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# Autotrophic component of soil respiration is repressed by drought more than the heterotrophic one in a dry grassland

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### Abstract

Summer droughts projected to increase in Central Europe due to climate change strongly influence the carbon cycle of ecosystems. Persistent respiration activities during drought periods are responsible for a significant carbon loss, which may turn the

<sup>5</sup> ecosystem from sink to source of carbon. There are still gaps in our knowledge regarding the characteristic changes taking place in the respiration of the different components of the ecosystem respiration in response to drought events.

Here, we combined a physical separation of soil respiration components with continuous measurements of soil CO<sub>2</sub> efflux and its isotopic (<sup>13</sup>C) signal at a dry grassland site in Hungary. The physical separation of soil respiration components was achieved by the use of inox meshes and tubes inserted into the soil. The root-excluded and root- and mycorrhiza-excluded treatments served to measure the isotopic signal of the rhizospheric, mycorrhizal fungi and heterotrophic components, respectively.

In the dry grassland investigated in this study the three components of the soil  $CO_2$ 

<sup>15</sup> efflux decreased at different rates under drought conditions. During drought the contribution made by the heterotrophic components was the highest. Rhizospheric component was the most sensitive to soil drying with its relative contribution to the total soil respiration dropping from 71 ± 4% (non-stressed) to 36 ± 12% under drought conditions. According to our results, the heterotrophic component of soil respiration is the major contributor to the respiration activities during drought events.

1 Introduction

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Grassland ecosystems strongly respond to drought events via substantial reduction of primary production (Hoover et al., 2014; Nagy et al., 2007; Parton et al., 2012; Reichstein et al., 2013). In contrast, below-ground respiration is not as strongly affected (van der Molen et al., 2011; Yang and Zhou, 2013), but tends to be reduced as well under drought (Balogh et al., 2011; Suseela and Dukes, 2013). According to climate change



scenarios, the frequency of droughts is expected to increase in Central Europe (Prudhomme et al., 2014), and thus understanding the mechanisms that control the net effect of droughts on  $CO_2$  effluxes is of key importance in dry grassland ecosystems representing one of the major land use forms in the region. Here we try to reveal which soil respiration component of a dry grassland could be responsible for the carbon losses during drought.

Soil organic matter (SOM) and litter derived respiration is considered to belong to the heterotrophic soil respiration component (Moyano et al., 2009). This decomposition is attributed mainly to soil bacteria and fungi. On the other hand, some of the soil fungi using recent photosynthetic assimilates are contributing to the autotrophic respiration component. Arbuscular mycorrhizal fungi (AME) are obligatory symbiont soil

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- piration component. Arbuscular mycorrhizal fungi (AMF) are obligatory symbiont soil fungi, forming intimate mutualistic associations in 70–90% of the plant species. About 10–20% of the assimilated C may be allocated to AMF (van der Heijden et al., 2015). Therefore soil respiration includes components of an autotrophic-heterotrophic contin-
- <sup>15</sup> uum from roots through the root-associated (rhizospheric and mycorrhizal) to non-rootassociated (heterotrophic) microbial components and the quantity of the soil produced CO<sub>2</sub> is highly dependent on plant carbon uptake (Högberg and Read, 2006).

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

- this are not fully revealed and the role of soil microbes is still not clear mainly due to the diversity of soil biota (Bardgett et al., 2008). Moreover, drivers of CO<sub>2</sub> production frequently interact with each other (Balogh et al., 2014; Vargas et al., 2010), hampering the partitioning of the total CO<sub>2</sub> efflux into components. Studies found a stronger effect of photosynthesis than that of temperature on root respiration (Gomez-Casanovas
- et al., 2012; Heinemeyer et al., 2012; Hopkins et al., 2013). Both autotrophic and heterotrophic components were shown to be sensitive to water shortage (Carbone et al., 2011; Moyano et al., 2013). Autotrophic component was found to be dominant over the heterotrophic one during drought periods in a Mediterranean woodland ecosystem



(Casals et al., 2011), but we have limited information from grasslands of shallow rooted herb species regarding the dominant source of carbon during drought periods.

Isotopic signatures of soil respiration have been used for estimating the contribution of the main components (Carbone et al., 2011; Hopkins et al., 2013). Diel patterns in

- $^{5}$   $\delta^{13}$ C may also be related to biases in measuring methods (Fassbinder et al., 2011; Midwood and Millard, 2011), while seasonal changes were expected to reflect the changes in the contributions of source components rather than the changes in the isotopic signal of the component itself (Knohl et al., 2005). However, SOM  $\delta^{13}$ C can also change during the year: fresh plant material is more depleted than the older SOM components
- <sup>10</sup> (Bowling et al., 2002), therefore fresh litter may contribute to the decreasing  $\delta^{13}$ C of the heterotrophic component. Drying of the surface layers can also modify  $\delta^{13}$ CO<sub>2</sub>, since heterotrophic respiration could be restricted to the deeper layers of the soil (Moyes et al., 2010).

Uncertainties in estimates about the contributions of soil respiration components could be reduced by a combination of different methodologies (Risk et al., 2012). Here, we ask the question which of the investigated soil respiration components (rhizospheric, mycorrhizal fungi and heterotrophic components) has the largest weight during drought. Our hypothesis was that autotrophic respiration component would be reduced linearly with photosynthesis, whereas heterotrophic respiration might not be affected as

strongly. To achieve our goals, we used an experimental setup of physical separation of soil respiration components combined with measurements of soil CO<sub>2</sub> efflux and its isotopic (<sup>13</sup>C) signal.

### 2 Methods

# 2.1 Site description

<sup>25</sup> The vegetation at the Bugac site (46.69° N, 19.6° E, 114 ma.s.l.) is a dry sandy grassland dominated by *Festuca pseudovina*, *Carex stenophylla* and *Cynodon dactylon* and



it has been under extensive management (grazing) for the last 20 years. Ten-year mean annual precipitation (2004-2013) was 575 mm, and the mean annual temperature reached 10.4 °C. The soil is a chernozem type sandy soil with high organic carbon content (Balogh et al., 2014).

