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Influence of defoliation on CO_2 efflux from soil and microbial activity in a Mediterranean grassland

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ABSTRACT

Defoliation of grasses affects carbon (C) input from plants into the rhizosphere and so may affect C turnover in soil. We examined the effect of grassland's defoliation on root-derived CO_2 efflux and microbial activity in Mediterranean Phaeozem. *In situ* partitioning of total CO_2 efflux into root-derived and microbial-derived CO_2 fluxes was performed by mesh-exclusion technique. Microbial basal respiration, N mineralization and the activity of enzymes involved in the cycling of C, N, P and S (also used to calculate microbial functional diversity) were measured in soils of defoliated and control plots. Cumulative CO_2 efflux in defoliated plots was 18% lower in 2006 and equal to control plots in 2007. The contribution of microbial CO_2 to total CO_2 efflux from soil ranged from 71% to 86% without significant differences between defoliated and non-defoliated plots. The lack of correlation between root-derived CO_2 and soil temperature after defoliation indicates that photoassimilate supply is the major determinant for root-derived CO_2. Microbial-derived CO_2 efflux was 20% lower in defoliated plots after accounting for temperature and humidity differences between the two treatments. Defoliation suppressed the activity of enzymes involved in the cycling of C, S and P and decreased basal respiration rates of soil microorganisms by 19%. In turn, defoliation stimulated activity of enzymes involved in the cycling of N, as indicated by the increase of potential nitrification rates and of leucine-aminopeptidase activity. Stimulation of N mineralization promotes a fast regeneration of defoliated plants. We confirm the presence of strong links between plant and microbial activity in a grassland community, as well as close coupling of aboveground photosynthetic activity with root-derived CO_2.

1. Introduction

Land-use changes are considered the most dominant component of global change in terms of impact on terrestrial ecosystems, being able to significantly change land cover, vegetation composition, biodiversity and biochemical cycles (Guo and Gifford, 2002; Walker et al., 1999). Land-use change and management practices can affect soil carbon (C) storage by altering the input of organic matter and by changing its decomposability (Cambardella and Elliott, 1992; Moscatelli et al., 2007).

In grassland ecosystems, containing about 12% of the earth's soil organic matter (SOM) (Schlesinger, 1977), a very little quantity of organic C is stored in the aboveground biomass and more than 90% is concentrated in roots and especially in soil organic matter (Burke et al., 1997; Parton et al., 1993). Because of the significant capacity of soil to store C, grasslands associated with a proper management are considered to be potential carbon sinks (Post et al., 1982; Degryze et al., 2004; Sun et al., 2004). Managed grasslands account for about 20% of the global terrestrial ice-free surface and, grazing and mowing regimes, are reported to induce changes of soil organic carbon. A large amount of data show that management based on defoliation may substantially influence the belowground food-web in terms of soil organic matter (SOM) transformation, nutrient cycling (Mikola et al., 2001; Bardgett et al., 1998) and biodiversity (Collins et al., 1998). Maintenance of SOM and soil fertility are the key factors in the sustainability of such ecosystems (Conant et al., 2001), insuring preservation of plant productivity and regrowth (Bending et al., 2004).

However, there is still a lack of clear information on the effect of defoliation of grassland plants on C and N cycling, soil biochemical properties and the role of soil organisms in aboveground-belowground feedbacks as different studies show contrasting results. Some ecological studies stated that mowing usually reduces CO_2 efflux from soil by 20–50% despite the increase of soil temperature (Bremer et al., 1998; Wan and Luo, 2003). This decrease of CO_2 efflux was mainly explained by the sensitivity of

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roots and microorganisms to reduction in photosynthetic C supply from aboveground and to decrease in rhizodeposition and thus in the amount of easily available C substrates for rhizosphere microorganisms (Craine et al., 1999; Bahn et al., 2006; Zhou et al., 2007a). However, Mawdsley and Bardgett (1997) concluded that defoliation of *Trifolium repens* increases the rhizosphere microbial biomass and activity, whereas defoliation of *Lolium perenne* had a little effect on soil organisms. Uhlírová et al. (2005), Zhou et al. (2007b) and Hamilton et al. (2008) report mowing as an improved grassland management as it stimulated rhizodeposition, increased soil microbial biomass, and consequently labile carbon pool. Soils under uncontrolled grazing on the contrary, tended to loose soil organic matter and reduce labile C availability (Lal, 2002; Stark and Kytoviita, 2006). Bardgett and Leemans (1995) and Bardgett et al. (1997) showed that sheep grazing positively influenced microbial biomass and activity and supported the hypothesis that heavy grazing favours "fast cycles" dominated by labile substrates. Furthermore grazing can result in higher soil C because of accelerated turnover of shoot and root material and changes in plant species composition (Reeder and Schuman, 2002; Bardgett et al., 1998).

For a complete understanding of the effect of defoliation practices on grassland community, ecological and biochemical studies should be combined. Monitoring and partitioning of soil CO_2 efflux *in situ* allow estimating the effect of defoliation on individual components of soil CO_2 efflux including root- and microbial-derived CO_2 in the short- and/or the long-term. This is especially important with regard to CO_2-driven greenhouse effect, as only CO_2 originated from microbial decomposition of SOM contributes to long-term changes in atmospheric CO_2 concentration, while root respiration and microbial decomposition of plant residues with their high turnover rates do not affect C sequestration in the short or long-term and mask the contribution of microbial-derived CO_2 when measuring CO_2 fluxes from planted soil (Kuzyakov, 2006). Therefore, the first aim of our study was to evaluate the effect of defoliation on components of CO_2 efflux from soil.

Biochemical analyses of soil properties allow further insights of changes in microbial pool size, its activity and functional diversity due to defoliation, providing additional information to interpret the changes observed in the respiratory fluxes. Beneficial and negative effects of soil management practices are strongly linked to the activity of microbial biomass and to soil enzymes because they regulate soil quality and functions due to their involvement in organic matter dynamics, cycling of main nutrients (C, N, P and S) and decomposition processes (Powlson et al., 1987; Rice et al., 1996; Tracy and Frank, 1998; Bending et al., 2004; Nannipieri et al., 2002). Indeed, enzymes provide an early indication of changes in organic matter status and turnover. Extracellular enzymes are considered integrative indicators of soil quality. They are relatively simple to analyze, show ecological significance, are sensitive to environmental stress and respond rapidly to changes in management (Dick, 1997; Yakovchenko et al., 1996; Turner et al., 2002), and CO_2 concentration in the atmosphere (Dorodnikov et al., 2009). However, the application of enzyme activities for monitoring soil quality requires information on activities from a wide range of soil types, land uses and managements under steady-state conditions. These data are still weakly presented in the literature. Hence, the second aim of our study was to link the enzymes activity changes induced by defoliation with changes of CO_2 efflux from soil.

