ecol. Lett.*,  
7 11(3), 296–310, doi:10.1111/j.1461-0248.2007.01139.x, 2008.

8 van der Heijden, M. G. A., Martin, F. M., Selosse, M.-A. and Sanders, I. R.: Mycorrhizal  
9 ecology and evolution: the past, the present, and the future, *New Phytol.*, 205, 1406–1423,  
10 doi:10.1111/nph.13288, 2015.

11 Heinemeyer, A., Tortorella, D., Petrovičová, B. and Gelsomino, A.: Partitioning of soil CO<sub>2</sub>  
12 flux components in a temperate grassland ecosystem, *Eur. J. Soil Sci.*, 63(2), 249–260,  
13 doi:10.1111/j.1365-2389.2012.01433.x, 2012.

14 Hiiesalu, I., Partel, M., Davison, J., Gerhold, P., Metsis, M., Moora, M., Öpik, M., Vasar, M.,  
15 Zobel, M. and Wilson, S. D.: Species richness of arbuscular mycorrhizal fungi: Associations  
16 with grassland plant richness and biomass, *New Phytol.*, 203(1), 233–244,  
17 doi:10.1111/nph.12765, 2014.

18 Hoover, D., Knapp, A. and Smith, M.: Resistance and resilience of a grassland ecosystem to  
19 climate extremes, *Ecology*, 95(9), 2646–2656, doi:10.1890/13-2186.1, 2014.

20 Hopkins, F., Gonzalez-Meler, M. a, Flower, C. E., Lynch, D. J., Czimczik, C., Tang, J. and  
21 Subke, J.-A.: Ecosystem-level controls on root-rhizosphere respiration., *New Phytol.*, 199(2),  
22 339–51 [online] Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23943914>, 2013.

23 Knohl, A., Werner, R. a, Brand, W. a and Buchmann, N.: Short-term variations in  $\delta^{13}\text{C}$  of  
24 ecosystem respiration reveals link between assimilation and respiration in a deciduous forest.,  
25 *Oecologia*, 142(1), 70–82, doi:10.1007/s00442-004-1702-4, 2005.

26 Koncz, P., Besnyői, V., Csathó, A. I., Nagy, J., Szerdahelyi, T., Tóth, Z., Pintér, K., Balogh,  
27 J., Nagy, Z. and Bartha, S.: Effect of grazing and mowing on the microcoenological  
28 composition of semi-arid grassland in Hungary, *Appl. Ecol. Environ. Res.*, 2014.

29 Lo, E.: Gaussian Error Propagation Applied To Ecological Data: Post-Ice-Storm-Downed  
30 Woody Biomass, *Ecol. Monogr.*, 75(4), 451–466, doi:10.1890/05-0030, 2005.

31 Midwood, A. J. and Millard, P.: Challenges in measuring the  $\delta^{13}\text{C}$  of the soil surface CO<sub>2</sub>  
32 efflux, *Rapid Commun. Mass Spectrom.*, 25(1), 232–242, doi:10.1002/rcm.4857, 2011.

33 van der Molen, M. K., Dolman, a. J., Ciais, P., Eglin, T., Gobron, N., Law, B. E., Meir, P.,  
34 Peters, W., Phillips, O. L., Reichstein, M., Chen, T., Dekker, S. C., Doubková, M., Friedl, M.  
35 a., Jung, M., van den Hurk, B. J. J. M., de Jeu, R. a. M., Kruijt, B., Ohta, T., Rebel, K. T.,  
36 Plummer, S., Seneviratne, S. I., Sitch, S., Teuling, a. J., van der Werf, G. R. and Wang, G.:  
37 Drought and ecosystem carbon cycling, *Agric. For. Meteorol.*, 151(7), 765–773,  
38 doi:10.1016/j.agrformet.2011.01.018, 2011.

39 Moyano, F., Kutsch, W. and Schulze, E.: Response of mycorrhizal, rhizosphere and soil basal  
40 respiration to temperature and photosynthesis in a barley field, *Soil Biol. Biochem.*, 39(4),  
41 843–853, doi:10.1016/j.soilbio.2006.10.001, 2007.