# 5 2.2 Spatial separation of soil CO<sub>2</sub> efflux components

In 2010, ten soil cores (160 mm in diameter and 800 mm in depth) were excavated, the roots have been removed and the root-free soil was packed layer by layer into PVC tubes. Five tubes were used to exclude both roots and mycorrhiza. Walls of another 5 tubes were partially removed and replaced by inox meshes (40 µm pore size) to exclude roots, while ensuring that the mycorrhiza filaments can grow into the tubes (Moyano et al., 2007). These root-free and root- and mycorrhiza-free soil cores were settled at a distance of 10 m from the eddy covariance tower to the south direction. The distance between the soil cores/tubes was 50 cm.

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

- undisturbed soil: total soil respiration,  $R_{soil}$ , 15

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- without roots and arbuscular mycorrhizal fungi = heterotrophic component only,  $R_{\rm rme}$ ,
- root-excluded soil = without roots, but with arbuscular mycorrhizal fungi,  $R_{re}$ .

#### 2.3 Gas exchange measuring systems

- Three different gas exchange systems were used in this study: eddy-covariance system 20 (EC), automated soil respiration measuring system (SRS) was connected to an isotopic CO<sub>2</sub> analyser (CRDS-technique). The size of the EC flux footprint area was larger by several orders of magnitude than the area covered by the SRS. Care was taken during the establishment of the experiment to select a part of the EC footprint area with the
- same average soil characteristics and vegetation composition and cover as found in 25



the plots where the SRS was installed. Hence, the NEE and ET estimates obtained in this way can be considered representative also for the small-scale SRS and isotope measurements.

Data from 15 May 2013 to 12 November 2013 (182 days) were analysed in this study.

### 5 2.3.1 Eddy covariance setup

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The EC system at the Bugac site has been measuring the  $CO_2$  and  $H_2O$  fluxes continuously since 2002. In dry years this grassland can turn into a net carbon source (Nagy et al., 2007), but the longer-term annual sums of net ecosystem exchange (NEE) show it to be a net sink, ranging from -171 to +106 gCm<sup>-2</sup> yr<sup>-1</sup> (Pintér et al., 2010) with a -100 gCm<sup>-2</sup> yr<sup>-1</sup> average.

The EC system consists of a CSAT3 sonic anemometer (Campbell Scientific, USA) and a Li-7500 (Licor Inc, USA) open-path infra-red gas analyser (IRGA), both connected to a CR5000 data logger (Campbell Scientific, USA) via an SDM (synchronous device for measurement) interface. Additional measurements used in this study were: <sup>15</sup> air temperature and relative humidity (HMP35AC, Vaisala, Finland), precipitation (ARG 100 rain gauge, Campbell, UK), global radiation (dual pyranometer, Schenk, Austria) incoming and reflected photosynthetically active radiation (SKP215, Campbell, UK), volumetric soil moisture content (CS616, Campbell, UK) and soil temperature (105 T, Campbell, UK). These measurements were performed as described in Nagy <sup>20</sup> et al. (2007) and Pintér et al. (2010). Fluxes of sensible and latent heat and CO<sub>2</sub> were processed using an IDL program after Barcza et al. (2003) adopting the CarboEurope

IP methodology. For a detailed description of data processing and gap-filling see Nagy et al. (2007) and Farkas et al. (2011).

## 2.3.2 Soil respiration system

<sup>25</sup> The 10 chamber automated soil respiration system was set up in July 2011. The system is an open dynamic one, consisting of an SBA-4 infrared gas analyser (PPSys-



tems, UK), pumps, flow meters (D6F-01A1-110, Omron Co., Japan), electro-magnetic valves, and PVC/metal soil chambers. The chambers were 10.4 cm high with a diameter of 5 cm, covering a soil surface area of 19.6 cm<sup>2</sup>. The flow rate through the chambers was 300 mL min<sup>-1</sup>, replacing the air in the chamber in 40 s. The PVC chambers
were enclosed in a white metal cylinder with 2 mm airspace in between to stabilize the chamber and to prevent warming by direct radiation. Four vent holes with a total area of 0.95 cm<sup>2</sup> were drilled in the top of the chambers. Vent holes also served to allow precipitation to drip into the chambers. The system causes minor disturbances in the soil structure and the spatial structure of the vegetation. It is applicable without cutting the leaves/shoots of the plants, so it is not disturbing transport processes taking place within the plant stems and roots. It is suitable for continuous, long-term unattended measurements of soil CO<sub>2</sub> efflux and has been used in previous experiments (Nagy et al., 2011). The soil respiration chambers contained no standing aboveground plant

 $_{15}$   $R_{soil}$  was measured by 6 SRS chambers and 3 of them were attached to the CRDS.  $R_{rme}$ , was measured by 2 SRS chambers and one of them was attached to the CRDS.

 $R_{\rm re}$  was measured by 2 SRS chambers and one of them was attached to the CRDS.

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

material.

- A Picarro G1101-i gas analyser (Picarro Inc., CA, USA) was attached to the soil respiration system from May to November in 2013. This CRDS system measured the isotopic composition of the reference air (in the grass canopy 10 cm above the surface) when the soil respiration system was idle (1 h idles after 1 h operation) and between two chamber measurements. The SRS had 10 chambers and one measurement lasted
- for 3 min on one chamber when the reference/analysis air was switched in every 7 s. The CRDS has much slower response, so similarly to the SRS, one chamber was measured for 3 min. After each chamber the isotopic signal of the reference air was measured for 3 min. Therefore every second chamber of the SRS was measured by



the CRDS giving a sequence of reference and analysis (soil  $CO_2$  efflux as sampled from the chamber) air for 3–3 min in one hour of operation.

### 2.4 Data processing and modelling

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

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

$$\delta^{13}C_R = \frac{\delta^{13}C_{\text{out}} \times c_{\text{out}} - \delta^{13}C_{\text{in}} \times c_{\text{in}}}{c_{\text{out}} - c_{\text{in}}}$$
(1)

where  $\delta^{13}C_{out}$  and  $\delta^{13}C_{in}$  are the isotopic signature of the outgoing and incoming air of the chamber and  $c_{out}$  and  $c_{in}$  are the CO<sub>2</sub> concentration of the of the outgoing and incoming air of the chamber, respectively.

$$\delta^{13}C = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \tag{2}$$

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

Individual measurements were filtered out by using a moving-window procedure if the investigated value (at the window center) was outside the range of the mean  $\pm$  median absolute deviation of the values in a 10 days moving window. This filtering



procedure left an overall data availability of 68–70 %. Daily averages were calculated using the remaining data.