Changes in microbial biodiversity are likely to be important in relation to maintenance of soil ecosystem functions (Nsamibana et al., 2004) because the microbial communities influence the soil potential for enzyme-mediated substrate catalysis (Kandeler et al., 1996). Characterization of microbial functional diversity may help to better understand and manipulate ecosystem processes, as the ability of an ecosystem to withstand serious disturbances may depend in part on the microbial component of the system. Therefore, the third aim was to verify if the expected changes in microbial activity are linked to changes in microbial functional diversity induced by defoliation.

In a previous study we focused on some chemical and biochemical properties (labile C pools and microbial indices) of soils in Mediterranean grassland located in central Italy and investigated the effects of different management practices (grazing and mowing) (Gavrichkova et al., 2008). The general aim of the present study is to investigate the effect of defoliation on different components of grassland C cycling by performing *in situ* measurements of CO_2 efflux from soil from various sources as well as to link changes in respiration rates with changes in microbial community activity and functional diversity. A two-year experiment on partitioning of soil CO_2 fluxes into root-derived and microbial-derived CO_2 was performed in the same experimental area. Microbial activity expressed as basal respiration, potential N mineralization and enzymes associated with the cycling of C, N, S and P were evaluated.

2. Materials and methods

2.1. Research area

The study was conducted in Amplero (AQ) – a Mediterranean grassland site, located in central Italy at 900 m a.s.l. Amplero site is a nearly flat to gently south sloping (2–3%) doline bottom with an average annual temperature of 10 °C and average annual precipitation of 1365 mm. The site is subjected to a long-term management, which consists in a once-a-year mowing during the peak of the growing season and the rest of the growing season the site is used as a pasture for cattle grazing (D: defoliated plots).

The soil is classified as Haplic Phaeozem (WRB classification, 2006) and contains 13% of sand, 33% of silt and 56% of clay, pH_{H_2O} of 6.6, total carbon (TC) 3.48% and total nitrogen (N) 0.28%. C and N determinations were performed in June 2006. The plant cover is mainly represented by the following families: *Caryophyllaceae* (19%), *Faseolaceae* (30%) and *Poaceae* (34%).

Four fenced areas (2.5 m × 2.5 m), which prevent the enclosed plots from cutting and grazing by large herbivores were established in 2002 (U: control, undefoliated plots).

2.2. CO_2 efflux from soil and its partitioning

Soil respiration and its partitioning was measured every two weeks or monthly in defoliated (D) and undefoliated control (U) plots during 2006 and 2007. Measurements were made with a closed dynamic system (LI-6400 09 (LI-COR Inc., Lincoln, NE, USA)) operated in the manual mode.

To avoid root damages, PVC soil collars of 11 cm in diameter were inserted into the soil for 2.5 cm only and stabilized with two iron legs to prevent its moving when the chamber was placed on top. Soil temperature was measured near each collar at 5 cm depth using LI-COR 6400 temperature probe. Soil water content ($m^3\ m^{-3}$) at depth of 5 cm was measured with a portable system (ThetaProbe ML2x, Delta-T devices Ltd., Cambridge, UK) at three points around each control collar plots and inside each collar of partitioning measurements plots (see below).

Partitioning measurements plots were installed both in D and U treatments. Inside each fence two complete partitioning measurements plots were placed: one in 2006 and one in 2007, the same was done outside the fences. In 2006 plots were established before the growing season have started (end of March) and the first measurements were done in the middle of May to ensure roots

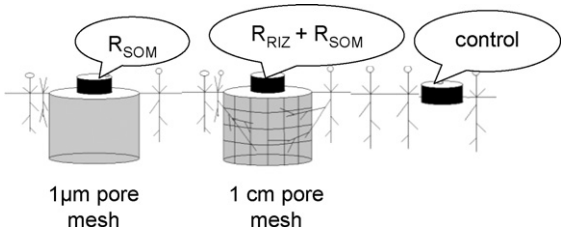

Fig. 1. Scheme of the modified root-exclusion technique. One complete partitioning plot is presented. Three collars to measure different components of soil respiration (control, R_s) are shown: root-derived (R_{RIZ}) and microbial-derived (R_{SOM}) CO_2.

in-growth inside the mesh bags. In early March 2007 new plots were placed and after one month the rate of respiration reached the value of the plots established in 2006. This period was assumed to be enough for reaching the roots in-growth equilibrium.

Root-exclusion technique modified after Leake et al. (2004) and Moyano et al. (2007, 2008) was used to partition CO_2 efflux from soil. A complete partitioning plot is shown in Fig. 1. In brief the preparation of the plots was the following:

- Soil cores, 20 cm in diameter and 30 cm deep were sampled; the soil was sieved through 4 mm mesh size and all roots were carefully removed.
- Half of the sampled soil was placed into the nylon bags with meshes of 1 μm pore size and returned to the plot. The CO_2 measured from these nylon bags was considered as respiration associated with microbial decomposition of SOM-microbial-derived CO_2 (R'_{SOM}).
- Another half of the sieved soil was placed back without any barriers for the roots growing (bags with 1.0 cm pore size). The CO_2 efflux from these bags was assumed as the sum of microbial decomposition of SOM and root-derived CO_2 ($R'_{SOM} + R_{RIZ}$).
- Root-derived CO_2 was calculated as a difference between these two treatments: $(R'_{SOM} + R_{RIZ}) - R'_{SOM}$. It integrates both, root respiration and respiration of rhizosphere microorganisms which could be hardly separated one from another.
- To consider the effect of sieving on microbial respiration, root-derived CO_2 was subtracted from total soil respiration (R_s) measured in the undisturbed soil ($R_s - R_{RIZ} = R_{SOM}$).

Summarizing, we applied following options to partition total CO_2 efflux from soil of different sources:

1 μm mesh pore size = microbial-derived CO_2 (R'_{SOM}), after disturbance.
1 cm mesh pore size = sum of microbial and root-derived CO_2 ($R'_{SOM} + R_{RIZ}$).
1 cm mesh pore size − 1 μm mesh pore size = root-derived CO_2 (R_{RIZ}).
No mesh = control for total CO_2 (R_s).
Control − R_{RIZ} = microbial-derived CO_2 (R_{SOM}), removing the effect of sieving.

