# 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 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 $CO_2$ efflux and its isotopic ($^{13}C$) 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 $CO_2$ efflux decreased at different rates under drought conditions. During drought the contribution made by the heterotrophic components was the highest (54±8%; mean±SE). Rhizospheric component was the most sensitive to soil drying with its relative contribution to the total soil respiration dropping from 66±7% (non-stressed) to 35±17% (mean±SE) under drought conditions. According to our results, the heterotrophic component of soil respiration is the major contributor to the respiration activities during drought events in this dry grassland ecosystem.

## 1 Introduction

Grassland ecosystems strongly respond to drought events via substantial reduction of primary production (GPP, Hoover et al., 2014; 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). Soil respiration is the second largest component of carbon cycling in grasslands and returns 50–90% of annual GPP back to the atmosphere (Bahn et al., 2008). Thus, the magnitude of soil respiration can turn the carbon budget from a net sink to a net source in dry years (Nagy et al., 2007). Here we address the question whether under drought this is primarily a function of autotrophic respiration which declines as GPP declines, whereas heterotrophic respiration remains largely unaffected.

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

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 and have an about 50% share in the total soil respiration in dry grasslands (Bao et al., 2010; Chen et al., 2009; Gomez-Casanovas et al., 2012). On the other hand, some of the soil fungi using recent photosynthetic assimilates are contributing to the autotrophic respiration 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 in exchange of acquisition of water and essential nutrients for plant productivity (van der Heijden et al., 2015). Therefore soil respiration includes components of an autotrophic-heterotrophic continuum from roots through the root-associated (rhizospheric and mycorrhizal) to non-root-associated (heterotrophic) microbial components.

Belowground $CO_2$ production by the 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 $CO_2$ production frequently interact with each other (Balogh et al., 2015; Vargas et al., 2010), hampering the partitioning of the total $CO_2$ 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.

The widely used separation techniques (trenching and girdling) are not considered suitable for grasslands (Epron, 2009), thus the physical separation of the soil $CO_2$ efflux components via root exclusion is hardly feasible without serious disturbance of soil structure and the root system. A viable option, however, is the use of stable isotopic signatures ($\delta^{13}C$) of soil respiration for estimating the relative contributions of the main components (Carbone et al., 2011; Hopkins et al., 2013). Although diel patterns in $\delta^{13}C$ may also be subject to biases in measuring methods (Fassbinder et al., 2011; Midwood and Millard, 2011), seasonal changes are 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 in $^{13}C$ than the older SOM components (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 $\delta^{13}CO_2$, since heterotrophic respiration could be restricted to the deeper layers of the soil (Moyes et al., 2010). Soil drying can also change the amount of $CO_2$ produced in the topsoil layer (Balogh et al., 2015) by allowing greater atmospheric invasion, therefore enriched $\delta^{13}C$ in soil air (Phillips and Nickerson, 2010). The disequilibrium between the measured isotopic composition and the isotopic composition of the respiratory source could be significant, especially in tracer experiments (Gamnitzer et al., 2011), but it is assumed to be less pronounced in open chamber measurements due to the steady-state diffusion (Nickerson et al., 2013).

Uncertainties in estimates about the contributions of soil respiration components could be reduced by a combination of different methodologies (Risk et al., 2012). The question we are asking is: which of the investigated soil respiration components (autotrophic - including

rhizospheric and mycorrhizal fungi - and heterotrophic components) of a dry grassland dominates during drought? Our hypothesis is that autotrophic respiration would be reduced linearly with photosynthesis, whereas heterotrophic respiration might not be affected as strongly, resulting in net C loss from the soil carbon. To achieve our goals, we used an experimental setup of physical separation of soil respiration components combined with measurements of soil $CO_2$ efflux and its isotopic ($^{13}C$) signal.

## 2 Methods

### 2.1 Site description

The vegetation at the Bugac site (46.69° N, 19.6° E, 114 m above sea level) 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., 2015).

### 2.2 Spatial separation of soil $CO_2$ efflux components

In 2010, ten soil cores (160 mm in diameter and 800 mm in depth, one of them 600 mm in diameter) were excavated. The roots were removed and the root-free soil was packed back - layer by layer - into PVC tubes with the same dimensions. Four tubes were used to exclude both roots and mycorrhiza. Walls of another 6 tubes were partially removed and replaced by inox mesh (40 μm mesh 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 6 m from the eddy covariance tower to the south direction (Fig. 1.). The distance between the soil cores/tubes was 50 cm.

