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Effect of clipping and shading on C allocation and fluxes in soil under ryegrass and alfalfa estimated by ^{14}C labelling

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ABSTRACT

Photosynthesis of higher plants drives carbon (C) allocation below-ground and controls the supply of assimilates to roots and to rhizosphere microorganisms. To investigate the effect of limited photosynthesis on C allocation, redistribution and reutilization in plant and soil microorganisms, perennial grass *Lolium perenne* and legume *Medicago sativa* were clipped or shaded. Plants were labelled with three ^{14}C pulses to trace allocation and reutilization of C assimilated before clipping or shading. Five days after the last ^{14}C pulse, plants were clipped or shaded and the total CO_2 and $^{14}CO_2$ efflux from the soil was measured. ^{14}C in above- and below-ground plant biomass and bulk soil, rhizosphere soil and microorganisms was determined 10 days after clipping or shading.

After clipping, 2% of the total assimilated ^{14}C originating mainly from root reserves were detected in the newly grown shoots. This corresponded to a translocation of 5 and 8% of total ^{14}C from reserve organs to new shoots of *L. perenne* and *M. sativa*, respectively. The total CO_2 efflux from soil decreased after shading of both plant species, whereas after clipping, this was only true for *L. perenne*. The $^{14}CO_2$ efflux from soil did not change after clipping of both species. An increased $^{14}CO_2$ efflux from soil under shading for both plants indicated that lower assimilation was compensated by higher utilization of the reserve C for root and rhizomicrobial respiration.

We conclude that C stored in roots is an important factor for plant recovery after limiting photosynthesis. This stored C is important for shoot regrowth after clipping, whereas after shading, it is utilized mainly for maintenance of root respiration. Based on these results as well as on a review of several studies on C reutilization for regrowth after clipping, we conclude that because of the high energy demand for nitrogen fixation, legumes use a higher portion (9–10%) of stored C for regrowth compared to grasses (5–7%). The effects of limited photosynthesis were of minor importance for the exudation of the reserve C and thus, have no effect on the uptake of this C by microorganisms.

1. Introduction

Below-ground translocation of carbon (C) by plants and its turnover in soils are important processes affecting the global C cycle. Thus, in the last decades, many studies have investigated the distribution and dynamics of assimilates in the plant–soil system, their utilization by microorganisms and contribution to carbon dioxide (CO_2) efflux. It has been shown that pasture plants translocate 30–50% of assimilated C below-ground. Approximately half of this C is incorporated into the root biomass, 12% remains in the soil and microbial biomass, and 36% is respired by roots or microorganisms, whereby about 5% of the fixed C is respired by mycorrhizas (Johnson et al., 2002; Kuzyakov and Domanski, 2000; Leake et al., 2006). Roots contribute 30–70% of the soil CO_2 efflux (Schlesinger, 1977; Subke et al., 2006), which is the second largest C flux in terrestrial ecosystems and account for 60–90% of ecosystem respiration (Goulden et al., 1996; Longdoz et al., 2000). Rhizodeposition is an important driver for many processes in terrestrial ecosystems, such as nutrient availability for plants, activity and turnover of microbes (Blagodatskaya et al., 2010) in addition to turnover of soil organic matter (Merbach et al., 1999).

The below-ground translocation of recently assimilated C is a very rapid process. The highest exudation rate of photosynthates by wheat roots is reached 2–3 h after fixation, declining to a third of the maximum after 5 h (Dilkes et al., 2004). Also for the grass *Nardus stricta* a fast transport of recent assimilates to soil and DOC has been reported (Johnson et al., 2011). In a tree girdling experiment, a large decrease in soil respiration was observed after

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disrupting assimilate transport to the roots (Högberg et al., 2001). These studies indicate that current photosynthesis and the supply of recent assimilates to roots are the main drivers for rhizodeposition and soil respiration (Kuzyakov and Gavrichkova, 2010). Thus, any alteration in environmental factors affecting photosynthetic activity, and thereby influencing availability of recent assimilates, is assumed to influence fast C pools and fluxes of plant-derived C, such as dissolved organic matter, soil CO_2 or microbial biomass. Defoliation by grazing (Detling et al., 1979) and shading are factors that reduce the photosynthesis rate due to lower leaf surface areas and less available light, respectively. It has been shown that defoliation increases the sink strength of regrowing leaves and, therefore, reduces C allocation below-ground (Detling et al., 1979; Mackie-Dawson, 1999). On the contrary, Holland et al. (1996) found a positive relationship between herbivory and below-ground C allocation for *Zea mays*. Defoliation by grazing affects plant biomass and soil respiration, depending on the grazing intensity, history and composition of vegetation (Cao et al., 2004; Milchunas and Lauenroth, 1993). Thus, grazing management can play an important role in C economy of grasslands.

Less is known about the effect of shading on the redistribution of C reserves. A rapid reduction of C reserves under low light conditions due to limited C supply has been observed (Merlo et al., 1994). Low light intensity decreased the root-to-shoot ratio (R:S) of *Z. mays* (Lambers and Posthumus, 1980), whereas an increase was observed for *Lolium perenne* (Hodge et al., 1997). To compensate temporary limited photosynthesis by defoliation or shading, plants are able to store C. Although both defoliation and reduced light intensity lead to reduced assimilation, it is assumed that because of the removal of plant biomass caused by defoliation, they have different impacts on the redistribution of stored C and thus on the C input into the soil and the C availability for soil microorganisms.