To account for differences in environmental conditions between D and U plots, total and microbial-derived CO_2 under different management practices were calculated for the same soil temperature (Ts) and soil water content (SWC). For these purposes CO_2 efflux from D plots was calculated at the same temperature as from U soil at non-limited SWC. In order to estimate the respiratory fluxes of D plots at same temperature and moisture of U plots a model regression as function of these two parameters was derived with all soil respiration measurements of the D plots over the whole course of the experiment.

For a better evaluation of the effect of defoliation on soil respiration originated from different C sources, root-derived and microbial-derived cumulated CO_2 fluxes were calculated. CO_2 efflux from soil between subsequent measurements was linearly interpolated. CO_2 sampling collected between 10:00 and 13:00 h being 90% of the observed diurnal averages, were assumed to be representative and suitable for calculation of annual cumulative CO_2 without application of any correction factor.

2.3. Soil biochemical properties

Soil was sampled twice in 2006: just after the mowing at the end of June and four months after the mowing in the beginning of October. For each sampling date 16 soil cores (0–20 cm depth) were collected: 2 soil samples inside each fenced area, 8 in total (U); and 2 soil samples outside the fences, 8 in total (D). Soil under defoliated plants was sampled at least 5 m away from the fences.

2.3.1. Soil basal respiration

Soil basal respiration was calculated following Badalucco et al. (1992): 20 g of soil sieved and carefully cleaned from roots (60% WHC) were weighed in a beaker and placed at the bottom of a 1 l jar. In another beaker 2 ml of 1 M NaOH were placed inside the jar in order to trap the CO_2 evolved during the incubation period (28 days). 4 ml of 0.375 M $BaCl_2$ was added to NaOH to precipitate CO_2 as $BaCO_3$. The excess of NaOH that did not react with the CO_2 was determined by titration with 0.1 M HCl after 1, 3, 7, 10, 14, 21 and 28 days. The mean values of the hourly CO_2 evolved after the 10 days of incubation were used as the basal respiration because after that period soil CO_2 production rate reached a constant value.

2.3.2. Activities of soil enzymes

Enzyme activity was measured according to Marx et al. (2001) and Vepsäläinen et al. (2001) based on the use of fluorogenic methylumbelliferyl (MUF) and (AMC) substrates. The soil was analysed for β-cellobiohydrolase (exo-1,4-β-glucanase, EC 3.2.1.91), N-acetyl-β-glucosaminidase (EC 3.2.1.30), β-glucosidase (EC 3.2.1.21), α-glucosidase (EC 3.2.1.20), acid phosphatase (EC 3.1.3.2), β-xylosidase (EC 3.2.2.27), arylsulphatase (EC 3.1.6.1) and leucine-aminopeptidase (EC 3.4.11.1). The respective substrates were 4-MUF-β-D-cellobioside, 4-MUF-N-acetyl-β-glucosaminide, 4-MUF-β-D-glucoside, 4-MUF-α-D-glucoside, 4-MUF-phosphate, 4-MUF-7-β-D-xyloside, 4-MUF-sulphate and L-leucine-7-amino-4-methylcoumarin. 2.0 g of fresh soil were homogenised in 100 ml of 0.5 M acetate buffer, pH 5.5, using an Ultra Turrax IKA for 3 min at 9600 rev min^{-1}. Aliquots of 100 μl of suspended soil were pipetted in a 96 multiwell plate with three replicates. Each substrate was added in each well in aliquots of 100 μl for a final concentration of 500 μM. Then the microplates were incubated at 30 °C for 3 h, with fluorescence readings occurring every 30 min. For the calibration curve 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 nmole of MUF or AMC, for leucine-aminopeptidase, were added to the same aliquots of soil suspension to take into account the quenching effect on fluorescence intensity (Freeman et al., 1995). Fluorescence readings (excitation 360 nm; emission 450 nm) were performed using a Fluoroskan Ascent (Thermo electron) fluorometer. The enzymatic specific activity was calculated as the ratio of each enzyme activity to microbial biomass. The synthetic enzymatic index (SEI) was calculated following Dumontet et al. (2001) and using the enzyme activities releasing the same reaction product (MUF).

From the activities of these seven enzymes, soil functional diversity was determined using the Shannon's diversity (H') ($H' = -\sum p_i \log_2 p_i$) and evenness ($J'$) ($J' = H'/\mathrm{Hmax} = H'/\log_2 S$) indexes (Bending et al., 2004), where p_i is the ratio of the activity of a particular enzyme to the sum of activities of all enzymes and S is the presence of the enzymatic activity. The value obtained for

each enzyme activity was divided by the highest value found for that specific activity in the whole set of samples, and then multiplied by 100 in order to have an equal weight for the different enzymes.

2.3.3. Soil N mineralization

Potential nitrification was measured after inhibition of $N–NO_2^-$ oxidation with sodium chlorate (10 mM) according to the short nitrification assay (SNA) reported by Hopkins et al. (1988). Briefly, 2 g of soil (60% WHC) were taken from each sample and were extracted by shaking during 24 h with $(NH_4)_2SO_4$ solution. Extracts were centrifuged 20 min at 2000 × *g*, then 1 ml of each sample was transferred to 50 ml flasks together with diazotizing and coupling reagents. Colorimetric readings were performed on the spectrophotometer (UV mini 1240, UV–vis spectrophotometer, Shimadzu). For calibration curve 0, 0.25, 0.5,1, 2, 3, and 4 ml were taken from 1 μg $N–NO_2$ ml^{-1} solution, transferred to 50 ml jars and then treated similarly to soil extracts.

2.4. Statistics

Each plot was considered as experimental replication. The replicate measurements for each date were averaged for further analyses. Standard error of mean was calculated to present the variation of CO_2 efflux. Linear and non-linear regression analyses were performed to estimate the effect of temperature and soil water content on CO_2 efflux from different sources. The significance of all regression coefficients was tested using STATISTICA (StatSoft).

Effects of management (defoliation and control) and sampling time were tested using two-way analysis of variance (ANOVA). Post hoc comparison was done using the Tukey's test. A statistical probability of $p < 0.05$ was considered as significant. For the regression analysis calculated between SEI and qCO_2, data for qCO_2 were taken from Gavrichkova et al. (2008).