Soil $CO_2$ efflux and its isotopic signal were measured in plots:

- with undisturbed soil (various positions, 36 positions in total within a ~4 $m^2$ plot): total soil respiration, $R_{soil}$, $\delta^{13}C_{Rsoil}$,
- without roots and arbuscular mycorrhizal fungi (4 spatial replications) = heterotrophic component only, $R_{rme}$, $\delta^{13}C_{Rrme}$,
- with root-excluded soil (6 spatial replications) = without roots, but with arbuscular mycorrhizal fungi, $R_{re}$, $\delta^{13}C_{Rre}$.

## 2.3 Gas exchange measuring systems

Three different gas exchange systems were used in this study: eddy-covariance system (EC), automated soil respiration measuring system (SRS), connected to an isotopic $CO_2$ analyser (cavity ring-down spectroscopy system, CRDS). The experimental area was in the EC footprint (Supplementary material Fig. S1), but 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 plot with the same average soil characteristics and vegetation cover as found in the EC footprint area. 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$^{th}$ May 2013 to 12$^{th}$ November 2013 (182 days) were analysed in this study.

### 2.3.1 Eddy covariance setup

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 g C $m^{-2}$ $yr^{-1}$ (Pintér et al., 2010) with a -100 g C $m^{-2}$ $yr^{-1}$ 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: 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 (105T, Campbell, UK). These measurements were performed as described in Nagy et al. (2007) and Pintér et al. (2010). Fluxes of sensible and latent heat and $CO_2$ 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

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 (PPSystems, 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 $cm^2$. The flow rate through the chambers was 300 ml $min^{-1}$, replacing the air in the chamber in 40 seconds. 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 $cm^2$ 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 $CO_2$ efflux and has been used in previous experiments (Nagy et al. 2011). The soil respiration chambers contained no standing aboveground plant material. Other studies (Nickerson et al., 2013; Risk et al., 2011) also used this chamber size, arguing that these chambers can be placed between the plants in grasslands, while larger chambers might create a non-representative surface due to the cutting necessary for placing the chambers on the ground (Risk et al., 2011).

$R_{soil}$ was measured by 6 SRS chambers.

$R_{rme}$ was measured by 2 SRS chambers.

$R_{re}$ was measured by 2 SRS chambers.

### 2.3.3 Isotopic ($^{13}CO_2$) measurements

A Picarro G1101-i gas analyser (CRDS, Picarro Inc., CA, USA) was attached to the soil respiration system from May to November in 2013. This CRDS system measured the isotopic composition inside the chambers and in the reference air 10 cm above the surface. The SRS sequentially measured each of the 10 chambers for 3 minutes. Every second chamber was additionally probed for isotopic signature measurements by the CRDS (3 minutes), followed by reference air measurements for another 3 minutes. Thus, 5 chambers for isotopic measurements took 30 minutes to measure in a single cycle. The CRDS integration time was set at 10 seconds, thus the CRDS provided 18 measurement points per chamber per cycle. Although the system response of the CRDS was clearly slower than the response of the SRS, the 3-minute duration was long enough to obtain robust results. Since CRDS followed the 3-minute intervals of SRS measurements, no additional grace time had to be considered for the isotopic measurements.

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

Since contributions by the different soil $CO_2$ efflux components were estimated for five different periods within the study period distinguished by NEE, SWC values and isotopic signals (see Results), data for each estimation originated from 2-3 spatial replications.

## 2.4 Data processing and modelling

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 $CO_2$ efflux were calculated using the isotopic mass balance approach in each plot:

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

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 $CO_2$ concentration of the of the outgoing and incoming air of the chamber, respectively.

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

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 ± 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 $CO_2$ 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. We defined the components following the terminology presented by Moyano et al. (2009):

Heterotrophic respiration= microbial respiration from litter and SOM decomposition.

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

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

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

$$\delta^{13}C_{Rsoil} = a \times \delta^{13}C_{Rrhizo} + (1-a) \times \delta^{13}C_{Rre} \quad (3)$$

$$\delta^{13}C_{Rsoil} = b \times \delta^{13}C_{Rmycrhiz} + (1-b) \times \delta^{13}C_{Rrme} \quad (4)$$

where $\delta^{13}C_{Rsoil}$ is the $\delta^{13}C$ of the total soil $CO_2$ efflux, $\delta^{13}C_{Rre}$ is the $\delta^{13}C$ of the root-excluded soil, $\delta^{13}C_{Rrme}$ is the $\delta^{13}C$ of the root- and mycorrhiza excluded soil (heterotrophic respiration), *a* is the fraction of the rhizospheric component ($R_{rhizo}$) and *b* is the fraction of the mycorrhizospheric component ($R_{mycrhiz}$) to the total soil efflux. According to these equations *1-b* represents the ratio of heterotrophic respiration component to the total soil efflux and *b-a* represents the ratio of mycorrhizal fungi component.