To determine the isotopic signature of the ecosystem respiration ( $R_{eco}$ ), Keeling plots were constructed by plotting the night-time  $\delta^{13}$ C values measured 10 cm over the surface against the inverse of the CO<sub>2</sub> concentration. The extrapolated *y*-intercept of the linear regression was used as  $\delta^{13}C_{Reco}$  values.

Total soil  $CO_2$  efflux was separated isotopically into its components. Two-source mixing models were used to estimate the fraction (*a*) of the rhizospheric (root and rhizospheric microbes) and mycorrhizospheric (root, rhizospheric microbes and mycorrhizal fungi) components (Moyano et al., 2009), based on the measured fractions:

$$\delta^{13}C_{\text{Rsoil}} = a \times \delta^{13}C_{\text{Rrhizo}} + (1-a) \times \delta^{13}C_{\text{Rre}}$$
(3)  
$$\delta^{13}C_{\text{Rsoil}} = b \times \delta^{13}C_{\text{Rmvcrhiz}} + (1-b) \times \delta^{13}C_{\text{Rrme}}$$
(4)

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where  $\delta^{13}C_{\text{Rsoil}}$  is the  $\delta^{13}C$  of the total soil CO<sub>2</sub> efflux,  $\delta^{13}C_{\text{Rre}}$  is the  $\delta^{13}C$  of the root-excluded soil,  $\delta^{13}C_{\text{Rrme}}$  is the  $\delta^{13}C$  of the root- and mycorrhiza-excluded soil (heterotrophic respiration), *a* is the fraction of the rhizospheric component and *b* is the fraction of the mycorrhizospheric component to the total soil efflux.  $\delta^{13}C_{\text{rhizo}}$  value was estimated by plotting  $\delta^{13}C_{\text{Rsoil}}$  values against the  $R_{\text{re}}/R_{\text{soil}}$  ratio. Since  $R_{\text{re}}/R_{\text{soil}}$  and  $R_{\text{rme}}/R_{\text{soil}}$  is hypothetically zero when only rhizospheric respiration is present, *y*-intercept of the linear correlation was assumed as  $\delta^{13}C_{\text{Rrhizo}}$ .  $\delta^{13}C_{\text{Rmyc}}$  was estimated 20 by plotting  $\delta^{13}C_{\text{Rre}}$  values against the  $R_{\text{re}}/R_{\text{rme}}$  fraction (Table 1).

Rhizospheric, mycorrhizal and heterotrophic respirations ( $R_{rhizo}$ ,  $R_{myc}$ ,  $R_{het}$ ) were calculated by multiplying the estimated daily contributions to total respiration with the total soil respiration ( $R_{soil}$ ). Cross-correlation was calculated between daily minimum half-hourly NEE (NEE<sub>min</sub>) and daily component respirations ( $R_{rhizo}$ ,  $R_{myc}$ ,  $R_{het}$ ).



## 2.5 Microbial investigations

Soil samples for the microbial investigations were taken after the gas exchange measurements in May 2014 to avoid the disturbance of the measurements by the soil sampling. Sampling date was chosen considering the maximum of the carbon sequestration capacity of the investigated grassland (Nagy et al., 2007). 5–5 samples were taken

from 5 soil layers (0–10, 10–20, 20–30, 30–40 and 40–50 cm) in each plot.
Determination of AM fungal hyphal length in the soil was based on the methods of Bååth and Söderström (1979) using separation by wet-sieving and centrifugation. The separated fungal hyphae were stained using agar solution (0.75%) containing trypan
<sup>10</sup> blue (0.05%) then dried for 24 h at 70°C. The hyphal length was measured in the dried agar film by the intersection method (Tennant, 1975) under a binocular microscope.

The fluorescein diacetate (FDA) hydrolysis assay was used to estimate the total microbial activity in soil samples and expressed as mg fluorescein released kg<sup>-1</sup> dry soil (Adam and Duncan, 2001).

#### 15 2.6 Uncertainty assessment

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Isotopic signal of soil respired  $CO_2$  has been studied extensively but several uncertainties related to the different methods have been revealed. Steady-state methods were found to provide more robust estimates than static chambers but still charged with biases (e.g. diffusive fractionation, Nickerson and Risk, 2009). Open systems have the advantage of unattended automatic measurement collecting large amount of data but are less sensitive to small isotopic differences (Midwood and Millard, 2011).

In our study  $\delta^{13}C_{\text{Reco}}$  estimates were independent of chamber related biases, using night-time  $\delta^{13}CO_2$  and  $CO_2$  concentration data of the free air over the surface for the calculation (Keeling-plot approach). This approach gave similar results to the chamber-based measurements, providing also partial verification of the latter ones. Moreover, instance measurements were independent on acil  $CO_2$  offlux measurements are

<sup>25</sup> based measurements, providing also partial verification of the latter ones. Moreover, isotopic measurements were independent on soil CO<sub>2</sub> efflux measurements, since IRGA and CRDS systems took different air samples from the same soil chambers.



Isotopic data together with  $CO_2$  efflux rates were collected during 1980 measurement cycles on 182 days in order to have robust estimates of isotopic signals.

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

Error propagation was done by parametric bootstrapping using the boot library in R (Canty and Ripley, 2014). The advantage of the bootstrapping method over the other methods to estimate uncertainty of a result of a complicated statistical equation is that it allows to estimate confidence intervals even with non-Gaussian distributed data and with complicated interactions that may not necessarily follow a Gaussian distribution. In practise, all components in an equation are perturbed by a random deviation from their initial value and the results are collected for thousands of runs (in our case we used 5000 sets of random deviations), and the standard error of the mean is derived from the statistical distribution of the outcomes of all simulation runs (Davison and Hinkley,

#### 3 Results

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1997; Wilks, 2006).

#### 20 3.1 Microbial biomass and activity

Hyphal length per g soil was significantly lower in the upper layers of root- and mycorrhiza-excluded soil compared to the undisturbed soil, but it was significantly higher in root-excluded plots at 10–20 cm depth. Hyphal length of the root-excluded soil was similar to undisturbed soil in the other soil layers. Fluorescein values were significantly lower in all soil layers of the root- and mycorrhiza-excluded plots compared



to the undisturbed soil. Fluorescein values of the root-excluded plots were also lower than in undisturbed soil, but this difference was not significant (Fig. 1).

