Title: Alterations in forest detritus inputs influence soil carbon concentration and soil respiration in a Central-European deciduous forest.

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Key words: soil CO_2, climate change, soil temperature, soil moisture, detritus manipulation, DIRT, oak forest

Paper type: Primary Research Article

Abstract

In a *Quercetum petraeae-cerris* forest in northeastern Hungary, we examined effects of litter input alterations on the quantity and quality soil carbon stocks and soil CO_2 emissions. Treatments at the Síkfőkút DIRT (**D**etritus **I**nput and **R**emoval **T**reatments) experimental site include adding (by doubling) of either leaf litter (DL) or wood (DW) (including branches, twigs, bark), and removing all aboveground litter (NL), all root inputs by trenching (NR), or removing all litter inputs (NI). Within 4 years we saw a significant decrease in soil carbon (C) concentrations in the upper 15 cm for root exclusion plots. Decreases in C for the litter exclusion treatments appeared later, and were smaller than declines in root exclusion plots, highlighting the role of root detritus in the formation of soil organic matter in this forest. By year 8 of the experiment, surface soil C concentrations were lower than Control plots by 32% in NI, 23% in NR and 19% in NL. Increases in soil C in litter addition treatments were less than C losses from litter exclusion treatments, with surface C increasing by 12% in DL and 6% in DW. Detritus additions and removals had significant effects on soil microclimate, with decreases in seasonal variations in soil temperature (between summer and winter) in Double Litter plots but enhanced seasonal variation in detritus exclusion plots. Carbon dioxide (CO_2) emissions were most influenced by detritus input quantity and soil organic matter concentration when soils were warm and moist. Clearly changes in detritus inputs from altered forest productivity, as well as altered litter impacts on soil microclimate, must be included in models of soil carbon fluxes and pools with expected future changes in climate.

1. Introduction

Changes in temperature and precipitation patterns that are predicted under scenarios of global climate change will have profound effects on species diversity and forest productivity (Langley and Megonigal, 2010; Wang et al., 2011; Rózsa and Novák, 2011; Williams et al., 2012), resulting in alteration of the quality and quantity of detritus inputs to soils. These changes can thus influence decomposition (Trofymow et al., 2002; Callesen et al., 2003; Chapin et al., 2009), thereby altering soil organic matter (SOM) content and dynamics (Carrillo et al., 2010; Kotroczó et al., 2014).

The role of forest soils in the global C balance is critical; although forests cover less than one-third of the earth's land surface, they provide 52-72% of global net primary biomass production (NPP) (Melillo et al., 1993, Roy et al., 2001; FAO 2010) and they contain approximately 80% of aboveground carbon pools (FAO 2005). Globally, soils are important components of global C stores, containing about two and a half times as much carbon as is found in vegetation (Batjes, 1998; Field and Raupach 2004). Globally CO_2-C emissions from soil are estimated to be 8×10^{16} g y^{-1} (Raich et al., 2002), more than 10 times the amount of C derived from fossil fuel combustion (Schlesinger and Andrews, 2000).

Anthropogenic alterations of soil respiration have substantial implications for the global C cycle, with rising atmospheric CO_2 levels resulting in a positive feedback to global warming (Raich and Tufekcioglu, 2000; Bernhardt et al., 2006). Alterations of detrital inputs can also affect soil microclimatic conditions, especially soil temperature and moisture content, which influences soil microbial activity and soil CO_2 emissions (Scott-Denton et al., 2006; Heimann and Reichstein, 2008; Lellei-Kovács et al., 2011). Where water is not a limiting factor, temperature is the primary abiotic driver of biological activity (Vogel et al., 2005). However, as soil moisture declines due to evapotranspiration or drought, moisture content becomes a more influential driver of respiration rates (Fierer and Schimel, 2002; Davidson et al., 2006a; Voroney, 2007). Temperature and soil moisture may also interact (Shen et al., 2009), so their impacts cannot be easily separated (Phillips et al., 2011).

Although much has been learned about abiotic controls on soil respiration, direct effects of changes in the quantity and sources of detritus on soil C balance or soil respiration remain poorly understood. Forests at the Síkfőkút International Long-Term Research (ILTER) Site are exhibiting climatological and compositional changes that are likely to affect leaf and root litter inputs, SOM content, and soil CO_2 emissions (Kotroczó et al., 2007; Fekete et al., 2011b). Long- term meteorological data indicate that the site has become drier and warmer over the past four decades, with annual precipitation decreasing by 15–20% in many Hungarian territories during the 20th century (Antal et al., 1997; Domonkos, 2003; Galos et al., 2009). Summer mean precipitation has not changed significantly over the last few decades, but the frequency of summer drought events increased during the 20th century; the Hungarian summer climate has shifted towards a more Mediterranean like climate (Domonkos, 2003; Bartholy et al., 2007).

Species composition and the structure of the Síkfőkút forest has changed significantly since the early 1970's; 68.4% of sessile oak (*Quercus petraea*) and 15.8% of Turkey oak (*Quercus cerris*) died, and field maple (*Acer campestre*) has increased in density from 0 to 131 stems ha^{-1} (Kotroczó et al., 2007). Leaf-litter production was 4060 kg ha^{-1}y^{-1} between 1972 and 1976, and 3540 kg ha^{-1}y^{-1} between 2003 and 2010 (Kotroczó et al., 2012). *Quercus cerris* and *Acer campestre* litter increased in relative importance as these species increased in dominance following the mortality of *Quercus petraea* (Kotroczó et al., 2012). Similar forest composition changes are also being observed in many areas throughout Europe (Somogyi, 2000; Thomas et al., 2002; Sonesson and Drobyshev, 2010). Differences in the chemistry of leaf and root litter can influence relative decomposition rates that control organic matter inputs to soil, thus influencing long-term C sequestration (Gholz et al., 2000). Furthermore, alterations in

vegetation composition can control SOM chemistry. For example, in a temperate deciduous forest, root-derived aliphatic compounds were a source of SOM that had greater stability than did leaf inputs. However, in a coniferous forest, aliphatic compounds derived from needles were preferentially preserved over aliphatic compounds derived from roots (Crow et al., 2009).