$\delta^{13}C_{Rrhizo}$ value was estimated by plotting $\delta^{13}C_{Rsoil}$ values against the $R_{re}/R_{soil}$ ratio (Supplementary material Fig. S3b). Since $R_{re}/R_{soil}$ is hypothetically zero when only rhizospheric respiration is present, y-intercept of the linear regression was assumed as $\delta^{13}C_{Rrhizo}$. $\delta^{13}C_{Rmycrhiz}$ was estimated using the same approach (Supplementary material Fig. S3a), $\delta^{13}C_{Rsoil}$ values were plotted against the $R_{rme}/R_{soil}$ ratio and y-intercept of the linear regression was assumed as $\delta^{13}C_{Rmycrhiz}$. Similarly, $\delta^{13}C_{Rre}$ values were plotted against the $R_{rme}/R_{re}$ ratio and y-intercept of the linear regression was assumed as $\delta^{13}C_{Rmyc}$ (Supplementary material Fig. S4c), but this value was not used in further calculations.

Contributions of rhizospheric, mycorrhizal fungi and heterotrophic respirations to total soil respiration were calculated by the mixing models applied on subsets (periods) of the dataset of the total study period.

## 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 cm, 10-20 cm, 20-30 cm, 30-40 cm 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 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 $kg^{-1}$ dry soil (Adam and Duncan, 2001).

## 2.6 Uncertainty assessment

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 $\delta^{13}C_{Reco}$ 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, isotopic measurements were independent on soil $CO_2$ 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 $CO_2$. 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. Isotopic signal of $CO_2$ efflux by the C4 plant was supposed to be -14‰.

In order to estimate the uncertainty of the measurements and estimated contributions by the different components to the total soil respiration, random errors of each factor ($CO_2$ concentrations, isotopic compositions, model fit errors and possible C4 contribution) were propagated by Gaussian error propagation (Lo, 2005).

# 3 Results

## 3.1 Meteorological conditions, NEE, ET, soil $CO_2$ efflux, $\delta^{13}C$ of $CO_2$ efflux

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. 1a). It rained only a few times from the end of June to 19$^{th}$ 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 $CO_2$ effluxes (Fig. 1c). There was a second active period following autumn rains, but $CO_2$ uptake and ET were smaller than in May or June.

$R_{soil}$ was the highest among the soil $CO_2$ effluxes, while $R_{rme}$ was the lowest, the average $CO_2$ effluxes in the whole study period were 5.0±2.1, 3.8±1.6 and 2.6±1.2 μmol $CO_2$ $m^{-2}$ $s^{-1}$ (mean±SD) in $R_{soil}$, $R_{re}$ and $R_{rme}$, respectively. $R_{re}$ was sometimes higher than $R_{soil}$, especially shortly after rain events. Lowest daily average total soil $CO_2$ efflux was measured in 15$^{th}$ August (2.22 μmol $CO_2$ $m^{-2}$ $s^{-1}$), while the lowest daily average $R_{re}$ and $R_{rme}$ values were observed in 2$^{nd}$ October (1.25 μmol $CO_2$ $m^{-2}$ $s^{-1}$) and 2$^{nd}$ November (1.04 μmol $CO_2$ $m^{-2}$ $s^{-1}$), respectively. Highest values of soil $CO_2$ effluxes were measured in May in all treatments ($R_{soil}$, $R_{re}$ and $R_{rme}$). Sudden increases in $R_{re}$ and $R_{rme}$ were observed shortly after rain events, but $R_{soil}$ showed slower (but more persistent) response to precipitation.

Isotopic signature of $R_{eco}$ was the lowest in May and June, increased in July and August and decreased again in October and November. $\delta^{13}C_{Reco}$ showed clear responses to precipitation pulses: sudden declines were observed during the rain events. Chamber-based $\delta^{13}C_{Rsoil}$ showed similar changes during the study period. $\delta^{13}C_{Rrme}$ and $\delta^{13}C_{Rre}$ showed large scatter during the whole study period with no clear and detectable trends. Differences between $\delta^{13}C_{Rsoil}$ and $\delta^{13}C_{Rrme}$ were 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 15th May to 20th June, a drying (stress development) period from 21st June to 22nd July, a drought period from 23rd July to 19th August, a wetting (stress release) period from 20th August to 16th September and a re-greening (recovery) period from 17th September to the end of the study period (11th November) (Fig. 1).