# 3.2 $\delta^{13}$ C of the respiration components

Figure 2 shows the measured and estimated  $\delta^{13}$ C values of the different soil CO<sub>2</sub> efflux <sup>5</sup> components.  $\delta^{13}C_{\text{Brme}}$  was the highest, while  $\delta^{13}C_{\text{Beco}}$  was the lowest, suggesting that rhizospheric respiration was the most substantially depleted, while heterotrophic respiration was the least depleted in <sup>13</sup>C. Mean of  $\delta^{13}C_{Beco}$ ,  $\delta^{13}C_{Bsoil}$ ,  $\delta^{13}C_{Bre}$  and  $\delta^{13}C_{Brme}$ were  $-27.9 \pm 0.5$ ,  $-26.8 \pm 1.3$ ,  $-26.4 \pm 1.8$  and  $-25.7 \pm 2\%$  (mean  $\pm$  SE), respectively. The estimated isotopic signal of the respiration of mycorrhizospheric, rhizospheric and mycorrhizal fungi components were  $-28.6 \pm 1.5$ ,  $-28.9 \pm 1.8$  and  $-27.2 \pm 2.3$ % (estimate  $\pm$  SE), respectively (Fig. 2).

#### Meteorological conditions, NEE, ET, soil CO<sub>2</sub> efflux, $\delta^{13}$ C of CO<sub>2</sub> efflux 3.3

The end of May and the beginning of June was the most productive period in the year due to good water availability, the lowest NEE (strongest carbon sink activity) and highest evapotranspiration (ET) values were measured in this period (Fig. 3a). 15 It rained only a few times from the end of June to 19 August (total precip: 10 mm) and the accompanying high temperature resulted in drought. Daily minimum NEE was around zero at the end of July and in August. Rain events after the drought period had significant effects on soil CO<sub>2</sub> effluxes (Fig. 3c). There was a second active period in autumn following rains, but CO<sub>2</sub> uptake and ET were lower than in May or June. 20

 $R_{\rm soil}$  was the highest among the soil CO<sub>2</sub> effluxes, while  $R_{\rm rme}$  was the lowest, the average CO<sub>2</sub> effluxes in the whole study period were  $5.0 \pm 2.1$ ,  $3.8 \pm 1.6$  and  $2.6 \pm 1.2 \mu$ mol  $CO_2 \text{ m}^{-2} \text{ s}^{-1}$  (mean ± SD) in  $R_{soil}$ ,  $R_{re}$  and  $R_{rme}$ , respectively.  $R_{re}$  was sometimes higher than  $R_{soil}$ , especially shortly after rain events.

Isotopic signature of  $R_{eco}$  was the lowest in May and June, increased in July and 25 August and decreased again in October and November.  $\delta^{13}C_{\text{Beco}}$  showed clear re-



sponses to precipitation pulses: sudden declines were observed during the rain events. Chamber-based  $\delta^{13}C_{\text{Rsoil}}$  showed similar changes during the study period.  $\delta^{13}C_{\text{Rrme}}$  and  $\delta^{13}C_{\text{Rre}}$  showed large scatter during the whole study period with no clear trends to be detected. Difference between  $\delta^{13}C_{\text{Rsoil}}$  and  $\delta^{13}C_{\text{Rrme}}$  was largest in the active period and smallest under drought conditions.

According to the NEE, SWC values and isotopic signals we distinguished 5 periods within the study period: an active period from 15 May to 20 June, a drying (stress development) period from 21 June to 22 July, a drought period from 23 July to 19 August, a wetting (stress release) period from 20 August to 16 September and a re-greening (recovery) period from 17 September to the end of the study period (11 November) (Fig. 3).

# 3.4 Ratio of the different components in total soil respiration during the vegetation period

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Two end-member mixing models (Eqs. 3 and 4) were used to estimate the relative
<sup>15</sup> contributions of rhizospheric, mycorrhizal fungi and heterotrophic components to total soil respiration during the study period. The autotrophic component (mycorrhizospheric component) of soil respiration showed significant decrease during the drying and drought periods. During the active period estimated rhizospheric contribution was lower than mycorrhizospheric and this difference was attributed to the mycorrhizal fungi
<sup>20</sup> component (Fig. 4). Average contribution (mean ± SE) made by the rhizospheric component decreased by 50 % as a response to drought from 71 ± 4 % in the active period to 36 ± 12 % during the drought period (Fig. 4). Relative mycorrhizal contribution was

- the highest  $(23 \pm 14\%)$ ; mean  $\pm$  SE) during the drought period. The estimated (relative) rhizospheric contribution increased after the rain events, even after a small amount of
- <sup>25</sup> precipitation (e.g. at the end of July). The estimated contribution by the heterotrophic component increased from about 20 to 60 % (data not shown) at some point during the drying period, when no precipitation occured.



Cross-correlation between daily  $R_{rhizo}$  and daily NEE<sub>min</sub> was the strongest at 4 days lag (R = -0.71) considering the whole study period. As for the different parts of the study period this relationship was significant in the active and drying periods, but the strongest correlation was found in the drying period (at 3 days lag, R = -0.42). Mycorrhizal respiration ( $R_{myc}$ ) showed no direct correlation with NEE<sub>min</sub> in the whole study period, but a significant negative correlation was found in drying and drought periods at 8 days lag. Heterotrophic respiration ( $R_{het}$ ) showed no direct correlations were found during in the whole study period either, but significant negative correlations were found during the drying and drought periods at 3 and 8 days lags, respectively.