Our research with the Síkfőkút (DIRT) is a part of the international DIRT effort to explore how changes in the quality and quantity of detritus inputs affect soil organic matter composition and content (Nadelhoffer et al., 2004). The aim of this work was to quantify changes in soil organic carbon (SOC) concentration and soil respiration in response to alterations in aboveground and belowground detritus inputs. We hypothesized that an increased litter input would enhance soil respiration and raise SOC. We also hypothesized that soil respiration would be the lowest in the root exclusion plots, as there is no root respiration, root exudates or fresh root detritus, which enhance heterotrophic respiration in other treatments. We also predicted that changes in litter inputs would modify soil microclimatic conditions resulting in different relationships between soil respiration and soil variables (temperature, moisture).

2. Material and methods
2.2. *Site description and experimental design*
We conducted our research in the Síkfőkút Experimental Forest in northeastern Hungary. The study area (27 ha) is located in the southern part of the Bükk Mountains at an average altitude of 325 m.a.s.l (47°55'N; 20°26'E). The area has been protected and has been part of the Bükk National Park since 1976. Mean annual temperature is 10 °C and mean annual precipitation is 553 mm (Antal et al., 1997), however, during our experiment (2001-2008) the mean annual temperature was 10.8 °C, and mean annual precipitation was 599 mm. Precipitation, averaged over 100 years in Eger near Síkfőkút, is seasonal (January - March: 93 mm; April - June: 192 mm; July - September: 171 mm; October - December: 145 mm. The growing season is from April to September. This forest (*Quercetum petraeae-cerris* community) has had no active management since 1976 (Jakucs, 1985), but has a legacy of intensive forest management that occurred before that time. In this previously coppiced forest, the sessile oak and turkey oak species that make up the overstory are approximately one hundred years old. The average amount of total aboveground dry detritus (including branches, twigs, fruits and buds) was 6572 kg ha^{-1} (2003-2005) (Tóth et al., 2007). Leaf litter is comprised of (in decreasing order): sessile oak (*Quercus petraea*), Turkey oak (*Quercus cerris*), Hedge maple (*Acer campestre*), and Cornelian cherry (*Cornus mas*) (Kotroczó et al., 2012). Soils are Cambisols, with a pH$_{H2O}$ in surface soils (0-15 cm) ranging between 4.85 and 5.50 depending on the detritus treatments (Tóth et al., 2013).

The experimental aboveground and belowground litter manipulation plots (Table 1) were established in November 2000. We established one control and five litter manipulation treatments each with three randomly located 7×7 m replicate plots established under complete canopy cover (Fekete et al., 2007). Plots with normal aboveground and belowground litter quantity were used as the Control (CO) treatment. There were two types of detritus addition treatments. Double Wood plots received double the annual input of wood detritus (branches, twigs and bark); in Double Litter (DL) plots, the annual amount of leaf litter was doubled. In three treatments, detritus inputs were removed. In the No Roots plots (NR), plots were trenched thus incising the living roots, thereby removing inputs from root litter and root exudates. In the No Litter plots (NL), annual aboveground litter (leaves, small twigs) was excluded, in the No Inputs (NI) treatment, both aboveground litter and roots were excluded (Nadelhoffer et al., 2004; Sulzman et al., 2005; Fekete et al., 2012) (Table 1). The surface solar radiation was approximately the same in all treatments.

Table 1. The DIRT (Detritus Input and Removal Treatments) treatments at the Síkfőkút LTER oak forest (Hungary).

Treatments	Description
Double Litter (DL)	Aboveground leaf-litter inputs are doubled by adding litter removed from NL plots annually after autumn senescence.
Double Wood (DW)	Aboveground wood debris inputs are doubled by adding wood to each plot annually after autumn senescence. Annual wood litter inputs were measured using (30 plastic boxes of 55.5 × 36.5 cm (0.2 m^2) size) collectors placed at the site.
Control (CO)	Normal leaf and root litter inputs are maintained.
No Litter (NL)	Aboveground inputs are excluded from plots. Leaf litter was removed by hand raking in autumn after senescence.
No Roots (NR)	Roots within the plots were severed from surrounding trees by excavation of trenches to the C horizon (1m); (the trenches were 0.4 m wide), impervious barriers (0.6 mm thick high density Rootproof Delta MS 500 PE foil) were inserted into the trenches which were then backfilled. Trees and shrubs in the plot were removed when the plot was established. Chemical weed control was also applied used by Medalon (agent: 480 g·l^{-1} glyphosate–ammonium) and dry plant residues were removed. Spraying was applied once or twice a year. The leaf litter and wood debris providing the trees in the surrounding plots was not removed. The amount of surface litter was similar to the control plots.
No Inputs (NI)	This is a combination of NL and NR treatments; both aboveground and belowground inputs are excluded.