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

Fig 2. shows the measured and estimated $\delta^{13}C$ values of the different soil $CO_2$ efflux components. $\delta^{13}C_{Rrme}$ was the highest, while $\delta^{13}C_{Reco}$ was the lowest, suggesting that rhizospheric respiration was the most substantially depleted, while heterotrophic respiration was the least depleted in $^{13}C$. Mean of $\delta^{13}C_{Reco}$, $\delta^{13}C_{Rsoil}$, $\delta^{13}C_{Rre}$ and $\delta^{13}C_{Rrme}$ were -27.9±0.5‰, -26.8±1.3‰, -26.4±1.8‰ and -25.7±2‰ (mean±SE), respectively. The estimated isotopic signal of the respiration of mycorrhizospheric ($\delta^{13}C_{Rmycrhiz}$), rhizospheric ($\delta^{13}C_{Rrhizo}$) and mycorrhizal fungi components ($\delta^{13}C_{Rmyc}$) were -28.6±1.6‰, -28.9±1.7‰ and -27.2±2.3‰ (estimate±SE), respectively (Fig. 2).

36% of the variation in $\delta^{13}C_{Rsoil}$ was explained by SWC ($\delta^{13}C_{Rsoil}$= -0.1267 x SWC- 25.537, $R^2$=0.36, P<0.0001), while only 3% of the variation of $\delta^{13}C_{Rrme}$ was explained by SWC and there was no correlation between $\delta^{13}C_{Rre}$ and SWC. Similar results were found between $T_s$ and isotopic signals, but the correlation was weaker ($\delta^{13}C_{Rsoil}$ = 0.1056 x $T_s$ - 28.588, $R^2$=0.11, P<0.0001).

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

Two end-member mixing models (eq. 3 and 4) were used to estimate the relative contributions of rhizospheric, mycorrhizal fungi and heterotrophic components to total soil respiration during the study period. The estimated contributions by the different components were 50±6%, 13±8% and 37±6% (mean±SE) for the rhizospheric, mycorrhizal fungi and

heterotrophic components, respectively. The autotrophic component (mycorrhizospheric component) of soil respiration showed significant decrease during the drying and drought periods. Rhizospheric component was the most sensitive to drying and drought. Average contribution by the rhizospheric component to total soil $CO_2$ efflux decreased from 66±7% (mean±SE) in the active period to 35±13% during the drought period (Fig. 3). After drought, rhizospheric contribution increased again and become dominant during the re-greening period in autumn 63±7% (mean±SE). During the transient (drying and wetting) periods the rhizospheric contribution to the total soil $CO_2$ efflux was 38±11% and 46±8%, respectively. Relative mycorrhizal contribution was between 8-21% during the whole study period, with the highest contribution (21±11%; mean±SE) during the wetting period. Heterotrophic contribution to soil respiration was lowest in the active period (21±7%) and highest under drought (54±13%) (Fig. 3).

Changes in soil $CO_2$ effluxes showed similar responses to drying and drought conditions as isotopic signals. Average $R_{soil}$ decreased by 60% (referenced to the average during the active period) as a response to drought, while $R_{re}$ and $R_{rme}$ showed declines of 56 and 52% respectively, suggesting declines in root respiration to be substantially larger than that in $R_{soil}$ (60%).

### 3.4 Microbial biomass and activity

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

## 4 Discussion

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

Ratio of the autotrophic component in total soil $CO_2$ efflux was 63±6% on average (rhizospheric and mycorrhizal fungi components, 50±6% and 13±8%, respectively) and it was

much higher than the ratio of the heterotrophic (37±6%) component, but the contributions by the different components showed significant changes during the growing season. In other studies, conducted in grassland ecosystems, the estimated yearly average ratio of the autotrophic component was found to be lower: 38-52% of the total soil respiration (Bao et al., 2010; Heinemeyer et al., 2012), while reaching 74% during the growing season in a prairie grassland (Gomez-Casanovas et al., 2012) and 60-74% in an arid perennial grassland (Carbone et al., 2008).

Soil $CO_2$ effluxes decreased in all treatments ($R_{soil}$, $R_{re}$, $R_{rme}$) under dry conditions, the largest decline was observed in total soil respiration ($R_{soil}$), therefore a strong response of the autotrophic component to drought could be assumed. The observed increase in $\delta^{13}C_{Reco}$ and $\delta^{13}C_{Rsoil}$ values during the drying period and during the drought also showed the decline of the autotrophic component. The same phenomenon was shown by the modelling results, with the smallest contribution by the rhizospheric component estimated for the drought period (35±13%; mean±SE), while the highest for the active period (66±7%; mean±SE). Fraction of the heterotrophic respiration were the highest during the drought (54±13% mean±SE) and mycorrhizal fungi respiration showed only a small decrease during drought compared to the active period (from 13±10%; to 11±18%;), 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 be moist enough for those organisms to thrive even under drought (Davidson et al., 2012). Since there was an absence in plant photosynthetic supply during drought period, mycorrhizal fungi component is expected to use stored carbon for respiration (van der Heijden et al., 2008).