#### 10 4 Discussion

# 4.1 Effect of drought on CO<sub>2</sub> effluxes and $\delta^{13}$ C values

All types of measured soil CO<sub>2</sub> effluxes ( $R_{soil}$ ,  $R_{re}$ ,  $R_{rme}$ ) decreased under dry conditions, but the biggest decline was observed in total soil respiration ( $R_{soil}$ ), therefore a strong response of the autotrophic component to drought could be assumed. The ob-<sup>15</sup> served increase in  $\delta^{13}C_{Reco}$  and  $\delta^{13}C_{Rsoil}$  values during the drying period and during the drought also suggested the decline of the autotrophic component. The same phenomenon was shown by the modelling results, with the lowest fraction of rhizospheric component estimated for the drought period ( $36 \pm 12\%$ ; mean  $\pm$  SE), while the highest for the active period ( $71 \pm 4\%$ ; mean  $\pm$  SE). Fraction of the heterotrophic respiration <sup>20</sup> and mycorrhizal fungi respiration were the highest during the drought ( $41 \pm 8\%$  and  $23 \pm 14\%$ , respectively; mean  $\pm$  SE), suggesting that the non root-associated microbes and mycorrhizal filaments were less sensitive to water shortages than the rhizosphere. Soil aggregates are expected to provide micro-habitats for soil organisms that should

<sup>25</sup> 2012). Since there was an absence in plant photosysnthetic supply during drought period, mycorrhizal fungi component is expected to use stored carbon for respiration



(van der Heijden et al., 2008). Estimated contributions by the soil components varied largely within the periods, governed by the precipitation pattern. Despite the drying of the surface layers, relative contribution by the heterotrophic component increased by 40% within the drying period, when it was not affected by precipitation.

We found a time-lagged correlation (4 days) between daily  $R_{rhiz}$  and NEE<sub>min</sub> in the 5 whole study period, but no correlation was found at any reasonable lag between the estimated activity by the other components ( $R_{het}$  and  $R_{mvc}$ ) and NEE<sub>min</sub>. Within the study period, rhizospheric respiration showed the strongest relationship with NEE<sub>min</sub> in the drying period. This result is similar to the findings by Gomez-Casanovas et al. (2012), who found a strong relationship between the autotrophic component and daytime NEE 10 with a time lag of 4-6 days in a tallgrass prairie. This relationship was stronger in periods when substrate supply could not meet the demands of the roots.

Low  $\delta^{13}C_{\text{Rsoil}}$  and  $\delta^{13}C_{\text{Reco}}$  were measured in the wetting and re-greening periods due to the drought-induced fall of the fresh litter to the surface as fresh plant material could be more depleted than the old litter (Bowling et al., 2002). The declines in 15  $\delta^{13}C_{\text{Bsoil}}$  and  $\delta^{13}C_{\text{Beco}}$  immediately after the rain events during drying and drought periods could also be explained by the wetting of the litter layer, exposing relatively fresh substrate to degradation for short periods. This phenomenon could also cause an overestimation in contributions made by the depleted components (rhizospheric) during rain events.

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Similar results were found in a tallgrass prairie by Gomez-Casanovas et al. (2012), where the autotrophic components were more sensitive to soil drying than the heterotrophic ones. For woodland ecosystems, on the other hand, different responses were reported: ratio of autotrophic components increased in response to drought but enriched signature of the recent photosynthetic supply during drought could also ex-25 plain the enrichment of the soil respired CO<sub>2</sub> (Casals et al., 2011). A drought induced increase in  $\delta^{13}$ C of root respiration of trees was also assumed in a recent study (Risk et al., 2012), suggesting that the isotopic signal of the assimilates, thereby the signal of the autotrophic component can also change. In our study,  $R_{\rm rme}/R_{\rm soil}$  showed significant



positive correlation with  $\delta^{13}C_{\text{Rsoil}}$  (the regression was used to estimate  $\delta^{13}C_{\text{Rrhizo}}$ ), so  $\delta^{13}C_{\text{Rsoil}}$  was high if the ratio of heterotrophic CO<sub>2</sub> efflux to the total soil CO<sub>2</sub> efflux was found to be high. Moreover,  $R_{\text{rme}}/R_{\text{soil}}$  ratios were the highest on the driest days, supporting the estimated decline of the autotrophic component.

 According to these studies and to our results we can assume that the different vegetation types can respond differently to drought: woodlands can increase the autotrophic ratio, while grasslands can decrease it (Casals et al., 2011; Gomez-Casanovas et al., 2012; Risk et al., 2012). Since plants with different rooting habits have different water availability during dry periods (van der Molen et al., 2011), this could explain the differences in the response to drought by the different ecosystems.

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

Measured and calculated  $\delta^{13}$ C values of the different respiration components showed similar differences to the ones reviewed by Bowling et al. (2008).  $\delta^{13}C_{\text{Reco}}$  (contain-<sup>15</sup> ing also the signal from above ground green biomass) was the most depleted, while  $\delta^{13}C_{\text{Rrme}}$  was the least depleted.  $\delta^{13}$ C of the root- and mycorrhiza-excluded respiration was similar to SOM  $\delta^{13}$ C measured in a previous study (Denef et al., 2013): –25 and –26‰ in the topsoil layers (without the litter layer). CO<sub>2</sub> effluxes from mycorrhizal fungi were expected to be more enriched in <sup>13</sup>C relative to the total soil respiration (about +3‰, Bowling et al., 2008). Estimated  $\delta^{13}$ C of mycorrhizal fungi component was –27.24±2.3‰ (estimate ± SE), which is 1.7‰ higher than the rhizospheric component (–28.9±1.8‰; estimate ± SE).

# 4.3 Microbial investigations

High hyphal density was maintained in  $R_{\rm re}$  plots and lower, but still significant microbial activities (SOM decomposition) were detected in  $R_{\rm rme}$  plots, therefore the measured  $\delta^{13}$ C values showed the sources of the root-free ( $\delta^{13}$ C<sub>Bre</sub>) and root- and mycorrhiza-



free ( $\delta^{13}C_{Rrme}$ ) soils. The fact that very high amounts of hyphae were found in the root-excluded treatment ( $R_{re}$ ) in the 10–20 cm layer proved that mycorrhizal fungi filaments were able to penetrate through the inox mesh and supported significant microbial activity. Grasses have extensive fibrous root systems with moderate to high levels

- <sup>5</sup> of mycorrhizal colonization (van der Heijden et al., 2015). The range of AM hyphal lengths found in this study (1.9–8.8 mg<sup>-1</sup> soil) is similar to those reported in the literature (e.g. Mummey and Rillig, 2008). The longer hyphal densities found in root-free soil might have been expected due to the higher availability of SOM-derived nutrients and to more space without the roots.
- <sup>10</sup> Values of fluorescein in root-excluded plots were similar to those measured in the undisturbed soil, probably because of the fact that hyphae of AM fungi provide an increased area for interaction with other microorganisms (hyphosphere, Andrade et al., 1997).