3. Results

3.1. Soil abiotic factors

Defoliation significantly affected the physical soil environmental conditions (Table 1). In comparison to undefoliated control, mean soil temperature at 5 cm depth and mean SWC under defoliated plants were much higher (by 2.4 °C, $p < 0.001$ and by 3.8%, $p < 0.01$, respectively for both growing seasons, Table 1).

3.2. In situ soil respiration and CO_2 partitioning

Cumulative CO_2 efflux from soil accounted for 736 and 878 g C m^{-2} in 2006 and 454 and 460 g C m^{-2} in 2007 in D and U soils, respectively (Table 2). Partitioning of CO_2 efflux showed that the average contribution of root-derived CO_2 to total CO_2 was 23% and 22%, respectively for D and U soils (average for 2006 and 2007, Table 2). It means that defoliation had no effect on relative contribution of the both CO_2 sources. Cumulative microbial and root-derived CO_2 were generally higher in 2006 in U plots, however the difference with D soil did not result significant for either respiration source (Fig. 2a, Table 2). In 2007 D soil showed higher microbial-derived CO_2 efflux before defoliation, after which the increment in cumulative CO_2 slowed down and resulted in no difference between the treatments. Root-derived CO_2 did not differ between managed and unmanaged plots in this year (Fig. 2b, Table 2).

Sensitivity of soil respiration to temperature was also influenced by defoliation (Fig. 3 and Table 3) being the effect

Table 1
Average soil temperature (Ts) and soil water content (SWC) measured in defoliated (D) and undefoliated (U) soils during the growing seasons 2006 and 2007 (May to November).

Parameters	2006		2007	
	D	U	D	U
Ts, °C	19.38^c	16.18^d	23.09^a	21.39^b
SWC, %	22.49^a	18.87^b	17.35^c	13.25^d

Different letters mean significant differences within each parameter.

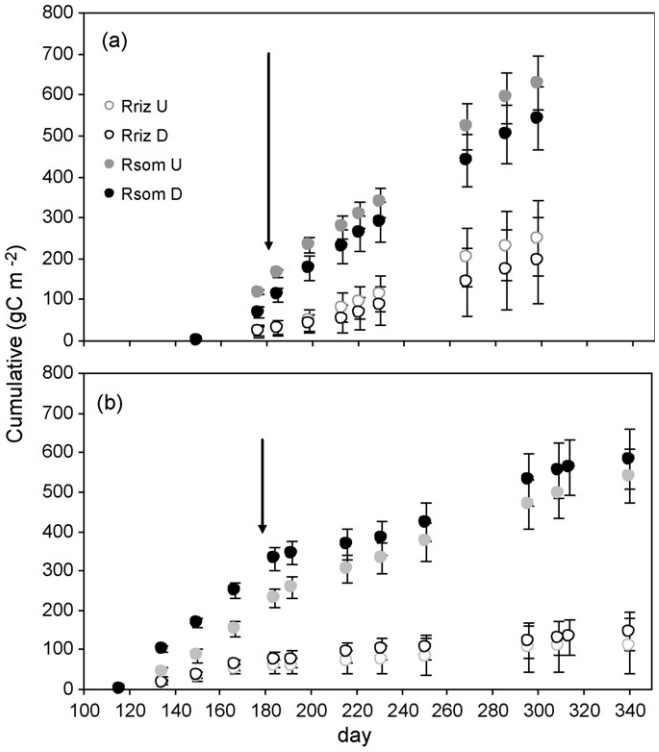

Fig. 2. Cumulative CO_2 efflux of root (R_{RIZ}) and microbial (R_{SOM}) origin measured in 2006 (a) and 2007 (b). Arrows indicate the day when mowing was performed. Bars represent standard error ($n = 8$).

Table 2
Cumulative total (R_s), root (R_{RIZ}) and microbial (R_{SOM})-derived CO_2 efflux from soil measured in defoliated (D) and undefoliated (U) soils in 2006 and 2007 (measurement period May to November).

Source	2006		2007	
	D	U	D	U
R_s, cum gC m^{-2}	$736.6^b \pm 32.9$	$878.2^a \pm 43.9$	$454.4^c \pm 61.9$	$460.5^c \pm 144.9$
R_{SOM}, cum gC m^{-2}	$541.6^a \pm 104.7$	$628.7^a \pm 92.9$	$363.0^b \pm 52.39$	$384.7^b \pm 53.2$
R_{RIZ}, cum gC m^{-2}	$195.0^a \pm 75.5$	$249.5^a \pm 64.7$	$91.5^b \pm 34.74$	$75.8^b \pm 45.1$
R_{RIZ}, %	26.5	28.4	20.1	16.5
R_{SOM}, %	73.5	71.6	80.0	83.7

±Standard error. Different letters mean significant differences within each respiration component.

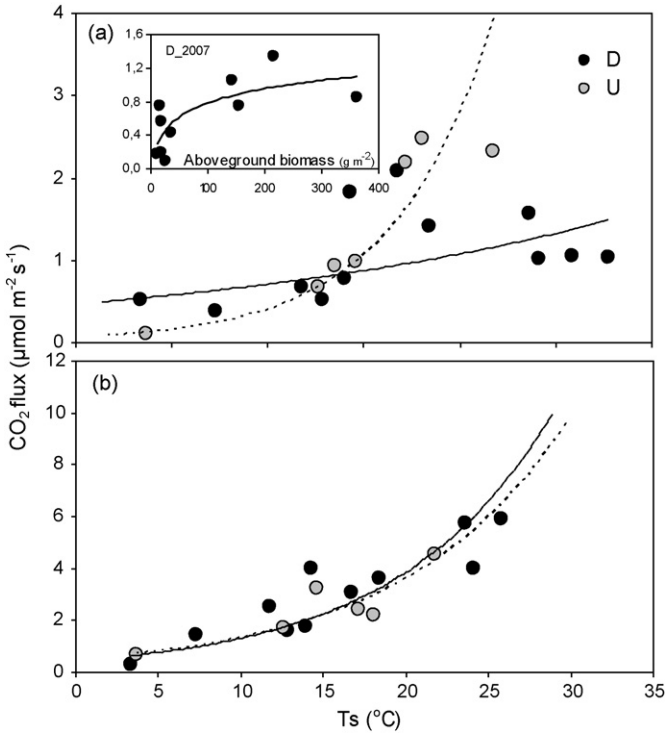

Fig. 3. (a) Root (R_{RIZ})-derived respiration and (b) microbial (R_{SOM})-derived respiration vs. changes in soil temperature (Ts) at 5 cm depth in defoliated (D) and undefoliated (U) plots measured in 2006 and 2007. Data at SWC < 20% were excluded from the analyses. Insertion – root-derived respiration vs. changes in aboveground biomass during the growing season 2007 in D plots.