42 Moyano, F., Atkin, O., Bahn, M., Bruhn, D., Burton, A., Heinemeyer, A., WL, K. and Wieser,  
43 G.: Respiration from roots and the mycorrhizosphere, in *Soil Carbon Dynamics: An*  
44 *Integrated Methodology*, edited by M. Bahn, A. Heinemeyer, and W. L. Kutsch, pp. 234–288,  
45 Cambridge University Press., 2009.

- 1 Moyano, F. E., Manzoni, S. and Chenu, C.: Responses of soil heterotrophic respiration to  
2 moisture availability: An exploration of processes and models, *Soil Biol. Biochem.*, 59, 72–  
3 85, doi:10.1016/j.soilbio.2013.01.002, 2013.
- 4 Moyes, A. B., Gaines, S. J., Siegwolf, R. T. W. and Bowling, D. R.: Diffusive fractionation  
5 complicates isotopic partitioning of autotrophic and heterotrophic sources of soil respiration.,  
6 *Plant. Cell Environ.*, 33(11), 1804–19, doi:10.1111/j.1365-3040.2010.02185.x, 2010.
- 7 Mummey, D. L. and Rillig, M. C.: Spatial characterization of arbuscular mycorrhizal fungal  
8 molecular diversity at the submetre scale in a temperate grassland, *FEMS Microbiol. Ecol.*,  
9 64(2), 260–270, doi:10.1111/j.1574-6941.2008.00475.x, 2008.
- 10 Nagy, Z., Pintér, K., Czóbel, S., Balogh, J., Horváth, L., Fóti, S., Barcza, Z., Weidinger, T.,  
11 Csintalan, Z., Dinh, N. Q., Grosz, B. and Tuba, Z.: The carbon budget of semi-arid grassland  
12 in a wet and a dry year in Hungary, *Agric. Ecosyst. Environ.*, 121(1-2), 21–29,  
13 doi:10.1016/j.agee.2006.12.003, 2007.
- 14 Nagy, Z., Pintér, K., Pavelka, M., Darenová, E. and Balogh, J.: Carbon balance of surfaces vs.  
15 ecosystems: advantages of measuring eddy covariance and soil respiration simultaneously in  
16 dry grassland ecosystems, *Biogeosciences*, 8, 2523–2534, doi:10.5194/bg-8-2523-2011, 2011.
- 17 Nickerson, N. and Risk, D.: A numerical evaluation of chamber methodologies used in  
18 measuring the  $\delta^{13}\text{C}$  of soil respiration, *Rapid Commun. Mass Spectrom.*, 23, 2802–2810,  
19 doi:10.1002/rcm, 2009.
- 20 Nickerson, N., Egan, J. and Risk, D.: Iso-FD: A novel method for measuring the isotopic  
21 signature of surface flux, *Soil Biol. Biochem.*, 62, 99–106, doi:10.1016/j.soilbio.2013.03.010,  
22 2013.
- 23 Parton, W., Morgan, J., Smith, D., Del Grosso, S., Prihodko, L., Lecain, D., Kelly, R. and  
24 Lutz, S.: Impact of precipitation dynamics on net ecosystem productivity, *Glob. Chang. Biol.*,  
25 18(3), 915–927, doi:10.1111/j.1365-2486.2011.02611.x, 2012.
- 26 Phillips, C. and Nickerson, N.: Soil moisture effects on the carbon isotope composition of soil  
27 respiration, *Rapid Commun. Mass Spectrom.*, 1271–1280, doi:10.1002/rcm, 2010.
- 28 Pintér, K., Balogh, J. and Nagy, Z.: Ecosystem scale carbon dioxide balance of two grasslands  
29 in Hungary under different weather conditions., *Acta Biol. Hung.*, 61 Suppl(2010), 130–5,  
30 doi:10.1556/ABiol.61.2010.Suppl.13, 2010.
- 31 Prudhomme, C., Giuntoli, I., Robinson, E. L., Clark, D. B., Arnell, N. W., Dankers, R.,  
32 Fekete, B. M., Franssen, W., Gerten, D., Gosling, S. N., Hagemann, S., Hannah, D. M., Kim,  
33 H., Masaki, Y., Satoh, Y., Stacke, T., Wada, Y. and Wisser, D.: Hydrological droughts in the  
34 21st century, hotspots and uncertainties from a global multimodel ensemble experiment.,  
35 *Proc. Natl. Acad. Sci. U. S. A.*, 111(9), 3262–7, doi:10.1073/pnas.1222473110, 2014.
- 36 R Core Team: R: A Language and Environment for Statistical Computing, R Foundation for  
37 Statistical Computing, Vienna, Austria., 2014.
- 38 Reichstein, M., Bahn, M., Ciais, P., Frank, D., Mahecha, M. D., Seneviratne, S. I.,  
39 Zscheischler, J., Beer, C., Buchmann, N., Frank, D. C., Papale, D., Rammig, A., Smith, P.,  
40 Thonicke, K., van der Velde, M., Vicca, S., Walz, A. and Wattenbach, M.: Climate extremes  
41 and the carbon cycle, *Nature*, 500(7462), 287–295, doi:10.1038/nature12350, 2013.
- 42 Risk, D., Nickerson, N., Creelman, C., McArthur, G. and Owens, J.: Forced Diffusion soil  
43 flux: A new technique for continuous monitoring of soil gas efflux, *Agric. For. Meteorol.*,  
44 151(12), 1622–1631, doi:10.1016/j.agrformet.2011.06.020, 2011.
- 45 Risk, D., Nickerson, N., Phillips, C. L., Kellman, L. and Moroni, M.: Drought alters respired