C allocation in plant and soil is also affected by plant properties. During plant development, the portion of C stored in shoots increases, leading to a decrease in below-ground translocation (Gregory and Atwell, 1991; Keith and Oades, 1986; Meharg and Killham, 1990). Furthermore, C allocation patterns differ between plant species. The relative below-ground translocation of C of perennial plants is higher compared to annual plants. This indicates a higher C storage in roots of perennial plants, whereas annual plants allocate more C in above-ground parts, especially grains (Kuzyakov and Domanski, 2000). Warembourg et al. (2003) investigated the C input into the rhizosphere of 12 Mediterranean plants. They found significant species-dependent differences in the below-ground allocation of assimilated C, with portions ranging from 41 to 76%. Among functional plant groups, legumes use the highest C portion for rhizosphere respiration compared to grasses and especially to non-legume forbs (Warembourg et al., 2003). This is because of the high energy requirement and consequently high C demand for N_2 fixation by symbiotic rhizobia (Phillips, 1980). Estimations give evidence that about 6 mg of C are necessary to fix 1 mg of nitrogen (N) (Vance and Heichel, 1991). The respiration losses tied to N_2 fixation can account for up to 70% of total root respiration (Witty et al., 1983). Thus, because of the high C costs for N_2 fixation, we hypothesized that changing rates of photosynthesis provoked different effects between a legume species (*Medicago sativa* L.) and a non-legume species (*L. perenne* L.) regarding the distribution of assimilates.

Using repeated $^{14}CO_2$ labelling of two plant species, *M. sativa* and a *L. perenne*, we investigated how defoliation (simulated grazing) and shading affected C allocation within the plant, below-ground C translocation and reutilization of stored C. The specific questions were:

(1) How does clipping and shading affect biomass production and ^{14}C distribution between various pools?
(2) Which plant parts provide C for growth of new shoots after clipping?
(3) How does limited photosynthesis after clipping or shading alter the redistribution of stored C in plant, soil, microorganisms and soil CO_2?
(4) Do clipping and shading induce different responses with respect to the redistribution of stored C in the plant and soil pools?

Table 1
Basic characteristics of the soil sampled from the A_p horizon of a haplic Luvisol near Göttingen (Germany) (Kramer et al., 2012).

Soil properties	
N_{tot} (mg g^{-1})	1.2
Org. C (mg g^{-1})	11.7
C/N	9.76
NO_3^- (mg g^{-1})	0.083
P (mg g^{-1})	0.160
S (mg g^{-1})	0.009
CEC (mmol$_c$ kg^{-1})	108
BS (%)	99.7
Texture[a] clay/silt/sand (% (w/w))	7.0/87.2/5.8
pH (H_2O)	6.6
pH ($CaCl_2$)	6.0

CEC: cation exchange capacity; BS: base saturation.
[a] Texture according to the German classification system.

2. Materials and methods

2.1. Soil properties and plant growing conditions

Plants were grown on an arable loamy haplic Luvisol developed on loess. This soil was collected near Göttingen (Germany, 51°33′36.8″N, 9°53′46.9″E) from the upper 10 cm of the A_p horizon. The basic characteristics of the soil are shown in Table 1.

Seeds of ryegrass (*L. perenne* L.) and alfalfa (*M. sativa* L.) were germinated on wet filter paper in Petri dishes. After 5 (*M. sativa*) and 8 days (*L. perenne*), the seedlings were transferred to pots (inner diameter 7 cm, height 20 cm), each filled with 700 g of air-dried, sieved (≤2 mm) soil. For *M. sativa*, each pot contained 3 plants and for *L. perenne* 5 plants, because of the lower biomass of *L. perenne*. In total 12 pots per plant species were prepared for the experiment. The pots were closed with a plastic lid with holes for shoots. The plants were grown at 26–28 °C day temperature and at 22–23 °C night temperature with a day-length of 14 h and a light intensity of approximately 211 μmol m^{-2} s^{-1}. Thus, the cumulative daily radiation was approximately in the range of field conditions. The soil water content was measured gravimetrically and adjusted daily to 70% of the available field capacity.

2.2. ^{14}C labelling procedure

Repeated ^{14}C pulse labelling was used to evaluate C reutilization and C input into the soil. All plants of one species (12 pots) were labelled simultaneously in a $^{14}CO_2$ atmosphere on days 35, 40 and 45 after planting. The day before the first labelling, holes in the plastic lids were sealed around the shoots with silicone paste (NG 3170, Thauer & Co., Dresden) and checked for air tightness. For labelling, the plants were placed in an acryl glass chamber. The chamber and the labelling technique are described in detail elsewhere (Werth and Kuzyakov, 2008). Briefly, the chamber was connected by tubing with a flask containing 10 ml of diluted $Na_2{}^{14}CO_3$ (ARC Inc., USA) solution with 1.67 MBq. $^{14}CO_2$ was released into the chamber by adding 3 ml of 5 M H_2SO_4 to the labelling solution. Plants were labelled during 3 h in the $^{14}CO_2$ atmosphere. Thereafter, the air from the chamber was pumped through 15 ml of 1 M NaOH solution for 2 h to trap the remaining unassimilated $^{14}CO_2$. Finally,

plants were removed from the chamber and grown under normal conditions until the next $^{14}CO_2$ pulse.