### 5 Conclusions

- <sup>15</sup> In the dry grassland investigated in this study all three components of the soil CO<sub>2</sub> effluxes decreased, following different dynamics under drought conditions. The strongest decrease in response to drought was seen in rhizospheric respiration (relative contribution to total respiration decreased from 71 ± 4% to 36 ± 12%; mean ± SE), while the relative contribution to the total soil respiration by mycorrhizal fungi and heterotrophic
- <sup>20</sup> components increased. During drought the contribution of the heterotrophic component was found to be the highest ( $41 \pm 8\%$ ; mean  $\pm$  SE), but similar to the contribution made by the rhizospheric component. According to the NEE measurements these respiration activities originated from carbon sources already stored, thereby decreasing the carbon content of the soil.
- <sup>25</sup> Drought events are expected to be more frequent in Central Europe in the future, and it is expected that the productivity of grassland ecosystems may strongly respond to projected dryness, influencing the carbon cycle of the ecosystems. Since poten-



tial productivity is generally linked to soil carbon content, a pronounced decrease in soil organic matter due to the enhanced activity of the heterotrophic component under drought may directly affect the long term productivity of grasslands.

*Author contributions.* J. Balogh, M. Papp, K. Pintér and Z. Nagy conceived and designed the experiment, M. Papp, K. Pintér and K. Posta performed the experiment, J. Balogh analyzed the data and wrote the paper, but all co-authors contributed to writing.

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#### References

10

20

- Adam, G. and Duncan, H.: Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils, Soil Biol. Biochem 33,943–951, 2001
- <sup>15</sup> Biochem., 33, 943–951, 2001.
  - Andrade, G., Mihara, K. L., Linderman, R. G., and Bethlenfalvay, G. J.: Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi, Plant Soil, 192, 71– 79, doi:10.1023/A:1004249629643, 1997.

Bååth, E. and Söderström, B.: The significance of hyphal diameter in calculation of fungal biovolume, Oikos, 33, 11–14, 1979.

Balogh, J., Pintér, K., Fóti, S., Cserhalmi, D., Papp, M., and Nagy, Z.: Dependence of soil respiration on soil moisture, clay content, soil organic matter, and CO<sub>2</sub> uptake in dry grasslands, Soil Biol. Biochem., 43, 1006–1013, doi:10.1016/j.soilbio.2011.017, 2011.

Balogh, J., Fóti, S., Pintér, K., Burri, S., Eugster, W., Papp, M., and Nagy, Z.: Soil CO2 efflux

- <sup>25</sup> and production rates as influenced by evapotranspiration in a dry grassland, Plant Soil, 388, 157–173, doi:10.1007/s11104-014-2314-3, 2014.
  - Barcza, Z., Haszpra, L., and Kondo, H.: Carbon exchange of grass in Hungary, Tellus B, 55, 187–196, doi:10.1034/j.1600-0889.2003.00014.x, 2003.



16903

- Bardgett, R. D., Freeman, C., and Ostle, N. J.: Microbial contributions to climate change through carbon cycle feedbacks, ISME J., 2, 805–814, doi:10.1038/ismej.2008.58, 2008.
- Bowling, D., McDowell, N., Bond, B., Law, B., and Ehleringer, J.: <sup>13</sup>C content of ecosystem respiration is linked to precipitation and vapor pressure deficit, Oecologia, 131, 113–124, doi:10.1007/s00442-001-0851-v. 2002.
- Bowling, D. R., Pataki, D. E., and Randerson, J. T.: Carbon isotopes in terrestrial ecosystem pools and CO<sub>2</sub> fluxes, New Phytol., 178, 24–40, doi:10.1111/j.1469-8137.2007.02342.x, 2008.

Canty, A. and Ripley, B. D.: Boot: bootstrap R (S-Plus) Functions, 2014.

5

<sup>10</sup> Carbone, M. S., Still, C. J., Ambrose, A. R., Dawson, T. E., Williams, A. P., Boot, C. M., Schaeffer, S. M., and Schimel, J. P.: Seasonal and episodic moisture controls on plant and microbial contributions to soil respiration, Oecologia, 167, 265–78, doi:10.1007/s00442-011-1975-3, 2011.

Casals, P., Lopez-Sangil, L., Carrara, A., Gimeno, C., and Nogués, S.: Autotrophic and het-

erotrophic contributions to short-term soil CO<sub>2</sub> efflux following simulated summer precipitation pulses in a Mediterranean dehesa, Global Biogeochem. Cy., 25, GB3012, doi:10.1029/2010GB003973, 2011.

Davidson, E. A., Samanta, S., Caramori, S. S., and Savage, K.: The Dual Arrhenius and Michaelis-Menten kinetics model for decomposition of soil organic matter at hourly to sea-

<sup>20</sup> sonal time scales, Glob. Change Biol., 18, 371–384, doi:10.1111/j.1365-2486.2011.02546.x, 2012.

Davison, A. C. and Hinkley, D. V: Bootstrap Methods and their Applications, Cambridge University Press, Cambridge, 1997.

Denef, K., Galdo, I., Venturi, A., and Cotrufo, M.: Assessment of soil C and N stocks and fractions across 11 european soils under varying land uses, Open J. Soil Sci., 3, 297–313,

- 25 fractions across 11 european soils under varying land uses, Open J. Soil Sci., 3, 297 2013.
  - Farkas, C., Alberti, G., Balogh, J., Barcza, Z., Birkas, M., Czobel, S., Davis, K. J., Fuehrer, E., Gelybo, G., Grosz, B., Kljun, N., Koos, S., Machon, A., Marjanovic, H., Nagy, Z., Peressotti, A., Pinter, K., Toth, E., and Horvath, L.: Methodologies, in: Atmospheric Greenhouse
- Gases: the Hungarian Perspective, edited by: Haszpra, L., Springer, New York, 65–90, 2011.
   Fassbinder, J. J., Griffis, T. J., and Baker, J. M.: Interannual, seasonal, and diel variability in the carbon isotope composition of respiration in a C<sub>3</sub>/C<sub>4</sub> agricultural ecosystem, Agr. Forest Meteorol., 153, 144–153, doi:10.1016/j.agrformet.2011.09.018, 2011.