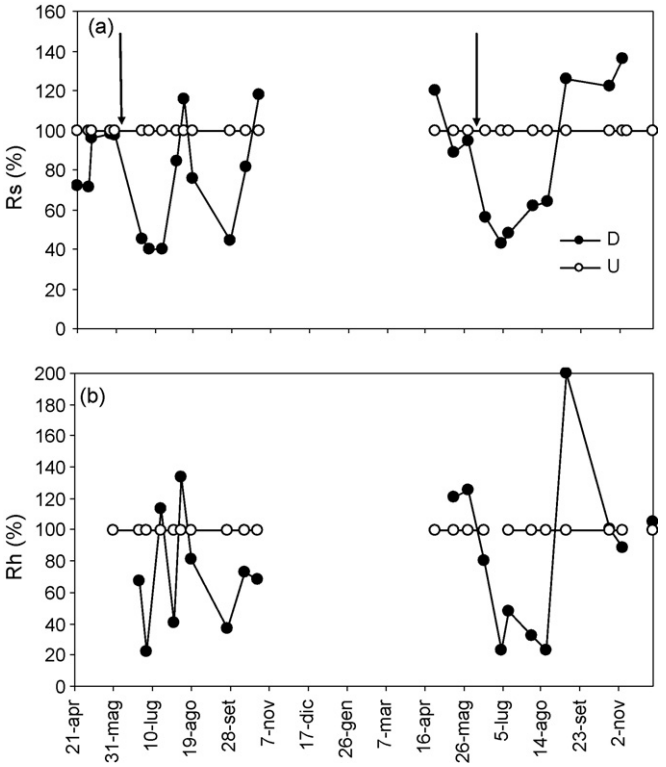

Fig. 4. (a) Cumulative CO_2 efflux (R_s) and (b) microbial-derived respiration (R_{SOM}) of defoliated (D) expressed as % of undefoliated plots (U) normalized to Ts and SWC. Arrows indicate the time of mowing.

especially pronounced for root-derived CO_2. Defoliation limited exponential increase of R_{RIZ}, decreased the Q_{10} (Table 3) and induced a loss of correlation with soil temperature, which was observed for non-defoliated soil.

When data were corrected for temperature and moisture, microbial-derived CO_2 was reduced by defoliation by 20% (average for 2006 and 2007, Fig. 4). After normalization to soil temperature at the reference of 15 °C, microbial-derived CO_2 in D and U plots responded differently to changes in SWC (Fig. 5). When soil moisture was below critical value of about 20% of WHC, CO_2 efflux increased with moisture, but it decreased when the soil moisture was higher than 35%.

3.3. Basal respiration, enzyme activities and N mineralization

Basal respiration of soil microorganisms was decreased by defoliation (−19% with respect to U plots, $p < 0.05$) in June (Table 4). Sampling time caused significant changes of basal respiration, both in D and U soils with the highest values in October.

Table 3
Q_{10} values for root (R_{RIZ}) and microbial (R_{SOM})-derived respiration in defoliated (D) and undefoliated (U) plots in 2006 and 2007.

Source	D	U
Q_{10}		
R_{RIZ}	$1.5^{(ns)}$	6.9^{**}
R_{SOM}	2.9^{*}	2.6^{*}

ns = not significant.
* $p < 0.05$.
** $p < 0.01$.

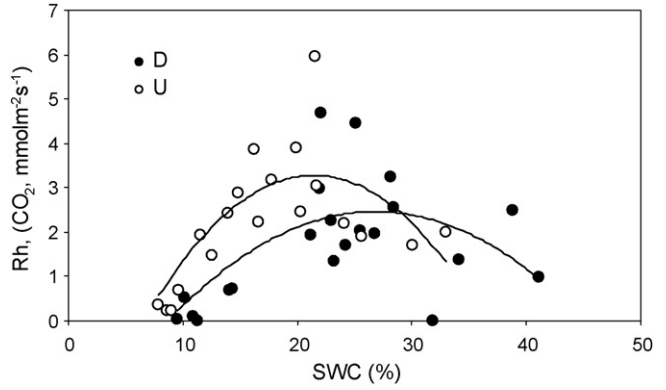

Fig. 5. Normalized microbial-derived respiration vs. soil moisture measured in 2006 and 2007.

The activities of almost all measured enzymes (except leucine-aminopeptidase) associated with microbial decomposition and involved in the cycles of C, P and S decreased significantly in June in D soils compared to U soils. The decrease ranged from −30% for cellulose to −8% for xylosidase (Fig. 6 and Table 5). Conversely leucine-aminopeptidase, involved in the N mineralization process, increased just after the mowing in June (+30%, $p < 0.05$). In October (four months after defoliation) no differences in the activities of most enzymes between treatments were registered.

N mineralization activity increased in D soils in June by 33% ($p < 0.05$), while in October no difference was observed between treatments (Table 4).

Significant linear positive relationships were observed between the synthetic enzymatic index (SEI) and the metabolic quotient (qCO_2, data from Gavrichkova et al., 2008) and between the N

Table 4
Basal respiration, N mineralization activity, Shannon's evenness of microorganisms measured in defoliated (D) and undefoliated (U) soils in June and October 2006.

Parameter month	Treatment	Basal respiration ($mg\ C–CO_2\ g^{-1}\ h^{-1}$)	Potential N mineralization ($\mu g\ N–NO_2\ g^{-1}\ 24\ h^{-1}$)	Shannon evenness index ($H'/ln\ S$)
June	U	$0.79^{b} \pm 0.08$	$11.05^{b} \pm 1.34$	$0.48^{b} \pm 0.00$
	D	$0.61^{c} \pm 0.04$	$14.66^{a} \pm 1.74$	$0.53^{a} \pm 0.00$
October	U	$1.11^{a} \pm 0.08$	$15.79^{a} \pm 2.48$	$0.49^{c} \pm 0.00$
	D	$1.12^{a} \pm 0.09$	$14.25^{a} \pm 0.86$	$0.49^{c} \pm 0.00$

±Standard error. Different letters mean significant differences within each estimated parameter.