2.3. Clipping and shading

Both plant species were subjected to shading or clipping 5 days after the last $^{14}CO_2$ pulse because it was assumed that after this period, the distribution of assimilated C between above- and below-ground pools was mostly complete (Domanski et al., 2001). Consequently, the translocated ^{14}C found in the various pools after shading or clipping was considered as remobilised reserve C. This is in agreement with Danckwerts and Gordon (1987) who found that assimilated ^{14}C reached its final destination within 4–6 days and termed this ^{14}C as reserve C. For clipping, the shoots were cut 4 cm above the soil surface for *L. perenne* and 8 cm for *M. sativa*. We used 4 replicates for each species. Different clipping heights were applied to achieve a similar stubble biomass of both plant species. Subsequently, plants continued growth under normal conditions. For shading, 4 planted pots of both species were exposed to a reduced light intensity of about 17 $\mu mol\, m^{-2}\, s^{-1}$ for 10 days. In addition, 4 pots per species were kept under normal conditions and used as controls with untreated plants (no shading and no clipping). All pots, including the controls, were harvested 10 days after the clipping or the beginning of shading.

2.4. Sampling

At harvest, above-ground biomass of all treatments was divided into 'shoot' (biomass above the cutting height of 4 or 8 cm) and 'stubble' (biomass between cutting height of shoots and soil surface). Furthermore, the shoots of the clipped plants were divided into 'clipped shoots' (the shoots already cut 5 days after labelling) and 'regrown shoot' (the shoots cut at harvest). Roots were separated from the soil by tweezers. To separate rhizosphere soil and bulk soil, the roots were slightly shaken and the remaining soil attached to the roots was accepted as rhizosphere soil.

To determine the impact of clipping and shading on the dynamics of soil CO_2 efflux, the soil air was trapped in 15 ml of 1 M NaOH solution by pumping with a membrane pump. Sampling of CO_2 started directly after the first $^{14}CO_2$ pulse. The NaOH solution was changed 3 times after each labelling (days 1, 3 and 5 after each labelling) and 6 times after clipping or the beginning of shading (days 1, 3, 5, 6, 8 and 10 after the treatments).

2.5. Sample analysis

All plant and soil samples were dried at 65 °C for 3 days, weighed and ground in a ball mill. Prior to liquid scintillation counting (LSC) for ^{14}C analyses, the solid samples (50 mg of plant material, 500 mg of soil) were combusted in an oxidizer unit (Feststoffmodul 1300, AnalytikJena, Germany) at 900 °C. The CO_2 released during combustion was trapped in 10 ml of 1 M NaOH. 2 ml aliquots of the NaOH solution were mixed with 4 ml of the scintillation cocktail Rotiszint Eco Plus (Carl Roth, Germany). After decay of chemiluminescence, the ^{14}C activity was measured by means of LSC (LS 6500 Multi-Purpose Scintillation Counter, 217 Beckman, USA). The ^{14}C activity of $^{14}CO_2$ trapped in NaOH solution during the experiment was measured in 1 ml aliquots added to 2 ml scintillation cocktail Rotiszint Eco Plus (Carl Roth, Germany) after decay of chemiluminescence. The ^{14}C measurements were carried out with an LSC (MicroBeta-TriLux, 205 Perkin Elmer Inc., USA). The total C content in trapped CO_2 was determined by titration of the NaOH solution with 0.01 M HCl against Phenolphthalein after addition of 1.5 M $BaCl_2$ solution.

Total C and ^{14}C incorporated into the microbial biomass in the bulk soil and rhizosphere soil during the experiment were analyzed by the chloroform-fumigation extraction method (CFE) (modified after Vance et al., 1987). 5 g of fresh soil were extracted with 20 ml of 0.05 M K_2SO_4 solution. Another 5 g of soil were first fumigated with ethanol-free chloroform for 24 h and then extracted in the same way. Both extracts were shaken for 1 h at 200 rpm and then centrifuged for 10 min at 3070 rpm. The extracts were frozen until analysis of total C and ^{14}C. The total C content in the extracts of the fumigated and unfumigated soil samples was measured using an N/C analyser (Multi N/C 2100, AnalytikJena, Germany). The ^{14}C activity of the extracts was measured by means of an LSC (LS 6500 Multi-Purpose Scintillation Counter, 217 Beckman, USA) as described for plant and soil material.