- Gomez-Casanovas, N., Matamala, R., Cook, D. R., and Gonzalez-Meler, M. A.: Net ecosystem exchange modifies the relationship between the autotrophic and heterotrophic components of soil respiration with abiotic factors in prairie grasslands, Glob. Change Biol., 18, 2532–2545, doi:10.1111/j.1365-2486.2012.02721.x, 2012.
- <sup>5</sup> Heinemeyer, A., Tortorella, D., Petrovičová, B., and Gelsomino, A.: Partitioning of soil CO<sub>2</sub> flux components in a temperate grassland ecosystem, Eur. J. Soil Sci., 63, 249–260, doi:10.1111/j.1365-2389.2012.01433.x, 2012.
  - Högberg, P. and Read, D. J.: Towards a more plant physiological perspective on soil ecology, Trends Ecol. Evol., 21, 548–554, doi:10.1016/j.tree.2006.06.004, 2006.
- <sup>10</sup> Hoover, D., Knapp, A., and Smith, M.: Resistance and resilience of a grassland ecosystem to climate extremes, Ecology, 95, 2646–2656, doi:10.1890/13-2186.1, 2014.
  - Hopkins, F., Gonzalez-Meler, M. A., Flower, C. E., Lynch, D. J., Czimczik, C., Tang, J., and Subke, J.-A.: Ecosystem-level controls on root-rhizosphere respiration., New Phytol., 199, 339–351, 2013.
- <sup>15</sup> Knohl, A., Werner, R. A., Brand, W. A., and Buchmann, N.: Short-term variations in δ<sup>13</sup>C of ecosystem respiration reveals link between assimilation and respiration in a deciduous forest., Oecologia, 142, 70–82, doi:10.1007/s00442-004-1702-4, 2005.
  - Koncz, P., Besnyői, V., Csathó, A. I., Nagy, J., Szerdahelyi, T., Tóth, Z., Pintér, K., Balogh, J., Nagy, Z., and Bartha, S.: Effect of grazing and mowing on the microcoenological composition of semi-arid grassland in Hungary, Appl. Ecol. Env. Res., 12, 563–575, 2014.
- of semi-arid grassland in Hungary, Appl. Ecol. Env. Res., 12, 563–575, 2014. Midwood, A. J. and Millard, P.: Challenges in measuring the  $\delta^{13}$ C of the soil surface CO<sub>2</sub> efflux, Rapid Commun. Mass Sp., 25, 232–242, doi:10.1002/rcm.4857, 2011.
  - Moyano, F., Kutsch, W., and Schulze, E.: Response of mycorrhizal, rhizosphere and soil basal respiration to temperature and photosynthesis in a barley field, Soil Biol. Biochem., 39, 843–
- <sup>25</sup> 853, doi:10.1016/j.soilbio.2006.10.001, 2007.
- Moyano, F., Atkin, O., Bahn, M., Bruhn, D., Burton, A., Heinemeyer, A., Kutsch, W. L., and Wieser, G.: Respiration from roots and the mycorrhizosphere, in: Soil Carbon Dynamics: an Integrated Methodology, edited by: Bahn, M., Heinemeyer, A., and Kutsch, W. L., Cambridge University Press, Cambridge, 234–288, 2009.
- Moyano, F. E., Manzoni, S., and Chenu, C.: Responses of soil heterotrophic respiration to moisture availability: an exploration of processes and models, Soil Biol. Biochem., 59, 72–85, doi:10.1016/j.soilbio.2013.01.002, 2013.



Moyes, A. B., Gaines, S. J., Siegwolf, R. T. W., and Bowling, D. R.: Diffusive fractionation complicates isotopic partitioning of autotrophic and heterotrophic sources of soil respiration., Plant Cell Environ., 33, 1804–19, doi:10.1111/j.1365-3040.2010.02185.x, 2010.

Mummey, D. L. and Rillig, M. C.: Spatial characterization of arbuscular mycorrhizal fungal

- <sup>5</sup> molecular diversity at the submetre scale in a temperate grassland, FEMS Microbiol. Ecol., 64, 260–270, doi:10.1111/j.1574-6941.2008.00475.x, 2008.
  - Nagy, Z., Pintér, K., Czóbel, S., Balogh, J., Horváth, L., Fóti, S., Barcza, Z., Weidinger, T., Csintalan, Z., Dinh, N. Q., Grosz, B., and Tuba, Z.: The carbon budget of semi-arid grassland in a wet and a dry year in Hungary, Agr. Ecosyst. Environ., 121, 21–29, doi:10.1016/j.agee.2006.12.003, 2007.
- Nagy, Z., Pintér, K., Pavelka, M., Darenová, E., and Balogh, J.: Carbon fluxes of surfaces vs. ecosystems: advantages of measuring eddy covariance and soil respiration simultaneously in dry grassland ecosystems, Biogeosciences, 8, 2523–2534, doi:10.5194/bg-8-2523-2011, 2011.

10

20

- <sup>15</sup> Nickerson, N. and Risk, D.: A numerical evaluation of chamber methodologies used in measuring the  $\delta^{13}$ C of soil respiration, Rapid Commun. Mass Sp., 23, 2802–2810, doi:10.1002/rcm, 2009.
  - Parton, W., Morgan, J., Smith, D., Del Grosso, S., Prihodko, L., Lecain, D., Kelly, R., and Lutz, S.: Impact of precipitation dynamics on net ecosystem productivity, Glob. Change Biol., 18, 915– 927, doi:10.1111/j.1365-2486.2011.02611.x, 2012.
  - Pintér, K., Balogh, J., and Nagy, Z.: Ecosystem scale carbon dioxide balance of two grasslands in Hungary under different weather conditions., Acta Biol. Hung., 61, 130–135, doi:10.1556/ABiol.61.2010.Suppl.13, 2010.