Table 5
Percentage effect of management regime (D vs. U) in both sampling dates and of season (June vs. October) in both treatments on soil enzymatic activities and on enzymatic specific activities.

	Pho	β-Glu	α-Glu	Cel	Chit	Aryl	Xyl	L-Leu
Enzyme activity								
% Effect June	−14.4**	−28.3*	−19.4*	−29.9*	−25.5*	−28.5	−7.7*	+30.6*
% Effect October	+2.8	+5.6	+27.1	+19.9	+37.9	−7.7	+14.0**	+11.7
% Seasonal variation D	5.9	+7.6	+12.5*	+24.4	+18.5	−3.2	+22.3*	+9.7
% Seasonal variation U	−11.8*	−27.0**	−28.7*	−27.3	−36.0**	−25.0	−1.0	+28.2
Enzyme specific activities								
% Effect June	−26.0*	−34.1*	−40.3*	−33.2	−35.4**	−37.7	−26.8*	−19.3
% Effect October	5.6	7.1	34.2	21.3	42.7	−8.3	16.2	13.4
% Seasonal variation D	−15.2	−15.6*	4.8	−4.7	−2.8	−26.3*	7.2	−5.5
% Seasonal variation U	−40.6***	−48.1**	−53.4**	−47.5**	−56.0***	−49.9**	−32.5**	−32.7

Pho = acid phosphatise; β-Glu = β-glucosidase; α-Glu = α-glucosidase; Cel = β-cellobiohydrolase; Chit = N-acetyl-β-glucosaminidase; Aryl = arylsulphatase; Xyl = β-xylosidase; L-Leu = leucine-aminopeptidase.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

mineralization and the leucine-aminopeptidase activity in June (Fig. 7a and b, respectively).

The Shannon's evenness index was increased (+11%, $p < 0.01$) by mowing with respect to U plots in June (Table 4). The effect was not evident in October. No changes of the J' values were observed with time.

4. Discussion

4.1. Partitioning of CO_2 efflux from soil

The average contribution of roots to total CO_2 efflux from soil is less than 38–40% and 43–56% as reported, respectively by Craine et al. (1999) and Wang et al. (2007) for temperate grasslands. Our estimate is consistent with the results obtained in semi-arid grazed grasslands (15–37% by Li et al., 2002). However in U plots the relative contribution of roots to total CO_2 efflux was the same as in the D one. Our result also agrees with the review of Subke et al. (2006) who found a higher contribution of microbial CO_2 in the ecosystems with relatively low annual R_s, which was observed for

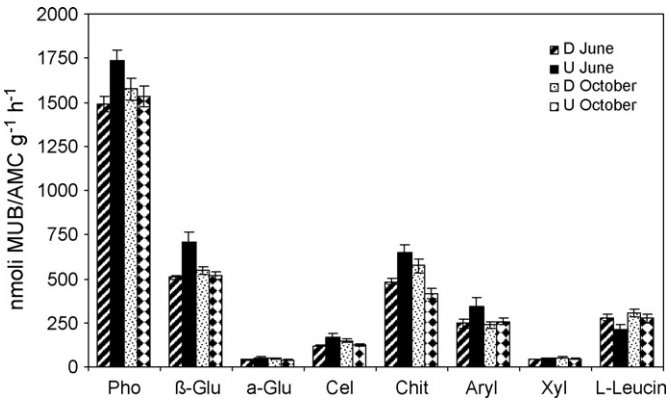

Fig. 6. Enzymatic activities measured in June and October 2006 in defoliated (D) and undefoliated (U) plots. Pho = acid phosphatise; β-Glu = β-glucosidase; α-Glu = α-glucosidase; Cel = β-cellobiohydrolase; Chit = N-acetyl-β-glucosaminidase; Aryl = arylsulphatase; Xyl = β-xylosidase; L-leucin = leucine-aminopeptidase. Bars represent standard error ($n = 8$).

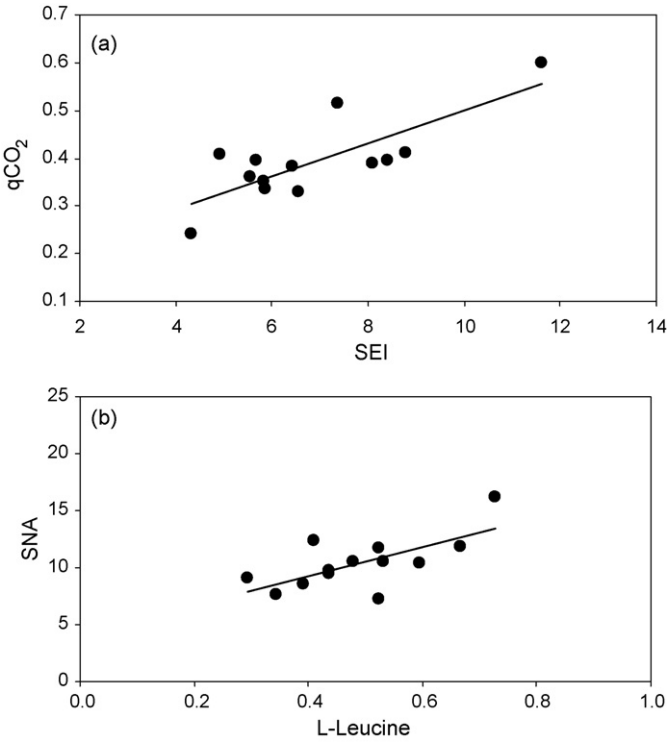

Fig. 7. (a) Relationship between synthetic enzymatic index (SEI) and metabolic quotient (qCO_2) in June; $R^2 = 0.60$, $p < 0.001$. For data related to qCO_2 see Gavrichkova et al. (2008). (b) Relationship between leucine-aminopeptidase activity and N mineralization (SNA) in June, $R^2 = 0.43$, $p < 0.001$, $n = 13$.

our site. The methods used for CO_2 partitioning (Kuzyakov, 2006) vary between different studies, each with its own shortcomings. The range of variation of methods used in literature make the comparison between studies particularly difficult.