2.3. Soil sampling and measurements

Soil samples were collected from 0-15 cm with a 20 mm diameter Oakfield soil corer (Oakfield Apparatus Company, USA) eight times from April 2001 through December 2008. Soil samples were collected randomly from 5 locations within the interior 5×5m portion of each plot. Samples were composited within plots and sieved to 2 mm. In 2008 we also separated soils into two depths: 0-5 cm and 5-15 cm. Soil samples were sieved, dried, ground, and pretreated with 10 % hydrochloric acid to eliminate inorganic carbonate content before organic carbon analysis by dry combustion (Matejovic, 1997) using a *Elementar Vario EL* CHNS elemental analyzer (ELEMENTAR Analysensysteme GmbH, Germany).

Soil temperature was measured with ONSET StowAway® TidbiT® type data loggers (Onset Computer Corporation, USA) placed at 10 cm depth in the middle of each plot. Data loggers have measured soil temperature hourly since March 2001. Soil moisture content at 12 cm depth was determined with a FieldScout® TDR 300 (Spectrum Technologies Inc., USA) whenever soil respiration was measured.

Soil respiration was measured 45 times with the soda lime method (Bowden et al., 1993; Grogan, 1998) from May 2002 until December 2007. Measurements were made monthly during the growing season and approximately every two months during the non-growing season period. On each sampling date, two replicate measurement chambers were used at each plot. Measurement chambers were plastic buckets 21 cm tall and 29 cm in diameter. On each plot, 29 cm diameter by 10 cm tall plastic rings were placed on the soil surface to prepare a good seal between soil and the measurement chambers and to minimize soil surface

disturbance on the actual day of measurement; rings were established 24 hours before the first measurement, and left in place between subsequent measurements. During each soil respiration measurement, CO_2 emitted from soil was absorbed continuously over a 24-hour sampling period, using 60 g of indicator-grade soda lime contained in 7.8 cm diameter by 5.1 cm tall soil tins. Prior to use, the tins of soda lime were oven-dried at 105 °C for 24 h and covered tightly. To measure soil CO_2 fluxes in the field, the plastic rings were removed from the soil surface, the tins of soda lime were uncovered and placed on the forest floor, the plastic buckets were placed over the tins, and approximately 4 kg weights were placed on the buckets to ensure a good seal between the buckets and the soil surface. After absorbing CO_2 emitted from soil for 24 h, the buckets were removed, the tins were covered, returned to the laboratory; the soda lime was oven-dried at 105°C, and reweighed (Raich et al., 1990; Grogan, 1998). Control tins of soda lime were used to correct for any CO_2 absorbed by the soda lime tins during transport to and from the laboratory and the field site. Oven-dried tins with soda lime were brought to the field, and opened and closed to expose the soda lime account for handling that resulted in CO_2 absorption attributable to actual soda lime. Weight gains by soda lime in these control tins were subtracted from the weights of the measurement tins.

2.4. Statistical analyses

Soil organic carbon concentrations among the treatments were compared by One-way ANOVA. A repeated measures ANOVA was used to test differences in soil respiration, temperature, and moisture among the treatments. Normality of the different variables was tested with the Kolmogorov - Smirnov test, and the homogeneity of the variances was examined by F_{max}-probe. Tukey's HSD post-hoc tests were applied to separate significantly different means. We tested the effects of soil moisture content and temperature on CO_2 emissions by applying Generalized Linear model analyses in Statisticatm. Three linear models were built, model I: $R_{emission}$ (mg carbon $m^{-2}h^{-1}$) = $\alpha + \beta_T$ Temperature (°C); model II: $R_{emission}$ (mg carbon $m^{-2}h^{-1}$) = $\alpha + \beta_H$ Soil moisture (% v/v); model III: $R_{emission}$ (mg carbon $m^{-2}h^{-1}$) = $\alpha + \beta_T$ Temperature (Co) + β_H Soil moisture (% v/v), where β_T, β_H is the regression coefficient of the given variable, α is the regression constant. To select the best statistical model we used the model's root mean squared error (RMSE) and the Akaike information criterion (AIC) values. When "p"≤ 0.05, examined values were considered to be significantly different.

3. Results
3.1. Changes in soil organic carbon (SOC) concentration

SOC was not different among the treatments during the first two years of the experiment, but by the third year, differences among treatments began to emerge (Table 2). After four years (in 12/2004), SOC was significantly lower in root exclusion treatments (NR, NI) than in the control ($F_{(5;12)}$= 5.88, p<0.05). After eight years (in 12/2008), SOC was lower in all the detritus exclusion plots (NL, NR, NI) than in the controls ($F_{(5;12)}$= 20.86, p<0.05, Tukey's test, p<0.05) (Table 2). In year 8, the litter addition (DL, DW) and control plots were not significantly different from one another. Soil carbon concentrations of the treatments differed among the years (p<0.05), however treatment differences remained consistent across the sampling years.

Table 2. SOC (means ± SE) from 2001 to 2008 detritus in the Síkfőkút DIRT treatments. SOC is expressed in g kg^{-1} dry soil. Means with the same letter within each sampling date are not significantly different.

Date	04.21.2001	05.14.2002	09.27.2002	04.04.2003	03.17.2004	12.14.2004	06.16.2005	12.10.2008
DL	44.1[a] ±	36.6[a] ±	36.5[ab] ±	36.3[a] ±	52.9[a] ±	38.9[b] ± 1	43.2[b] ± 2	44.1[b] ±