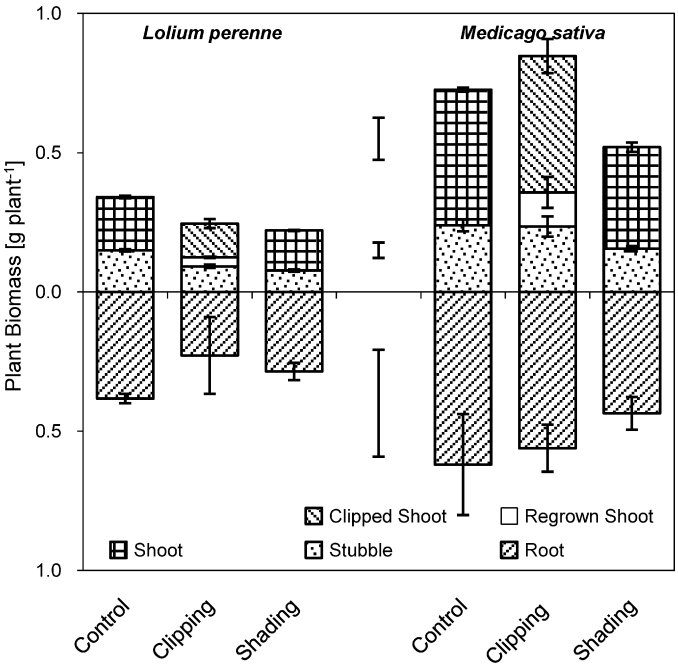

Fig. 1. Above-ground and below-ground plant dry mass (mean ± SE) of 60 days old *L. perenne* and *M. sativa* 10 days after clipping or shading. LSD values ($p < 0.05$) are presented as whisked segments.

2.6. Calculations and statistics

The ^{14}C activity in shoots, stubbles, roots, bulk soil, rhizosphere soil, microbial biomass and in CO_2 efflux is presented as percentage of total recovered ^{14}C. Specific ^{14}C activities are expressed as $kBq\, g^{-1}$ dry weight for shoots, stubbles, roots and soil samples, and as $kBq\, g^{-1}$ C for CO_2 and microbial biomass. The total C and ^{14}C in microbial biomass was calculated by dividing the microbial C flush (difference between extractable C from fumigated and unfumigated soil samples) with a k_{EC} factor of 0.45 (Wu et al., 1990).

The experiment was conducted with 4 replicates for all treatments. All results are presented as mean values with standard errors of the mean. If the standard error exceeded the mean by more than 10%, the replicate with the highest deviation was not considered. Significances between the treatment and the plant species were obtained by a two-factor analysis of variance (ANOVA) in combination with a post hoc Newman–Keuls test as least significant differences between the means (LSD; $p < 0.05$).

3. Results

3.1. Plant biomass production

Plants of *M. sativa* produced significantly more shoot biomass as well as stubble biomass compared to *L. perenne* (Fig. 1). Only after

Table 2
Root-to-shoot (R:S) ratio (mean ± SE) of *Lolium perenne* and *Medicago sativa* 10 days after clipping and shading. The statistical analyses showed no significant differences between the results.

	R:S ratio	
	L. perenne	*M. sativa*
Control	1.12 ± 0.06	0.85 ± 0.25
Clipping	1.00 ± 0.60	0.61 ± 0.10
Light reduction	1.23 ± 0.14	0.84 ± 0.12

shading the stubble biomass was the same for both plant species. *M. sativa* had slightly higher root biomass compared to *L. perenne*, resulting in a slightly higher R:S ratio by *L. perenne* (Table 2).

Clipping caused an increase in shoot biomass (including clipped shoots) of *M. sativa* after 10 days of regrowth. These results indicate faster regrowth of *M. sativa* compared to *L. perenne*. For the stubble biomass, a significant decrease after clipping was observed only for *L. perenne*, while there was no change for *M. sativa*. Shading for 10 days reduced the biomass of the stubbles of both plant species (Fig. 1). The amount of root biomass showed no significant differences between the different treatments, and thus, also the R:S ratio was unaffected (Table 2).

3.2. Distribution of ^{14}C in plant and soil pools

The amount of C allocated into shoots, stubbles, roots, bulk soil and rhizosphere soil was determined as percentage of total ^{14}C recovery and as ^{14}C specific activity. The ^{14}C specific activity of a pool allowed comparison of C allocation with respect to the pool size, while ^{14}C recovery within this pool showed the allocation of total C after the start of labelling and thus reflected the effect of clipping and shading.

About 50% of the recovered ^{14}C was found in the above-ground biomass for both plant species (Fig. 2). Except for the control plants, where the ^{14}C recovery in the shoots was higher for *M. sativa* than *L. perenne* there was no difference in the shoot ^{14}C recovery between both plant species. The ^{14}C recovery in the roots reached about 20% for *M. sativa* and, depending on the treatment, between 6 and 15% for *L. perenne* (Fig. 2). ^{14}C recovery for the stubbles was nearly identical for both species as well as between the treatments and ranged from about 10 to 15%.

Translocation of reserve C to newly grown shoots after clipping was measured by ^{14}C in the regrown shoots. The reserve C used for shoot regrowth contributed about 2% of total ^{14}C recovery for both plants. After clipping, there was no significant change of ^{14}C recovery and ^{14}C specific activity in the stubbles and in the roots (Figs. 2 and 3). However, a relative ^{14}C decrease in the roots of *L. perenne* was observed, indicating that roots are a probable source of reused C reserves after clipping.

There was no effect of shading on the ^{14}C recovery as compared to the controls (Fig. 2). However, due to lower amounts of above-ground biomass (Fig. 1) and a lower assimilation of new C compared to plants grown under control conditions, ^{14}C specific activity of the stubble and shoots of shaded *L. perenne* was higher than under normal light conditions. For *M. sativa*, however, this increase was only observed for the stubbles (Fig. 3). There was no change in the ^{14}C specific activity in roots.