The mesh-exclusion technique used to partition total CO_2 efflux is associated with various limitations and assumptions, which result in a possible overestimation of microbial-derived CO_2 and underestimation of root-derived CO_2 fluxes. Namely, the shortcomings of the mesh-exclusion are: disturbance of the soil structure by sieving, lateral diffusion of CO_2 (Jassal and Black, 2006) to the mesh bags from the surrounding soil and failure to separate root respiration from rhizomicrobial one. The advantage of mesh-exclusion technique over other trenching and root-exclusion methods is that it permits to exclude the roots in-growth inside the bags and in the same time allows the exchange of water and other substances with an exterior soil.

We minimised the effect of the soil disturbance on CO_2 fluxes by installing an additional mesh of 1 cm, permitting roots in-growth inside and obtaining the root-derived CO_2 flux as a difference between two meshes with equally disturbed soil. However, here we assumed that there is no difference between non-disturbed soil and previously sieved soil in terms of roots in-growth patterns. Microbial-derived CO_2 flux was calculated further from the difference between control (non-disturbed soil) plots and root-derived CO_2 fluxes. The effect of the lateral diffusion of CO_2 from the surrounding soil is difficult to estimate. Moyano et al. (2008) reported a value of ca. 10%. It could result in a systematic overestimation of the microbial-derived CO_2. Here we made the second assumption: the effect of the lateral diffusion of CO_2 is constant during the growing season and equal between all soil cores with different meshes, thus this effect should be minimized by installation of the additional mesh of 1 cm.

4.2. Microbial-derived CO_2

Defoliation by altering plant transpiration rates and soil exposure to sun increased significantly soil temperature and soil water content. Numerous studies observed increases in soil CO_2 efflux in response to warming (Peterjohn et al., 1994; Rustad et al., 2001; Melillo et al., 2002; Niinisto et al., 2004; Zhou et al., 2007a). The warming-induced response of soil respiration may be regulated by acclimatization of respiration (Luo et al., 2001), phenological and physiological adjustments of plants and microorganisms (Melillo et al., 2002), extension of growing season (Dunne et al., 2003; Wan et al., 2005; Zhou et al., 2007a), changes in net N mineralization (Wan et al., 2005) and stimulation of C_4 plant productivity (Wan et al., 2005). Our study showed that warming of the defoliated soil significantly increased microbial respiration, resulting in an increase of the total CO_2 efflux from soil. This increase likely resulted from enhanced mineralization of soil organic matter in warmed plots. Warming of the soil under defoliated plants cancelled the potential effect of defoliation on reducing respiration and resulted in the absence of difference in the cumulative soil respiration and its components between treatments. Differences in temperature and moisture between D and U plots should thus be taken into account when interpreting data of soil respiration.

Many studies reported that the removal of aboveground biomass by clipping, trenching, shading or girdling reduces the supply of current photoassimilates to roots and micorrhizal fungi, usually resulting in a decrease in soil CO_2 efflux by 19–49% at a short-term period (several days to month) (Bremer et al., 1998; Boone et al., 1998; Craine et al., 1999; Högberg et al., 2001; Zhou et al., 2007a; Bahn et al., 2006). In our study, at the same soil temperature and moisture, defoliation reduced microbial-derived CO_2 by ca. 20% during the growing season. A reduction of microbial respiration after clipping was also found for grassland communities (Craine et al., 1999; Zhang et al., 2005; Zhou et al., 2007a and Bahn et al., 2006). The main explanation of this effect was a depletion of available C from rhizodeposition. Our results confirm the decrease of microbial respiration but we argue that the reason for this was exactly the opposite. Defoliation of plants provoked an increased rhizodeposition process from roots which resulted in a general decrease of C mineralization activity since other easily available C substrates were present in soil. This was confirmed by a significant increase in soil soluble C forms in D soils found just after the mowing in June (Gavrichkova et al., 2008) and by other similar results reported in literature (Uhlírová et al., 2005). However we were not able to study the effect of defoliation also on the rhizomicrobial respiration as the experimental set-up did not allow to separate it from the root-derived respiration. Furthermore we cannot completely exclude an increase of root mortality in D soils although Chapin III and Slack (1979), Richards (1984), Ferraro and Oesterheld (2002), and McNaughton et al. (1998) reporting a possible reduction of root growth rates in response to defoliation, found no changes in root mortality rates.

4.3. Root-derived CO_2 efflux

It was not possible to account for the dynamics of root-derived CO_2 at the same environmental conditions in D and U plots as this component of soil CO_2 efflux was not related to changes in soil temperature. Root-derived CO_2 in U plots was characterized by exponential increase with Ts and high Q_{10} values, whereas in plots subjected to defoliation practice the Q_{10} was considerably reduced and the dependence on changes in soil temperature was lost. Data obtained after mowing were out of the general trend, indicating that other factors rather than temperature are responsible for changes in root-derived respiration. We did not observe any difference in belowground root biomass between D and U treatments one month after defoliation (data not shown), so one of the main factors which could affect patterns of root-derived CO_2 is the supply of current photoassimilates (Kuzyakov and Cheng, 2001). The dependence of root-derived CO_2 fluxes by photosynthesis is often masked by temperature, as the aboveground plant biomass usually peaks at the high temperatures and favourable soil water conditions as, in fact, this was observed in U plots. After defoliation aboveground biomass does not follow any more increments in soil temperature and thus result also in the uncoupling of root-derived CO_2 efflux with temperature. In fact, changes in aboveground biomass was the only factors which explained well variations in root-derived CO_2 efflux from soil in mowed and grazed plots (Fig. 3). Some studies (Kuzyakov et al., 2002; Bahn et al., 2006; Zhou et al., 2007a) reported no changes in root-derived CO_2 under experimental clipping and girdling, suggesting that carbohydrates stored into grass organs may buffer the effect of reduced assimilate supply and sustain root metabolism for some days or even weeks. In our study the first soil respiration measurements were performed a couple of days after the mowing, so we are not able to account for an immediate effect of defoliation.

Summarizing, our results show that factors affecting the carbohydrate supply to roots are evident on the short-term (days) and long-term (months) both on root and microbial respiration components. The effects were however, buffered by increased soil temperature and soil water content under defoliation.

To get deeper insight into the effects of defoliation on soil microbial biomass and activity some biochemical analyses were performed.

4.4. Basal respiration

Our results prove a significant effect of mowing and grazing on microbial biomass activity. A significant decrease of basal

respiration of microorganisms just after the mowing was in fact observed in D soils.