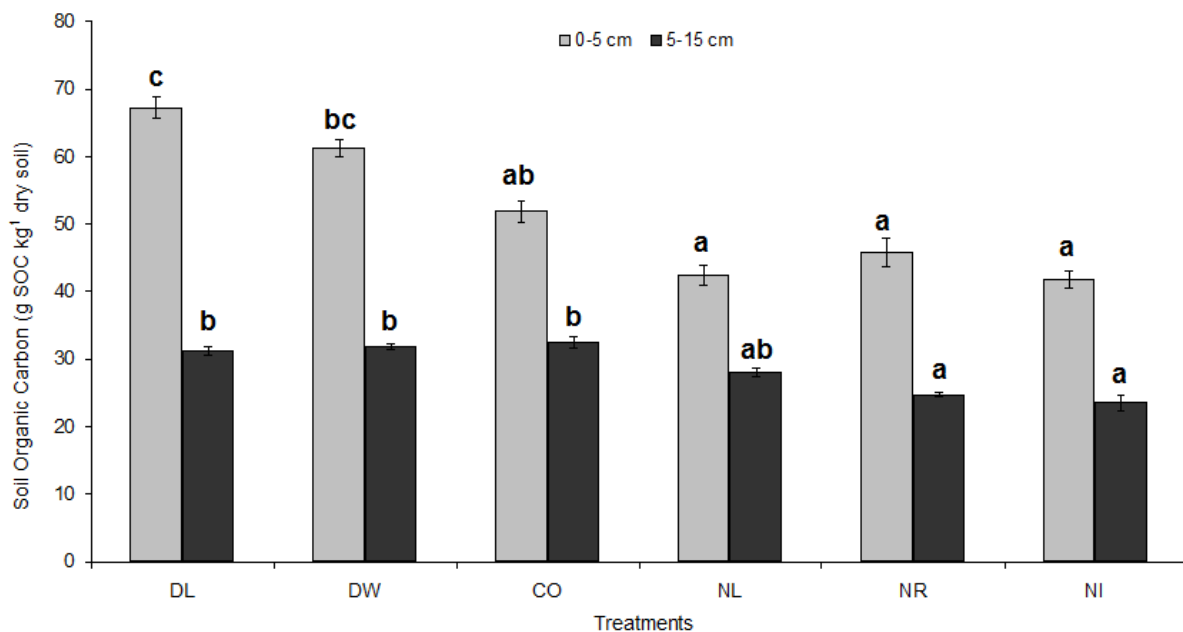|								
	0.8	0.3	2.9	3.6	3.3	.5	.6	1.6
DW	44.4^a ± 2.1	37.2^a ± 3.8	33.2^{ab} ± 2.6	37.8^a ± 4.3	41.6^a ± 5.5	36.8^b ± 4.2	41.3^b ± 0.6	41.6^b ± 1.2
CO	41.0^a ± 3.5	39.6^a ± 1.8	39.6^b ± 2.0	35.0^a ± 2.3	44.8^a ± 6.6	38.2^b ± 0.8	41.4^b ± 3.1	39.0^b ± 1.8
NL	44.9^a ± 3.0	35.6^a ± 2.8	38.0^{ab} ± 3.8	30.7^a ± 1.1	38.0^a ± 2.7	33.0^{ab} ± 0.9	34.9^{ab} ± 2.8	32.8^a ± 1.6
NR	46.7^a ± 0.7	34.2^a ± 1.5	29.2^{ab} ± 1.0	31.3^a ± 1.1	35.9^a ± 7.8	27.4^a ± 1.1	32.0^a ± 0.4	31.7^a ± 1.6
NI	41.9^a ± 2.8	32.9^a ± 1.6	27.2^a ± 2.3	31.1^a				

Fig. 1. SOC in the 0–5 and 5–15 cm soil layers in 2008 in the Síkfőkút DIRT treatments. SOC is expressed in g kg^{-1} dry soil. Means with the same letter within each soil layer are not significantly different.

In 2008, the 0–5 cm soil layer in the litter additions had significantly greater SOC concentrations ($F_{(5;12)}$=14.92; $p<0.05$) than litter exclusion treatments. DL values were also significantly higher than controls (Tukey's test, $p<0.05$). Carbon concentrations decreased in order of litter inputs: DL>DW>CO>NR>NL>NI. In the 5-15 cm soil layer, concentrations in the CO, DW and DL treatments were significantly higher than those in root exclusion treatments ($F_{(5;12)}$=9.6; $p<0.05$) (Figure 1).

3.2. Soil CO$_2$ emissions

A repeated mesures ANOVA was used to test the effect of both season and treatment on soil CO$_2$ release. Season has significant effect on CO$_2$ release (($F_{(9, 520)}$=64.883, $p<0.05$) with the highest rates in summer, while treatments alone have no significant effect. On an annual basis, detrial treatments had no significant effect on soil CO$_2$ emissions (Table 3), however, there were significant differences ($F_{(5, 29)}$=21.91, $p<0.05$) among treatments in winter (Figure 2). Emission rates were greater in litter addition treatments compared to the three detritus exclusion treatments, and rates were significantly higher in CO than in NL and NI (Tukey's

test, $p<0.05$). In contrast, rates of soil CO_2 release during summer months followed the order of NR, NI, DW, CO, DL and NL (Figure 2).

Table 3. Soil properties and aboveground litter inputs in the Síkfőkút DIRT treatments. Means with the same letter within each sampling date are not significantly different.