In the control and the shaded plants, higher portions of ^{14}C were recovered in the rhizosphere of *L. perenne* compared to *M. sativa* (Fig. 4). Clipping and shading showed no significant effects on ^{14}C recovery in the soil pools of both plants compared to their respective control plants (Fig. 4). ^{14}C recovery and specific activity in the microbial biomass was similar for both plant species and was unaffected by clipping and shading (Fig. 4).

3.3. Total CO_2 and ^{14}C efflux from soil

The cumulative CO_2 efflux from soil under *L. perenne* was highest for the control treatments (Fig. 5). The reduced availability of assimilates after clipping or shading decreased the CO_2efflux, with a larger decrease after clipping. For *M. sativa*, soil CO_2 efflux was also reduced after clipping or shading (Fig. 5). However, after

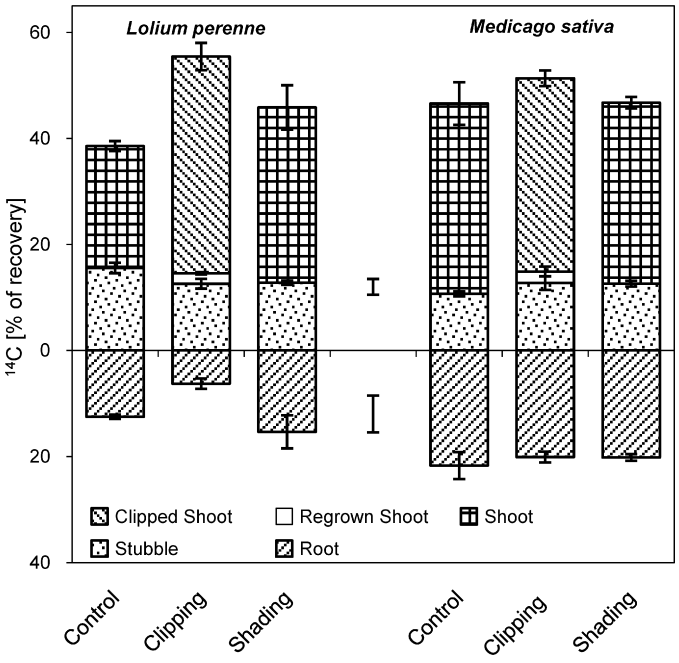

Fig. 2. ^{14}C recovery (mean ± SE) in the above- and below-ground plant parts 10 days after clipping or shading of 60 days old *L. perenne* and *M. sativa* presented as portions of ^{14}C recoveries. LSD values ($p < 0.05$) are presented as whisked segments.

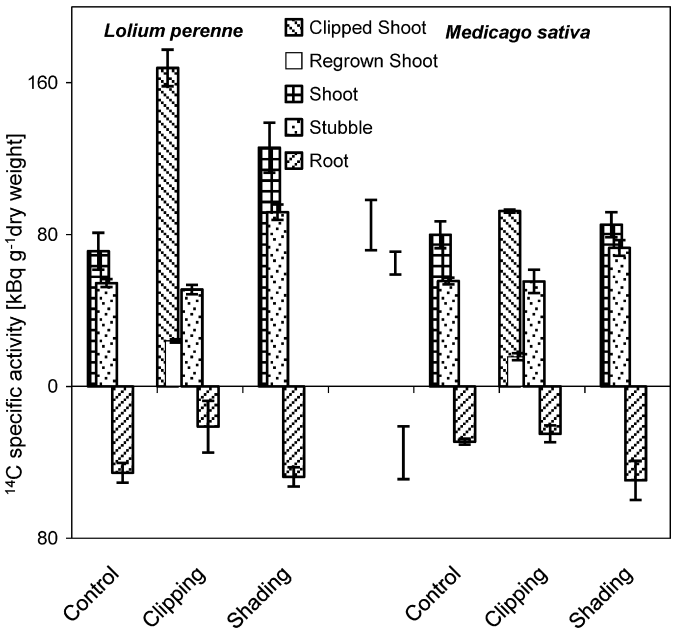

Fig. 3. ^{14}C specific activity (mean ± SE) of above-ground and below-ground plant parts for different treatments 10 days after clipping or shading. LSD values ($p < 0.05$) are presented as whisked segments.