Low $\delta^{13}C_{Rsoil}$ and $\delta^{13}C_{Reco}$ 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 $\delta^{13}C_{Rsoil}$ and $\delta^{13}C_{Reco}$ 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. Since the rhizospheric contribution estimated for the re-greening period was high, it is supposed that this result was obtained partly due to the increased amount of fresh litter. 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. In contrary, Carbone et al. (2008) found

more sensitive response by the heterotrophic component in an arid (<150 mm annual precipitation) perennial grassland. Ratio of autotrophic components were reported to increase in response to drought in a woodland ecosystem, suggesting signature of the recent photosynthetic supply became enriched during drought and that could also explain the enrichment of the soil respired $CO_2$ (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 might also increased. In our study, $R_{rme}/R_{soil}$ showed significant positive correlation with $\delta^{13}C_{Rsoil}$ (the regression was used to estimate $\delta^{13}C_{Rrhizo}$, Supplementary material Fig S4), so $\delta^{13}C_{Rsoil}$ was high if the ratio of heterotrophic $CO_2$ efflux to the total soil $CO_2$ efflux was found to be high. According to these studies and to our results we can assume that the different vegetation types may respond differently to drought: woodlands may increase the autotrophic contribution, while in grasslands it may decrease (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 differences similar to the ones as reviewed by Bowling et al. (2008). $\delta^{13}C_{Reco}$ (containing also the signal from above ground green biomass) was the most depleted, while $\delta^{13}C_{Rrme}$ (heterotrophic components only) 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). $CO_2$ effluxes from mycorrhizal fungi were expected to be more enriched in $^{13}C$ relative to the total soil respiration (about +3‰, Bowling et al. 2008). Estimated $\delta^{13}C$ of mycorrhizal fungi component was -27.2±2.3‰ (estimate±SE), which is 1.7‰ higher than the rhizospheric component (-28.9±1.7‰; estimate±SE).

In our study, neither $\delta^{13}C_{Rrme}$ values (heterotrophic respiration), nor $\delta^{13}C_{Rre}$ values (heterotrophic+mycorrhizal fungi respiration) showed correlation with SWC, but $\delta^{13}C_{Rsoil}$ (total soil respiration) showed significant negative correlation with SWC. We can assume that $\delta^{13}C$ of heterotrophic respiration was not influenced by SWC changes during the growing season, as it was found also by other studies (Phillips and Nickerson, 2010; Risk et al., 2012).

Further, the lack of correlation by the present study also suggests that soil moisture induced changes in diffusivity (disequilibrium effect due to changing soil moisture) were not large enough to affect the measured $\delta^{13}C$ values.

### 4.3 Microbial investigations

High hyphal density was maintained in $R_{re}$ plots and low, but still significant microbial activities (SOM decomposition) were detected in $R_{rme}$ plots, therefore the measured $\delta^{13}C$ values characterized the sources of the root-free ($\delta^{13}C_{Rre}$) and root- and mycorrhiza-free ($\delta^{13}C_{Rrme}$) soils. The fact that very high amounts of hyphae were found in the root-excluded soil 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 of mycorrhizal colonization (van der Heijden et al., 2015). The range of AM hyphal lengths found in this study (1.9–8.8 m $g^{-1}$ soil) are similar to those reported in the literature (e.g. Mummey and Rillig 2008). The higher hyphal densities found in root-free soil might have been related to the higher availability of SOM-derived nutrients and to more space without the roots (i.e. lack of competition). According to our results, significant amount of $CO_2$ was respired from mycorrhizal filaments in the undisturbed soil, having 12-31% share in the respiration by the autotrophic component.

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), but were much lower in root- and mycorrhiza excluded soil. These results support the component estimations showing the significant activity of root-associated microorganisms.

## 5 Conclusions

In the dry grassland investigated in this study all three components of the soil $CO_2$ effluxes decreased, following different dynamics under drought conditions. Both measured $CO_2$ effluxes and isotopic signals showed similar results regarding component responses. The strongest decrease in response to drought was seen in rhizospheric respiration (relative contribution to total respiration decreased from 66±7% to 35±13%; mean±SE), while the relative contribution to the total soil respiration by the heterotrophic components increased during soil drying. During drought the contribution of the heterotrophic component was found to be the highest (54±8%; mean±SE). Mycorrhizal fungi respiration had its higher share in

soil respiration (21±11%; mean±SE) in the wetting period after drought. According to these results, the carbon source activities identified by NEE measurements originated from carbon sources already stored, thereby decreasing the carbon content of the soil.