0-15 cm	DL	DW	CO	NL	NR	NI
Litter inputs (kg C ha^{-1} year^{-1}) (2003-2008)	4298±167	3963±477	2754±206	0	2506±187*	0
CO_2 emissions (g C m^{-2} year^{-1}) (2002-2007)	618^a±46.9	657^a±56.7	639^a±56.3	550^a±45.4	623^a±49.9	586^a±48.2
Soil moisture (% v/v) (2002-2007)**	24.1^a±1.52	26.2^a±1.57	25.0^a±1.57	25.8^a±1.51	37.0^b±1.24	34.7^b±1.38
Soil temperature (°C) (2002-2007)**	11.3^a±0.77	11.0^a±0.82	11.0^a±0.81	11.2^a±0.94	11.5^a±0.95	11.2^a±0.98
Differences between the winter and summer mean temperature values (°C)***	14.1^a±0.48	15.2^{ab}±0.65	15.0^{ab}±0.65	16.9^{bc}±0.67	17.2^{bc}±0.62	18.0^c±0.70

*NR has no shrub litter production. According to Tóth et al. 2007, shrubs provide 9% of the total leaf litter production at the Síkfőkút site. **Soil moisture content and temperature was determined whenever soil respiration was measured. ***The mean summer (1 June–31 August) as well as the mean winter (1 December–28 February) temperatures from the daily mean temperature values 2001–2008.
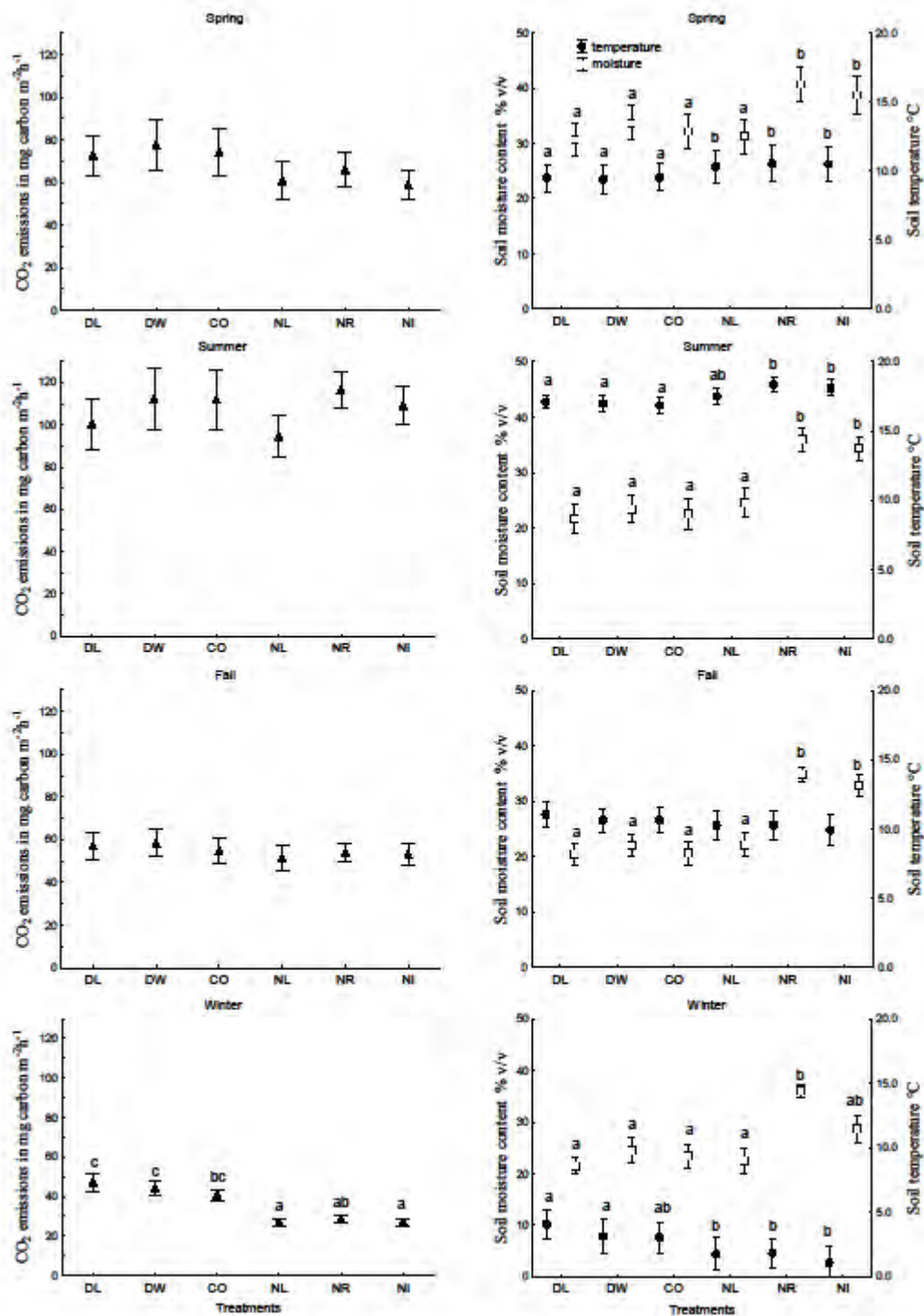
Fig. 2. Soil CO_2 emissions, soil moisture content and temperature in the Síkfőkút DIRT treatments. Means with the same letter within each sampling date are not significantly different.

3.3. Soil temperature and soil moisture

Soil microclimate was significantly influenced by litter treatments. In summer soil temperature was significantly higher ($F_{(5;77)}=25.8$; $p<0.05$) in the detritus exclusion than in the CO and litter addition treatments (Figure 2). In contrast, in winter temperatures in the aboveground litter exclusion treatments (NL and NI) were significantly lower ($F_{(5;30)}=5.39$; $p<0.05$) than the CO and litter addition treatments. There were not large differences in annual temperature ranges among treatments, with larger differences between mean winter and summer soil temperatures in the NL, NR and NI treatments (Table 3). Soil moisture was significantly higher in root exclusion treatments than in the other treatments ($F_{(5;245)}=13.36$; $p<0.05$) (Table 4). Differences were especially notable during the summer ($F_{(5;77)}= 65.59$; $p<0.05$) and fall ($F_{(5;71)} = 45.57$; $p<0.05$) (Figure 2). Root exclusion treatments showed smallest differences between the driest and wettest soil moisture values (Table 5).