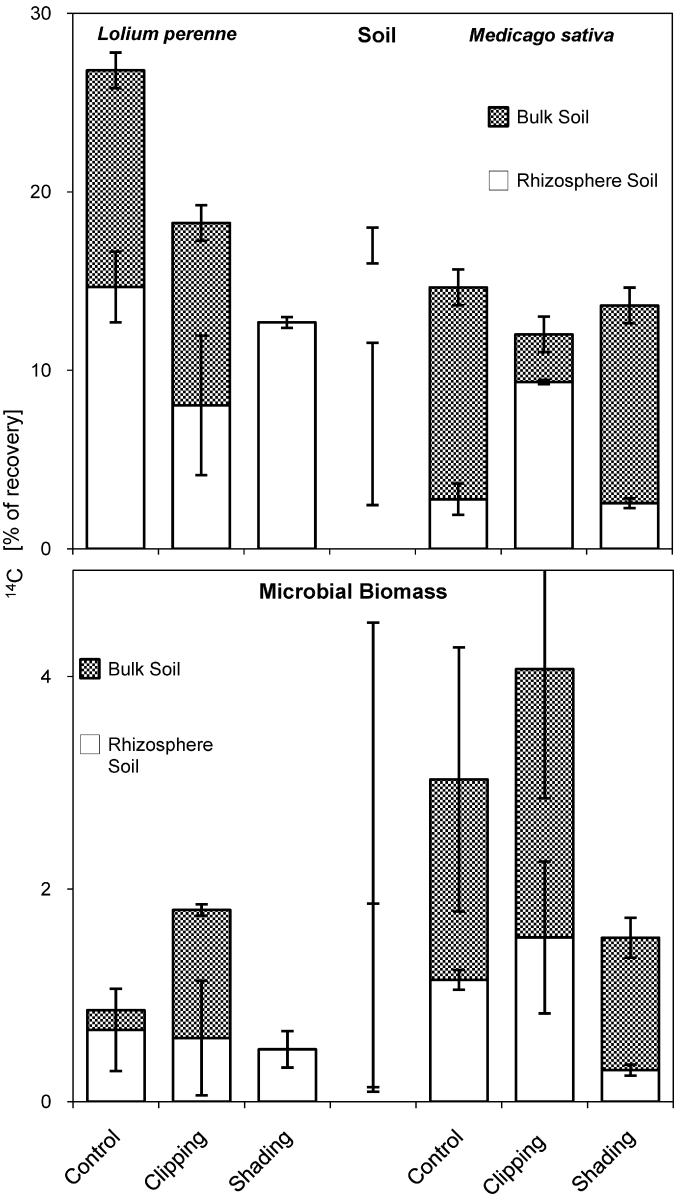

Fig. 4. ^{14}C recovery (mean ± SE) in soil (top) and microbial biomass (bottom) under *L. perenne* and *M. sativa* 10 days after clipping or shading. LSD values ($p < 0.05$) are presented as whisked segments. Soil of shaded *L. perenne* was completely rooted and therefore no data for bulk soil are available.

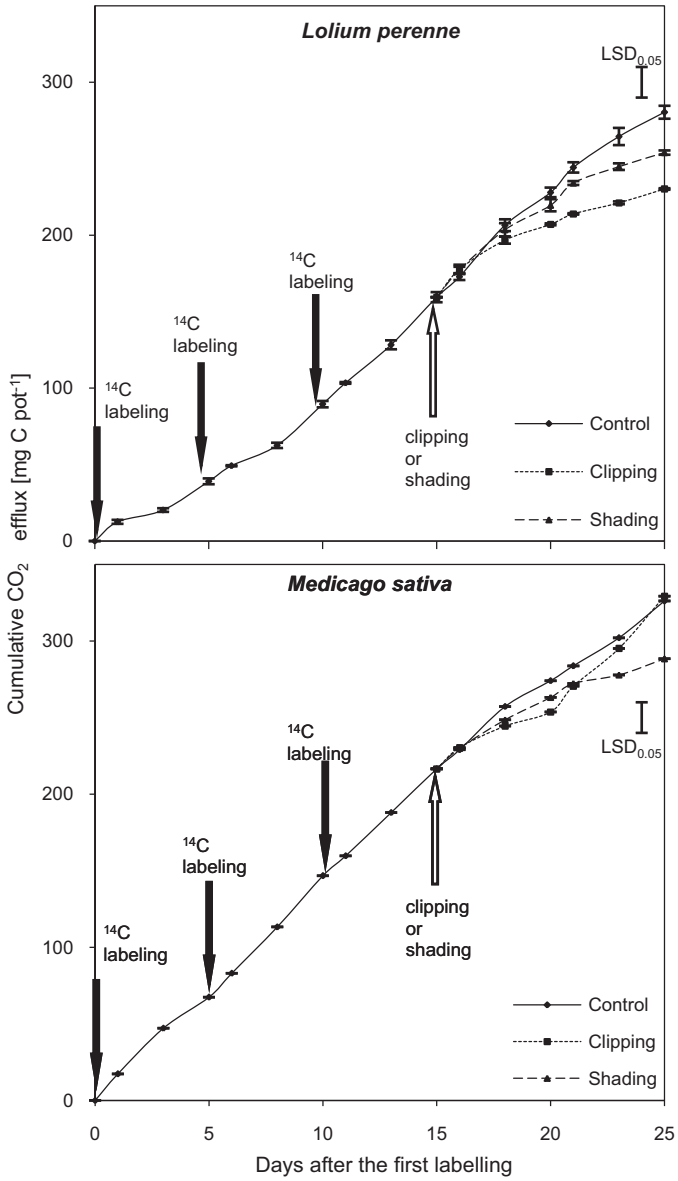

Fig. 5. Cumulative CO_2 efflux (mean ± SE) from soil under *L. perenne* (top) and *M. sativa* (bottom) beginning after the first ^{14}C labelling and the effect of clipping and shading on the CO_2 efflux. LSD value ($p < 0.05$) for the last day of the experiment is presented as whisked segment.

clipping this was only observed for 5 days and after 10 days, it reached the same level as that of control plants. The lowest amounts of soil CO_2 for *M. sativa* were observed after shading. Comparing both plant species, total soil CO_2 efflux was higher for *M. sativa* than for *L. perenne*.