1  $\delta^{13}\text{CO}_2$  from autotrophic, but not heterotrophic soil respiration, *Soil Biol. Biochem.*, 50, 26–  
2 32, doi:10.1016/j.soilbio.2012.01.025, 2012.

3 Suseela, V. and Dukes, J.: The responses of soil and rhizosphere respiration to simulated  
4 climatic changes vary by season, *Ecology*, 94(2), 403–413, doi: abs/10.1890/12-0150.1, 2013.

5 Tennant, D.: A test of a modified line intersect method of estimating root length, *J. Ecol.*, 63,  
6 995–1001, 1975.

7 Vargas, R., Detto, M., Baldocchi, D. D. and Allen, M. F.: Multiscale analysis of temporal  
8 variability of soil  $\text{CO}_2$  production as influenced by weather and vegetation, *Glob. Chang.*  
9 *Biol.*, 16(5), 1589–1605, doi:10.1111/j.1365-2486.2009.02111.x, 2010.

10 Yang, F. and Zhou, G.: Sensitivity of temperate desert steppe carbon exchange to seasonal  
11 droughts and precipitation variations in Inner Mongolia, China., *PLoS One*, 8(2), e55418,  
12 doi:10.1371/journal.pone.0055418, 2013.

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14

1 Table 1 Measured and estimated CO<sub>2</sub> effluxes and isotopic signals in this study

	CO <sub>2</sub> efflux	Isotopic signals
measured	$R_{\text{eco}}, R_{\text{soil}}, R_{\text{re}}, R_{\text{rme}}$	$\delta^{13}\text{C}_{\text{Reco}}, \delta^{13}\text{C}_{\text{Rsoil}}, \delta^{13}\text{C}_{\text{Rre}}, \delta^{13}\text{C}_{\text{Rrme}}$
estimated	$R_{\text{rhizo}}, R_{\text{myc}}, R_{\text{het}}$	$\delta^{13}\text{C}_{\text{Rmycrhiz}}, \delta^{13}\text{C}_{\text{Rrhizo}}, \delta^{13}\text{C}_{\text{Rmyc}}$

2

3



1 Table 2 Mean measured (undisturbed soil and tubes) and estimated (heterotrophic,  
 2 mycorrhizal fungi and rhizospheric) respiration rates for the different periods ( $\mu\text{mol CO}_2 \text{ m}^{-2}$   
 3  $\text{s}^{-1}$ ) with propagated uncertainties.