Table 4. The results of Generalized Linear Model (GLM) analyses of the effects of soil moisture content and temperature on soil CO2 emissions. Three linear models were built, model I: $R_{emission}$ (mg carbon $m^{-2}h^{-1}$) = $\alpha + \beta_T$ Temperature(^{o}C); model II: $R_{emission}$ (mg carbon $m^{-2}h^{-1}$) = $\alpha + \beta_m$ Soil moisture (v/v%); model III: $R_{emission}$ (mg carbon $m^{-2}h^{-1}$) = $\alpha + \beta_T$ Temperature (^{o}C) + β_m Soil moisture (v/v%), where β_T, β_m is the regression coefficient of the given variable, α is the regression constant. The root mean squared error (RMSE), Adjusted R-Squared and the Akaike information criterion (AIC) values of these models are also presented.

Treatment (n=45)		α	β_T	β_m	Ratio of β_m/β_T	Adjusted R^2	p	RMSE	AIC
DL	model I.	34.20	3.44			0.19	<0.01	33	395
	model II.	32.59		1.84		0.18	<0.01	33.4	396
	model III.	-35.18	4.28	2.32	0.54	0.5	<0.01	26.1	377
DW	model I.	34.40	3.91			0.19	<0.01	40	411
	model II.	23.99		2.39		0.23	<0.01	39	409
	model III.	-68.67	5.48	3.23	0.59	0.62	<0.01	27.6	382
CO	model I.	30.20	4.2			0.22	<0.01	39.2	409
	model II.	29.6		2.05		0.16	<0.01	40.7	412
	model III.	-64.26	5.6	2.89	0.52	0.56	<0.01	29.5	387
NL	model I.	25.15	3.59			0.34	<0.01	28.4	383
	model II.	20.67		1.89		0.22	<0.01	31	390
	model III.	40.01	4.02	2.21	0.55	0.66	<0.01	20.5	358
NR	model I.	15.21	5.03			0.61	<0.01	24.1	370
	model II.	36.08		1.39		0.05	0.201	37.9	406
	model III.	-64.60	5.22	1.76	0.36	0.73	<0.01	20.2	357
NI	model I.	20.50	4.33			0.52	<0.01	25.9	375

model II.	28.88		1.42		0.08	0.09	35.9	402
model III.	-38.57	4.42	1.56	0.35	0.63	<0.01	22.6	366

Table 5. Soil CO_2 emissions, soil moisture content and soil temperature (means ± SE). Two groups were used, when soil moisture content was below 16 % v/v in Control plots (#); and soil moisture content was above 16 % v/v in Control plots (^). Means with the same letter within each sampling date are not significantly different.

Treatments	CO_2 emissions (mg C m^{-2}h^{-1})		Moisture (%)		Temperature (^{o}C)	
	<16%#	>16%^	<16%#	>16%^	<16%#	>16%^
DL	49.0^a±5.35	85.8^a±7.00	12.9^a±0.69	27.0^a±1.51	16.0^a±0.7	11.8^a±0.92
DW	49.8^a±4.44	91.4^a±8.27	14.0^a±0.81	29.7^a±1.43	16.1^a±0.8	11.5^a±0.95
CO	47.6^a±4.56	90.8^a±8.63	12.8^a±0.78	28.4^a±1.39	16.1^a±0.78	11.4^a±0.93
NL	53.5^{ab}±6.32	75.5^a±6.82	13.9^a±1.03	29.0^a±1.37	16.6^a±0.95	11.6^a±1.06
NR	80.1^c±7.08	81.7^a±7.82	28.1^b±1.68	39.7^a±1.15	17.0^a±0.96	12.1^a±1.13
NI	72.9^{bc}±7.46	77.2^a±7.52	26.5^b±1.85	37.2^a±1.45	17.0^a±1.07	11.8^a±1.15

3.4. Effects of soil moisture and temperature on soil CO_2 emissions
The precipitation was the same in every plot, but microclimatic conditions were different (e.g. evapotranspiration). The Control treatment was used for standard; and we used two moisture period groups. One for drier period when soil moisture content in Control was below 16 % v/v and another one for wetter period when soil moisture content in Control was above 16 % v/v. During drier periods soil CO_2 emissions differed among treatments ($F_{(5;54)}$=5.49; $p<0.05$) (Table 5). NR rates were significantly greater than NL, DW, DL and CO rates. Soil CO_2 emissions in NI were significantly greater than in CO ($p<0.05$), and were marginally greater than in DL and DW ($p<0.1$). A one-way ANOVA revealed significant differences in soil moisture content among the treatments ($F_{(5;54)}$=41.85; $p<0.05$); the root exclusion treatments had significantly greater soil moisture contents than control or litter addition treatments. During wetter periods soil CO_2 emissions rates were not significantly different among the treatments, but the mean emission rates of the litter addition treatments were 5-21% greater than detritus exclusion treatments (Table 5).
The Generalized Linear model illustrates the effects of temperature and moisture on soil respiration rates, revealing remarkable differences between the treatments. Characteristic differences were observed between the treatments in Model III (Table 4). The effect of soil temperature on soil CO_2 emission was greatest in the root exclusion treatments (adjusted $R^2_{(NR)}$ = 0.61; $R^2_{(NI)}$ =0.52 in model I) compared to other treatments (adjusted $R^2_{(DL)}$ =0.19; $R^2_{(DW)}$ =0.19; $R^2_{(CO)}$ =0.22; $R^2_{(NL)}$ =0.34). In contrast, the effect of soil moisture was smaller in root exclusion treatments (adjusted $R^2_{(NR)}$ = 0.05; $R^2_{(NI)}$ =0.08 in model II) compared to other treatments (adjusted $R^2_{(DL)}$ =0.18; $R^2_{(DW)}$ =0.23; $R^2_{(CO)}$ =0.16; $R^2_{(NL)}$ =0.22). The β_m, β_T, and β_m/β_T values also show these above effects in model III as $\beta_{m(NR)}$: 1.76; $\beta_{m(NI)}$: 1.56 compared to ex. $\beta_{m(DW)}$:3.23 or $\beta_{m(Co)}$: 2.89. The ratios of β_m/β_T in NR and NI are 0.36 and 0.35, remarkably lower than in treatments where roots were not excluded (Table 4).