The percentage of ^{14}C recovery in the CO_2 efflux increased in response to clipping under *L. perenne*, whereas it showed no significant change after shading (Fig. 6). ^{14}C specific activity, calculated as mean of the time between the beginning of treatment and harvest, was higher under *M. sativa* than under *L. perenne* for clipped and shaded plants (Fig. 6). Clipping increased the ^{14}C specific activity of the soil CO_2 efflux under *M. sativa*, whereas there was no effect under *L. perenne*. After shading, an increase in ^{14}C specific activity of CO_2 was observed for both plant species. In contrast to clipping, the remobilization of reserve C may play a more important role in maintaining respiration after shading.

4. Discussion

4.1. C allocation by L. perenne and M. sativa

The biomass of the above-ground plant parts and roots was higher for *M. sativa* than for *L. perenne* (Fig. 1). These results are in accordance with the higher ^{14}C recovery found in shoots of the control of *M. sativa* compared to *L. perenne* (Fig. 2). The lower R:S ratio of *M. sativa* showed that this legume allocates more C in its above-ground biomass, whereas C allocation in roots is higher for the non-legume *L. perenne*. This is also supported by the higher specific ^{14}C activity of the roots of *L. perenne*. The higher ^{14}C recovery found in the soil under *L. perenne* compared to *M. sativa* (Fig. 4) can be explained by a higher investment of *L. perenne* for rhizodeposition since an enhanced rhizodeposition leads to increased nutrient availability for roots (Kuzyakov, 2002), which is of more importance for non-legumes than for legumes. On the other hand, legumes have higher C costs for N_2 fixation estimated as between 4 and 12% of

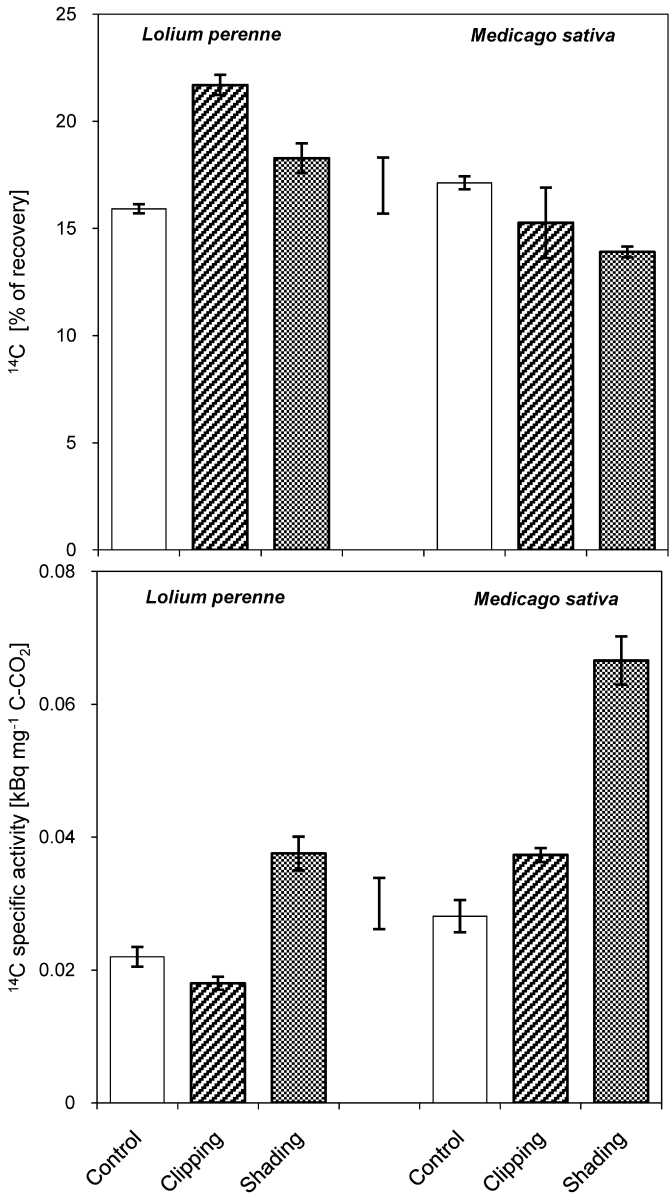

Fig. 6. ^{14}C recovery (mean ± SE) in CO_2 efflux from soil under *L. perenne* and *M. sativa*, calculated from the cumulated ^{14}C efflux (top), and mean value of ^{14}C specific activity (mean ± SE) of the soil CO_2 under *L. perenne* and *M. sativa* measured from clipping or shading until harvest (bottom). LSD value ($p < 0.05$) is presented as whisked segment.

photosynthesis (Lambers, 1987), resulting in higher root and rhizomicrobial respiration. Thus, the higher soil CO_2 efflux of *M. sativa* compared to the non-legume *L. perenne* (Fig. 5) can be explained by higher root respiration to maintain N_2 fixation.