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 potential 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, Sz, Fóti, W. Eugster and Z. Nagy analyzed the data and wrote the paper, but all co-authors contributed to writing.

## Acknowledgements

The authors gratefully acknowledge the financial support of the projects OTKA-PD 100575, OTKA-PD 105944, Research Centre of Excellence (9878/2015/FEKUT) and KTIA_AIK_12-1-2012-0012. János Balogh and Szilvia Fóti acknowledge the support of the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. Isotopic measurements were supported by Gábor Baross Programme (REG-KM-09-1-2009-0029).

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

| | $CO_2$ efflux | Isotopic signals |
|---|---|---|
| measured | $R_{eco}$, $R_{soil}$, $R_{re}$, $R_{rme}$ | $\delta^{13}C_{Reco}$, $\delta^{13}C_{Rsoil}$, $\delta^{13}C_{Rre}$, $\delta^{13}C_{Rrme}$ |
| estimated | $R_{rhizo}$, $R_{myc}$, $R_{het}$ | $\delta^{13}C_{Rmycrhiz,}$ $\delta^{13}C_{Rrhizo}$, $\delta^{13}C_{Rmyc}$ |

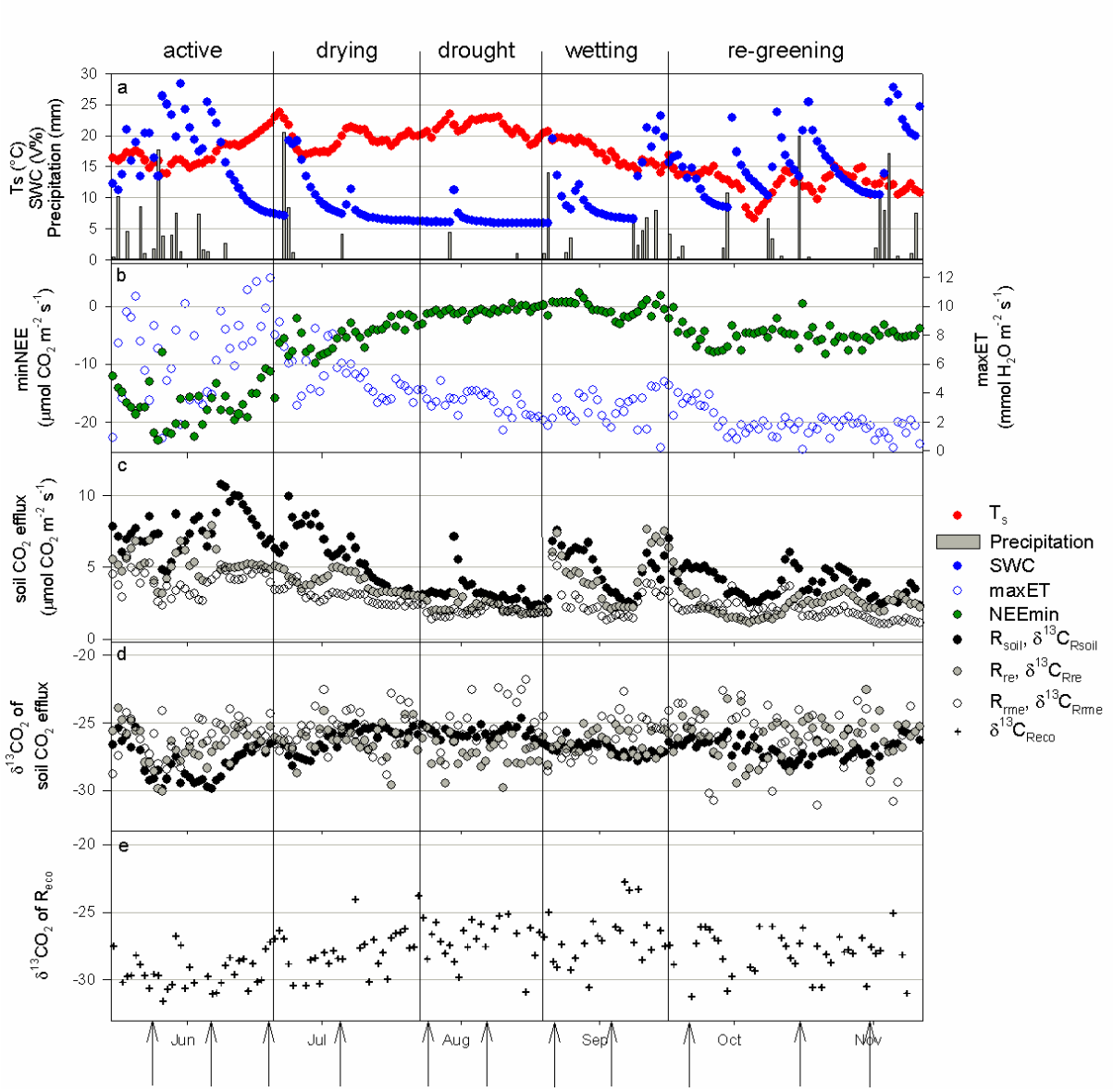