4. Discussion
4.1. Changes in soil total carbon concentration

In the first year after establishment of the Síkfőkút DIRT plots, soil SOC concentration was highest in NR compared to other treatments, probably as a result of the decomposition of severed tree roots that remained in the plots. The carbon concentration of NR plots in the first year began to decline markedly by the second year, likely due to the reduction of substrate supply (Kalyn and Van Rees, 2006). After two years, significantly lower soil C concentrations were measured in the root exclusion treatments, and after eight years (in 2008), significantly lower concentrations were observed in all detritus exclusion treatments. This is similar to results found in a temperate deciduous DIRT site in Massachusetts (USA), where C concentrations decreased within the first decade of experimental litter reductions (Nadelhoffer et al., 2004), and which were more pronounced after two decades (Lajtha et al., 2013a). Similar soil C reductions were observed in two other DIRT experiments in deciduous temperate forests in Allegheny College Bousson Environmental Research Reserve (USA) and University of Wisconsin Arboretum (USA) (Bowden et al., 2013, Lajtha et al., 2013b).

The two root exclusion treatments showed greater losses of soil C than those observed in the NL treatment in the 5-15 cm soil layer, demonstrating the critical role of belowground C supply to controlling soil C accumulation in this forest, as has been demonstrated elsewhere (Makkonen and Helmisaari, 2001; Rasse et al., 2005; Fekete et al., 2011a). Decreased soil C could also be due, at least in part, to elevated rates of decomposition enhanced by higher soil moisture in plots without roots (and thus without transpirational water losses) in these dry forests.

We expected woody debris to have a greater effect on soil C levels than leaf litter, given that leaves are likely to decompose more quickly than woody tissue (Harmon et al., 1986) and enter the soil C pool (Berg et al., 1982), however, leaf litter addition (DL) increased SOC concentration in the upper soil layer to a greater extent than did a similar amount of woody debris (branches, twigs, bark). The effect of wood debris, therefore, might not be observed in the first decade.

The upper 0-5 cm and the 5-15 cm layers of soil reacted in different ways to detritus manipulations. After eight years, there were significant differences between the litter addition treatments and the detritus exclusion treatments in the upper 0-5 cm layer. These differences were driven primarily by alterations in aboveground detritus inputs. In the root exclusion treatments, the absence of roots caused significant differences in the deeper mineral soil layer, but did not alter upper soil SOC concentration, presumably due to the continued input of aboveground detritus, and perhaps to the vertical distribution of roots within the soil profile.

Several studies have suggested that the majority of organic carbon in soil is derived from belowground inputs, with aboveground litter inputs having a limited influence on soil SOC storage (Rasse et al., 2005; Schmidt et al., 2011). However, in DIRT experiments in North American temperate forests, aboveground litter inputs are equally or more important in maintaining soil C stocks. After 20 years, soil C in litter exclusion and root exclusion plots showed similar declines in an oak forest (Harvard Forest, Massachusetts, USA (Lajtha et al., 2013b)). Soil C reduced to a greater extent in a litter exclusion treatment than in a root exclusion treatment in a black cherry-sugar maple forest (Bousson Environmental Research Reserve, Pennsylvania, USA) (Bowden et al., 2013).

It is not clear what controls the relative importance of aboveground and belowground litter contributions to soil C. Root and leaf litter decompose at different rates (Hobbie et al., 2010), and may produce different organic compounds that undergo different rates of chemical (Hassink, 1997) and physical (Six et al., 2002, Pronk et. al., 2013) protection.

In contrast to soil C losses in response to litter exclusion, litter addition at Síkfőkút resulted in increased SOC concentration in the upper 0-5 cm layer within the first few years of the experiment. This is in contrast to results of other DIRT sites. For example, after five years of doubled litter additions at the H.J. Andrews DIRT site (Oregon, USA) soil C concentrations

were not increased (Crow et al., 2009), and even after two decades of doubled litter inputs, the Harvard Forest and Bousson sites did not display increases in soil C. At all of these sites, soil CO_2 production is elevated in response to litter additions (Bowden et al., 1993; Sulzman et al., 2005; unpublished data) thus reducing the amount of organic matter that might enter the soil C pool. A priming effect (Kuzyakov, 2010) was observed at the at the H.J. Andrews DIRT site (Sulzman et al., 2005; Crow et al., 2009) and may have reduced SOC early in the experiment; priming in concert with elevated rates of soil respiration would explain the lack of SOC increase with long-term doubled litter inputs. At Síkfőkút, soil respiration was not increased in DL or DW plots, but the upper soil layer of DL treatments showed significantly higher carbon concentrations than CO treatment. We cannot determine if priming had occurred. These differences between the American and Hungarian DIRT sites may be explained by climate factors that control decomposition. First, annual precipitation at Síkfőkút is much lower than that at the US DIRT sites (Andrews: 2370 mm yr^{-1}; Harvard: 1120 mm yr^{-1}; Bousson: 1050 mm yr^{-1}), and rainfall is more seasonally distributed (Sulzman et al., 2005; Crow et al., 2009). Moreover, the annual mean temperature at Síkfőkút is higher than that at the US DIRT sites. Even with significantly lower precipitation, litter fall at Síkfőkút (2754 kg C ha^{-1} yr^{-1}) is 31% greater than at Bousson (2100 kg C ha^{-1} yr^{-1}, (Bowden et al., 1993)), 459% greater than at Andrews (600 kg C ha^{-1} yr^{1}, (Sulzman et al., 2005)) and 26% greater than at Harvard Forest (2190 kg C ha^{-1} yr^{-1}, (Savage and Davidson, 2001)). These conditions, with lower precipitation and higher temperatures, create conditions that will hinder decomposition, and thus reduce soil CO_2 production.