Similar results were reported by Guitian and Bardgett (2000) who found significant reductions of basal respiration, increases of microbial biomass and a decrease of metabolic quotient in defoliated plots suggesting changes in exudation patterns of defoliated and undefoliated plants and thus a higher C use efficiency of soil microbes. Uhlírová et al. (2005) also reported a lower carbon mineralization to decomposition ratio for the mowed plots indicating the use of different carbon sources than those used at untreated plots (soluble compounds and cellulose, respectively) and resulting again in higher carbon use efficiency in the mowed plot.

An increased supply of soluble carbon in the rhizosphere of defoliated plants derived from roots via increased exudation and/or root death is widely reported (Paterson, 2003; Hamilton III et al., 2008; Gavrichkova et al., 2008). An increasing number of studies have shown that herbivory increases the short-term flux of photoassimilate carbon belowground which, in turn, increases root exudation and the provision of soluble carbon for microbial growth (Bardgett et al., 1998; Holland et al., 1996).

The importance of increased rhizodeposition for recovery of the mowed grasses is well summarized in the review of Paterson (2003). The release of C substrates from roots most commonly result in an excess of available C and shortage of N (Merckx et al., 1987; Hamilton III et al., 2008), which promotes further microbial N mineralization. The turnover of the microbial biomass in the rhizosphere is rapid (order of hours to days) thus roots, having longer life-spans (order of months or years), have repeated opportunity to compete for mineralized N as it is cycled through the soil and components of the microbial community.

4.5. Activity of soil enzymes, N mineralization and microbial functional diversity

Almost all enzymes had different activity levels under different management regimes. Enzyme activities related to C, S and P decreased in mowed plots. The likely increased root exudation (Gavrichkova et al., 2008) induced a general suppression of C mineralization activity just after the mowing. A significant positive relationship between the SEI (synthetic enzymatic index) and the metabolic quotient (qCO_2) could be used as an extra proof of the efficient energy use of bacterial community. The specific enzyme activity represents a measure of the microbial physiological capacity, and has been found to be more closely related to community composition than total enzyme activity (Waldrop et al., 2000). Furthermore it is a valid indicator of microbial efficiency in utilization of energy and the degree of substrate limitation for soil microorganisms (Schjonning et al., 2002). Soil specific enzymatic activities were lower in D plots (−31% as an average value for June, Table 5). Therefore the increase of easily available C in the rhizosphere of defoliated plants decreased the maintenance energy requirements, shifted the energy from maintenance to growth promoting a higher C use efficiency as also reported by Guitian and Bardgett (2000) and Uhlírová et al. (2005).

The increased N mineralization activity just after the mowing and the increase in the activity of the enzyme involved in N cycle (N-leucine-aminopeptidase) confirm that defoliation stimulated the flow of C into the soil and thus further increased the demand for additional N. A positive significant relationship between leucine-aminopeptidase and N mineralization was also found. Defoliation often increases soil N cycling (McNaughton et al., 1997; Hamilton III et al., 2008) which in turn can result in the improved N availability to plants. In fact, productivity of the grazed and/or mowed grasslands depends on the rate of plant recovery after defoliation. Stimulation of microbial activity by root exudation may be an important mechanism promoting a fast recovery of grazed plants.

The production of chemically diverse root exudates in response to defoliation (Leake et al., 2006; Hamilton III et al., 2008) can sustain a vast array of microorganisms. Mowing practice enhances plant biodiversity (Collins et al., 1998; Marriot et al., 2004; Isselstein et al., 2005), which in turn promotes an increase of the variety of organics release by roots into the soil (Carney and Matson, 2006). In our study defoliation induced a significant increase of microbial functional diversity as shown by the Shannon's evenness index (J') in June. Additionally, changes in soil water content (Bossio and Scow, 1998), pH (Fierer and Jackson, 2006) and nutrient supply have all been shown to affect the composition of soil microbial communities. Changes in specific microbial community's structures under defoliation practices were confirmed by Patra et al. (2005, 2006) for the microorganisms involved in the N cycle.

The observed changes in the C and N mineralization activities induced by defoliation are thus related to shifts in the structure and/or functional diversity of the microbial community. The decrease in basal respiration and the specific enzyme activity indicate that the microbial community is characterized by a higher C use efficiency. This is also further confirmed by the significant increase of the microbial quotient (the ratio of microbial biomass to total organic C, $q$mic) and decrease of metabolic quotient (qCO_2) in D soils in June (Gavrichkova et al., 2008). Furthermore $q$mic has been widely used as an indicator of future positive changes in organic matter status, very important for grasslands sustainability, due to alterations in land use and management, cropping systems and tillage practices (Sparling, 1997).

4.6. Seasonal effect

Different sampling time lead to significant variations in some microbial parameters showing higher values from D plots in October than in June and lower values for U plots (Tables 4 and 5, Fig. 6). Different seasonal patterns of soil microbial parameters are common in grasslands (Uhlírová et al., 2005; Tracy and Frank, 1998; Moscatelli et al., 2005). On the other hand the differences due to defoliation were mostly evident in June. This is connected with the fact that soil was sampled few days after the mowing and thus microorganisms were affected by the availability of soluble C released from roots (Guitian and Bardgett, 2000). A moderate grazing pressure was still present in October but did not affect much soil biochemical properties as the annual mowing possibly because the livestock distribution in the field is generally not homogenous and thus the defoliation is selective. Furthermore the different climatic conditions occurring in Autumn may have influenced the exudation patterns and thus microbial metabolism.

Other studies, investigating the belowground response to aboveground defoliation have shown that the effect can vary depending on plant species composition (Guitian and Bardgett, 2000; Hokka et al., 2004; Mikola et al., 2005), on the timing of defoliation during plant development (Ilmarinen et al., 2005) and on other biotic and abiotic factors.

4.7. Conclusions

Root and microbial activity in soil were affected by defoliation. The defoliation effect was clearly highlighted in both field measurements and laboratory analyses. The response of soil CO_2 efflux (root- and microbial-derived) to defoliation was masked by fluctuations in abiotic factors, mainly temperature. However, both CO_2 efflux components when corrected for soil temperature and moisture differences were significantly decreased by defoliation.

The decrease of microbial C mineralization activity was confirmed by soil biochemical analyses showing that defoliation decreased C cycling (potential microbial respiration and C-related enzymes), but favoured a microbial community characterized by a higher C use efficiency and diversity level. At the same time defoliation increased N mineralization activity which ultimately could result in a faster recovery of grazed plants.

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