period	measured (mean $\pm$ SD)			estimated (mean $\pm$ SE)		
	R <sub>soil</sub>	R <sub>re</sub>	R <sub>rme</sub>	R <sub>het</sub>	R <sub>myc</sub>	R <sub>rhizo</sub>
active	7.7 $\pm$ 1.6	5.1 $\pm$ 1.5	3.9 $\pm$ 1.1	1.7 $\pm$ 1.1	1.0 $\pm$ 1.2	5.1 $\pm$ 1.1
drying	5.7 $\pm$ 2.0	3.8 $\pm$ 1.5	2.9 $\pm$ 0.6	2.9 $\pm$ 0.7	0.6 $\pm$ 0.9	2.1 $\pm$ 0.8
drought	3.2 $\pm$ 1.1	2.3 $\pm$ 0.4	1.9 $\pm$ 0.4	1.7 $\pm$ 0.5	0.3 $\pm$ 0.6	1.1 $\pm$ 0.5
wetting	4.8 $\pm$ 1.7	4.3 $\pm$ 1.5	2.6 $\pm$ 1.2	1.5 $\pm$ 0.6	1.0 $\pm$ 0.7	2.3 $\pm$ 0.7
re-greening	3.8 $\pm$ 1.0	2.4 $\pm$ 0.8	2.0 $\pm$ 1.1	1.1 $\pm$ 0.4	0.3 $\pm$ 0.5	2.4 $\pm$ 0.4
total study period	5.0 $\pm$ 2.1	3.8 $\pm$ 1.6	2.6 $\pm$ 1.2	1.8 $\pm$ 0.6	0.7 $\pm$ 0.8	2.6 $\pm$ 0.7

4

1

2 Fig. 1 (a) Daily averages of soil temperature ( $T_s$ ), soil water content (SWC) at 5 cm depth and  
3 daily sum of precipitation, (b) daily minimum half-hourly NEE (NEE<sub>min</sub>) and maximum  
4 half-hourly ET (maxET), (c) daily averages of CO<sub>2</sub> efflux in undisturbed soil ( $R_{soil}$ ), root-  
5 excluded soil ( $R_{re}$ ) and root- and mycorrhizal fungi excluded soil ( $R_{rme}$ ), (d) daily averages of  
6  $\delta^{13}C$  of soil CO<sub>2</sub> efflux in undisturbed soil ( $\delta^{13}C_{Rsoil}$ ), root-excluded soil ( $\delta^{13}C_{Rre}$ ) and root-  
7 and mycorrhizal fungi excluded soil ( $\delta^{13}C_{Rrme}$ ) and (e) daily averages of  $\delta^{13}C$  of ecosystem  
8 respiration ( $\delta^{13}C_{Reco}$ ) during the study period in 2013, at Bugac site. Arrows indicate the  
9 positions changes of the soil chambers. Gray horizontal lines show Y major values.

10

11 Fig. 2 Measured ( $R_{eco}$ ,  $R_{soil}$ ,  $R_{re}$ ,  $R_{rme}$ ) and estimated ( $R_{mycrhiz}$ ,  $R_{rhizo}$ ,  $R_{myc}$ )  $\delta^{13}C$  values of the  
12 respiration components. Horizontal black lines in boxes show medians and dashed whiskers  
13 show data extremes. Open circles and solid whiskers show means $\pm$ propagated standard errors.  
14 Gray horizontal lines show Y major values.

15

16 Fig. 3 Relative contributions made by rhizospheric, mycorrhizal fungi and heterotrophic  
17 components to the total soil respiration in the different parts of the vegetation period  
18 (15/05/2013-12/11/2013) at Bugac site. Propagated uncertainties of each estimate are shown  
19 in the lower panel. Gray horizontal lines show Y major values.

20

21 Fig. 4 (a) Mean hyphal length ( $m\ g^{-1}$  dry soil) and (b) mean microbial activity expressed as  
22 fluorescein released ( $mg\ kg^{-1}$  dry soil) in the undisturbed soil, root-exclusion and root- and  
23 mycorrhiza exclusion in different soil depths. Asterisks denote significant differences from  
24 undisturbed soil determined by the Tukey honest significant difference test.

25