Prudhomme, C., Giuntoli, I., Robinson, E. L., Clark, D. B., Arnell, N. W., Dankers, R.,

- Fekete, B. M., Franssen, W., Gerten, D., Gosling, S. N., Hagemann, S., Hannah, D. M., Kim, H., Masaki, Y., Satoh, Y., Stacke, T., Wada, Y., and Wisser, D.: Hydrological droughts in the 21<sup>st</sup> century, hotspots and uncertainties from a global multimodel ensemble experiment, P. Natl. Acad. Sci. USA, 111, 3262–3267, doi:10.1073/pnas.1222473110, 2014. Reichstein, M., Bahn, M., Ciais, P., Frank, D., Mahecha, M. D., Seneviratne, S. I., Zscheisch-
- <sup>30</sup> Ier, J., Beer, C., Buchmann, N., Frank, D. C., Papale, D., Rammig, A., Smith, P., Thonicke, K., van der Velde, M., Vicca, S., Walz, A., and Wattenbach, M.: Climate extremes and the carbon cycle, Nature, 500, 287–295, doi:10.1038/nature12350, 2013.



- Risk, D., Nickerson, N., Phillips, C. L., Kellman, L., and Moroni, M.: Drought alters respired  $\delta^{13}CO_2$  from autotrophic, but not heterotrophic soil respiration, Soil Biol. Biochem., 50, 26–32, doi:10.1016/j.soilbio.2012.01.025, 2012.
- Suseela, V. and Dukes, J.: The responses of soil and rhizosphere respiration to simulated climatic changes vary by season, Ecology, 94, 403–413, 2013.

5

15

- R Core Team: R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2014.
- Tennant, D.: A test of a modified line intersect method of estimating root length, J. Ecol., 63, 995–1001, 1975.
- Van der Heijden, M. G. A., Bardgett, R. D., and van Straalen, N. M.: The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems., Ecol. Lett., 11, 296–310, doi:10.1111/j.1461-0248.2007.01139.x, 2008.
  - Van der Heijden, M. G. A., Martin, F. M., Selosse, M.-A., and Sanders, I. R.: Mycorrhizal ecology and evolution: the past, the present, and the future, New Phytol., 205, 1406–1423, doi:10.1111/nph.13288, 2015.
- Van der Molen, M. K., Dolman, A. J., Ciais, P., Eglin, T., Gobron, N., Law, B. E., Meir, P., Peters, W., Phillips, O. L., Reichstein, M., Chen, T., Dekker, S. C., Doubková, M., Friedl, M. A., Jung, M., van den Hurk, B. J. J. M., de Jeu, R. A. M., Kruijt, B., Ohta, T., Rebel, K. T., Plummer, S., Seneviratne, S. I., Sitch, S., Teuling, A. J., van der Werf, G. R., and
- <sup>20</sup> Wang, G.: Drought and ecosystem carbon cycling, Agr. Forest Meteorol., 151, 765–773, doi:10.1016/j.agrformet.2011.01.018, 2011.
  - Vargas, R., Detto, M., Baldocchi, D. D., and Allen, M. F.: Multiscale analysis of temporal variability of soil CO<sub>2</sub> production as influenced by weather and vegetation, Glob. Change Biol., 16, 1589–1605, doi:10.1111/j.1365-2486.2009.02111.x, 2010.
- <sup>25</sup> Wilks, D. S.: Statistical methods in the atmospheric sciences, Academic Press, 2006.
- Yang, F. and Zhou, G.: Sensitivity of temperate desert steppe carbon exchange to seasonal droughts and precipitation variations in Inner Mongolia, China, PLOS One, 8, e55418, doi:10.1371/journal.pone.0055418, 2013.



Table 1. Measured and estimated  $CO_2$  effluxes and isotopic signals in this study.

	CO <sub>2</sub> efflux	Isotopic signals
measured estimated	$R_{ m eco}, R_{ m soil}, R_{ m re}, R_{ m rme}$ $R_{ m rhizo}, R_{ m myc}, R_{ m het}$	$ \begin{array}{c} \delta^{13} C_{Reco},  \delta^{13} C_{Rsoil},  \delta^{13} C_{Rre},  \delta^{13} C_{Rrme} \\ \delta^{13} C_{Rmycrhiz},  \delta^{13} C_{Rrhizo},  \delta^{13} C_{Rmyc} \end{array} $

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**Figure 1. (a)** Mean hyphal length  $(mg^{-1} dry soil)$  and **(b)** mean microbial activity expressed as fluorescein released  $(mgkg^{-1} dry soil)$  in the undisturbed soil, root-exclusion and rootand mycorrhiza-exclusion in different soil depths. Asterisks denote significant differences from undisturbed soil.





**Figure 2.** Measured ( $R_{eco}$ ,  $R_{soil}$ ,  $R_{re}$ ,  $R_{rme}$ ) and estimated ( $R_{mycrhiz}$ ,  $R_{rhizo}$ ,  $R_{myc}$ ) $\delta^{13}$ C values of the respiration components. Horizontal lines in boxes show medians and dashed whiskers show data extremes. Open circles and solid whiskers show means ± propagated standard errors.







**Figure 3. (a)** Daily averages of soil temperature ( $T_s$ ), soil water content (SWC) at 5 cm depth and daily sum of precipitation, (**b**) daily minimum half-hourly NEE and maximum half-hourly ET, (**c**) daily averages of CO<sub>2</sub> efflux in undisturbed soil ( $R_{soil}$ ), root-excluded soil ( $R_{re}$ ) and root- and mycorrhizal fungi-excluded soil ( $R_{rme}$ ), (**d**) daily averages of  $\delta^{13}$ C of soil CO<sub>2</sub> efflux in undisturbed soil ( $\delta^{13}C_{Rsoil}$ ), root-excluded soil ( $\delta^{13}C_{Rre}$ ) and root- and mycorrhizal fungiexcluded soil ( $\delta^{13}C_{Rsoil}$ ), root-excluded soil ( $\delta^{13}C_{Rre}$ ) and root- and mycorrhizal fungiexcluded soil ( $\delta^{13}C_{Rrme}$ ) and (**e**) daily averages of  $\delta^{13}$ C of ecosystem respiration ( $\delta^{13}C_{Reco}$ ) during the study period in 2013, at Bugac site.



**Figure 4.** Relative contributions made by rhizospheric, mycorrhizal fungi and heterotrophic components to the total soil respiration in the different parts of the vegetation period (15 May 2013–12 November 2013) at Bugac site. Propagated uncertainties of each estimate are shown in the lower panel.