Fig. 1 (a) Daily averages of soil temperature ($T_s$), soil water content (SWC) at 5 cm depth and daily sum of precipitation, (b) daily minimum half-hourly NEE and maximum half-hourly ET, (c) daily averages of $CO_2$ efflux in undisturbed soil ($R_{soil}$), root-excluded soil ($R_{re}$) and root- and mycorrhizal fungi excluded soil ($R_{rme}$), (d) daily averages of $\delta^{13}C$ of soil $CO_2$ efflux in undisturbed soil ($\delta^{13}C_{Rsoil}$), root-excluded soil ($\delta^{13}C_{Rre}$) and root- and mycorrhizal fungi excluded 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. Arrows indicate the positions changes of the soil chambers.

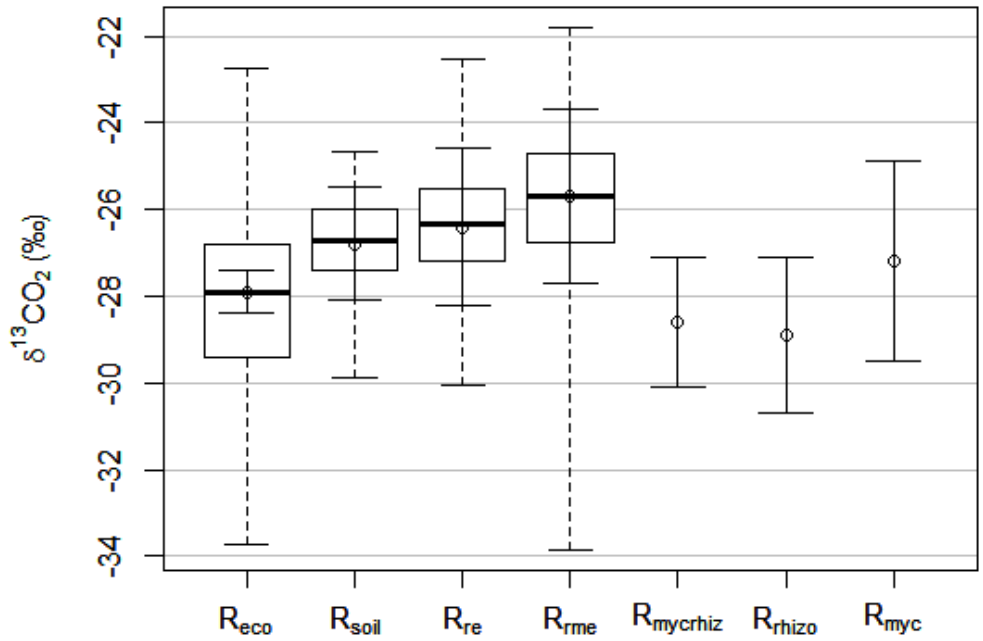


Fig. 2 Measured ($R_{eco}$, $R_{soil}$, $R_{re}$, $R_{rme}$) and estimated ($R_{mycrhiz}$, $R_{rhizo}$, $R_{myc}$) $\delta^{13}C$ values of the respiration components. Horizontal lines in boxes show medians and dashed whiskers show data extremes. Open circles and solid whiskers show means±propagated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