4.2. Soil temperature and moisture

The differences in soil CO_2 emissions among treatments during winter and summer periods are likely due to altered insulation and soil moisture conditions, which influence soil microclimate and biological processes both directly and indirectly (Sayer, 2006; Veres et al., 2013). Numerous studies have documented the dominant role of temperature in controlling decomposition and soil CO_2 emissions (Chen et al., 2000; Knorr et al., 2005; Kotroczó et al., 2008; Bond-Lamberty and Thomson, 2010; Smith and Fang, 2010). At our site, soil moisture was a critical controller of soil respiration during periods that were warm and dry. During dry periods, soil moisture was higher in the root exclusion plots than it was in the other treatments. Even though soil respiration in the root exclusion treatments were driven only by heterotrophic contributions, soil CO_2 emissions during dry periods were greater in these treatments than it was in the other treatments, where both heterotrophic and autotrophic sources contributed to total soil respiration. Our results are similar to what others have observed (e.g. Bowden et al., 1998; Davidson and Janssens, 2006b; Almagro et al., 2009; Matías et al., 2012). Our results also show that under dry conditions, detritus inputs have little direct effect on soil CO_2 production. Elevated soil temperature can enhance soil CO_2 production only if soil moisture content is favorable for microbial processes. In drought periods, the metabolism of the decomposing microorganisms, as well as nutrient transport, become slower (Sardans and Peñuelas, 2005; Füzy et al., 2008; Fekete et al., 2012), thus reducing soil respiration.

4.3. Summary

To sum up, we can state that detritus exclusion caused a significant reduction in SOM concentration, while detritus addition did not entail any significant increase in the 0-15 cm soil layer. However, SOM concentration showed significant increase in the 0-5 cm soil layer in DL. Soil temperature and moisture concentration influenced soil respiration to various extents in different treatments. In NR and NI treatments, where soil moisture content was higher, a much weaker correlation was observed between soil respiration and soil moisture

content than in the other treatments. In the root exclusion treatments the role of temperature was more important. The main findings of Generalized Linear model analyses were that in root exclusion treatments, where moisture is not a limiting factor, temperature alone has great effect on soil CO_2 release. When moisture is limited the further increase of temperature has not remarkable risen the soil CO_2 release. We found the most significant decrease in SOM concentration in the root exclusion treatments. The reason for this could be the more intensive soil respiration due to the higher moisture content and the decrease in litter inputs.

Our results suggest changes in ecosystem productivity that alter litter production can alter total soil C through both direct effects due to changing litter C inputs, as well as indirectly through altered microclimate regimes that influence C dynamics. Carbon sequestration in forest soils is now recognized as being driven not only by amount of litter production, but also by the rate of various detritus (leaf, wood, root).

5. References

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Tables and Figures captions

Table 1. The DIRT (Detritus Input and Removal Treatments) treatments at the Síkfőkút LTER oak forest (Hungary).

Table 2. SOC (means ± SE) from 2001 to 2008 detritus in the Síkfőkút DIRT treatments. SOC is expressed in g kg^{-1} dry soil. Means with the same letter within each sampling date are not significantly different.

Table 3. Soil properties and aboveground litter inputs in the Síkfőkút DIRT treatments. Means with the same letter within each sampling date are not significantly different.

Table 4. The results of Generalized Linear Model (GLM) analyses of the effects of soil moisture content and temperature on soil CO_2 emissions. Three linear models were built, model I: $R_{emission}$ (mg carbon m^{-2}h^{-1}) = $\alpha + \beta_T$ Temperature(°C); model II: $R_{emission}$ (mg carbon m^{-2}h^{-1}) = $\alpha + \beta_m$ Soil moisture (v/v%); model III: $R_{emission}$ (mg carbon m^{-2}h^{-1}) = $\alpha + \beta_T$ Temperature (°C) + β_m Soil moisture (v/v%), where β_T, β_m is the regression coefficient of the given variable, α is the regression constant. The root mean squared error (RMSE), Adjusted R-Squared and the Akaike information criterion (AIC) values of these models are also presented.

Table 5. Soil CO_2 emissions, soil moisture content and temperature (means ± SE). Two groups were used, when soil moisture content was below 16 % v/v in Control plots (#); and soil moisture content was above 16 % v/v in Control plots (^). Means with the same letter within each sampling date are not significantly different.

Figure 1. SOC in the 0-5 and 5-15 cm soil layers in 2008 in the Síkfőkút DIRT treatments. SOC is expressed in g kg^{-1} dry soil. Means with the same letter within each soil layer are not significantly different.

Figure 2. Soil CO_2 emissions, soil moisture content and temperature in the Síkfőkút DIRT treatments. Means with the same letter within each sampling date are not significantly different.