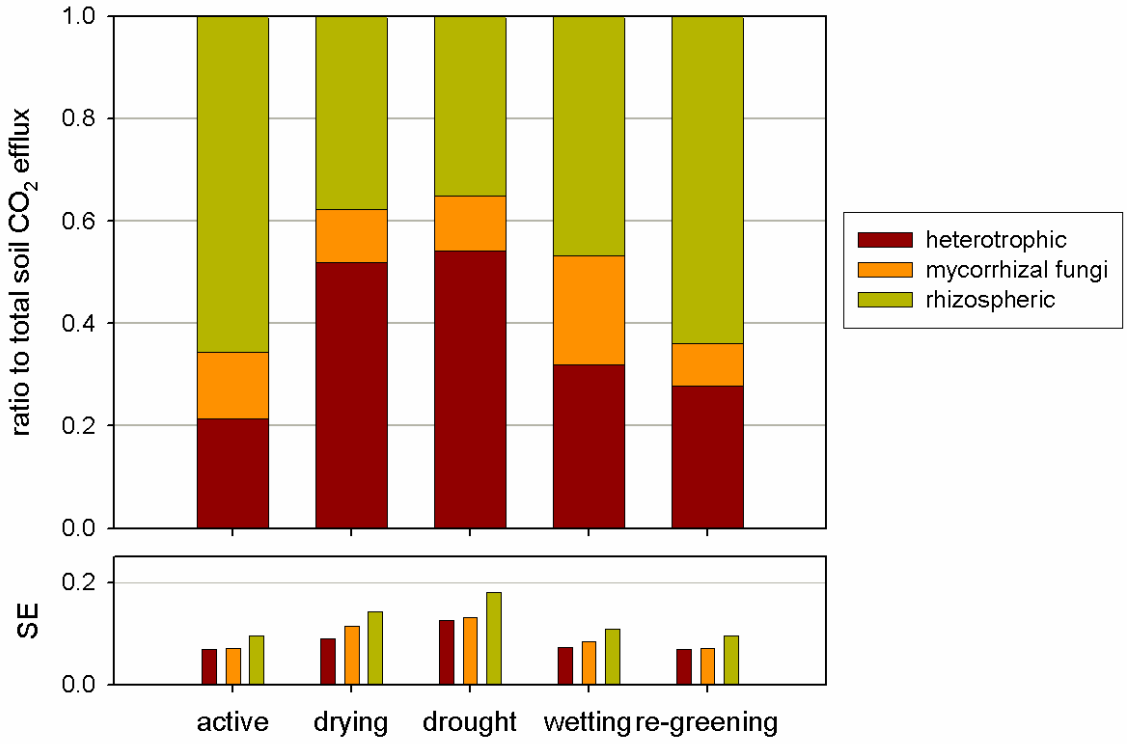


Fig. 3 Relative contributions made by rhizospheric, mycorrhizal fungi and heterotrophic components to the total soil respiration in the different parts of the vegetation period (15/05/2013-12/11/2013) at Bugac site. Propagated uncertainties of each estimate are shown in the lower panel.

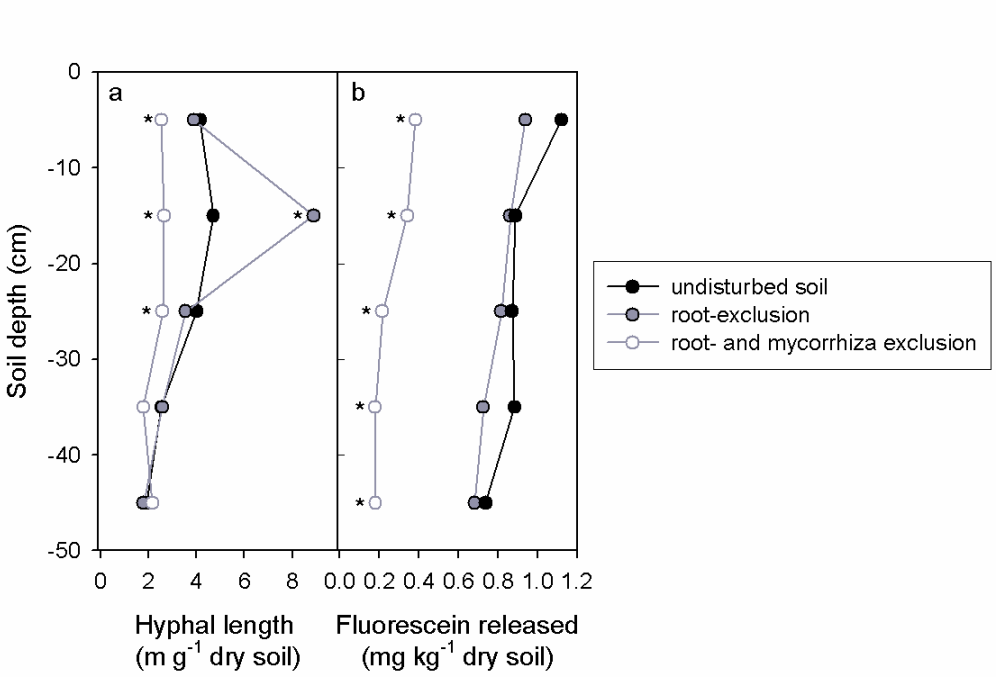


Fig. 4 (a) Mean hyphal length (m $g^{-1}$ dry soil) and (b) mean microbial activity expressed as fluorescein released (mg $kg^{-1}$ dry soil) in the undisturbed soil, root-exclusion and root- and mycorrhiza exclusion in different soil depths. Asterisks denote significant differences from undisturbed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