4.2. Redistribution of stored C in plant pools

The results of 28 studies investigating the effect of defoliation on growth of grasses and herbs were reviewed by Ferraro and Oesterheld (2002). Most plant species decrease their biomass production after defoliation, depending on (a) the recovery period after the last defoliation, (b) the time interval between defoliation events and (c) N availability. In our study, the above-ground biomass (including clipped shoots) of *L. perenne* was reduced after clipping, whereas that of *M. sativa* was increased (Fig. 1). A trend of biomass reduction of *L. perenne* roots was observed after clipping because of higher herbivory tolerance of *L. perenne* compared to *M. sativa* (Counce et al., 1984). For herbivory-tolerant grass species, defoliation-induced reduction of root growth was a consequence of allocation of assimilates to support shoot regrowth (Guitian and Bardgett, 2000). The decreased R:S ratio of both plant species indicated assimilate translocation from roots to shoots after clipping (Table 2).

^{14}C was found in the newly grown shoots of both species. This is supported by many other studies that have labelled grasses with ^{14}C or ^{13}C (Johansson, 1993; Kuzyakov et al., 2002; Morvan-Bertrand et al., 1999). The ^{14}C in the shoot must have been translocated from the stubbles or roots left after clipping. The translocation of C is very important for the growth of new tissue since 91% of the C in these plant parts is derived from reserves (Morvan-Bertrand et al., 1999). Five and 8% of ^{14}C in *L. perenne* and *M. sativa*, respectively, were translocated from storage pools to newly grown shoots. The remobilization was, however, too low to cause significant changes in ^{14}C recovery in the stubble or roots. A greater use of stored C by *M. sativa* can be explained by a faster growth of the new shoots compared to *L. perenne*. However, higher ^{14}C specific activity in newly grown shoots of *L. perenne* indicated a higher use of stored C related to biomass increase compared to *M. sativa.* Since *L. perenne* is more herbivory-tolerant, it is better adapted to the removal of biomass by means of a higher ability to use reserve C as compared to *M. sativa*. A trend for reduced portion of recovered ^{14}C was determined in roots of *L. perenne* but not in its stubbles, indicating remobilization of stored C from roots rather than from the stubble. In contrast, no difference in ^{14}C recoveries was observed between clipped and control treatments, neither in roots nor in stubbles of *M. sativa* (Figs. 2 and 3). The results of *M. sativa* were surprising since no source of the ^{14}C in the new shoot could be found. However, a decrease in reserve C in the root by translocation to the shoots could be counterbalanced by a reduced proportion of reserve C in root respiration (discussed below).

We reviewed several studies focusing on the effects of clipping (simulated grazing) on the portion of C translocated to the newly grown shoots of grassland species (Table 3). Legumes use significantly higher portion of C (10%) for support of the new shoots as compared to grasses (7%). However, the reviewed studies did not allow conclusions about the absolute amount of C reutilization since the amount of stored C was neither measured nor presented. The source of C reutilized by grasses and legumes for shoot regrowth was mainly roots (Table 3). The relative amount of translocated reserve C in newly grown shoots depends on the period after defoliation (Briske et al., 1996). During the first 3 days after defoliation, the most important C source for the elongation and maturation zone is stored C (Schnyder and De Visser, 1999). However, when comparing the reviewed studies, plant species and clipping height is more important than the time of regrowth.

Shading allows the sole investigation of the effect of limited photosynthesis on the redistribution of reserve C, without the effect of C translocation to support shoot regrowth, as is the case after clipping. This study showed that shading reduced the amount of dry matter in above-ground biomass and roots but had no effect on the R:S ratio of *M. sativa* and *L. perenne* (Fig. 1 and Table 2). This indicates that the C stored in shoots and roots was used for maintenance proportional to the weight of the plant parts. A positive relationship between plant biomass and light intensity has also been observed in many other studies (Lambers and Posthumus, 1980; Zagal, 1994). In comparison to clipped plants, plants grown under low light showed a higher R:S ratio and the ^{14}C recovery in roots was higher after shading for *L. perenne*. Thus, clipped plants rely more on translocated C for regrowth compared to shaded plants. ^{14}C specific activity in the above-ground biomass of *L. perenne* was higher after shading compared to control plants and clipped plants (Fig. 3). This is because the lower photosynthesis after shading led to less dilution of ^{14}C by unlabeled assimilates. For *M. sativa*,

Table 3
Review of sources and amounts of C relocated to the newly grown shoots after clipping of legumes and grasses.

Plant species	Approach	Source of retranslocated C	Days after cutting	Clipping height	C portion retranslocated	Reference
Medicago sativa	^{13}C pulse	Roots (taproots, lateral roots), stubble stem	30	6 cm	5%	Avice et al. (1996)
	^{14}C pulse	Roots (stubbles were not measured)	28	5 cm	12%	Ta et al. (1990)
	^{14}C pulse	np[a]	28		19%	Pearce et al. (1969)
	^{14}C continuous	Stubbles and roots		5 cm	9%	Smith and Marten (1969)
	Repeated ^{14}C pulse	Roots	10	8 cm	8%	This study
	^{14}C + ^{13}C continuous	Stubbles	23	5 cm		Crawford et al. (2000)
Medicago truncatula	^{14}C pulse for a single leaf	In the beginning stolons and after 5 days roots	10	Removing of all meristems and leaves	11%	Danckwerts and Gordon (1989)
Trifolium repens		np		Removing of all meristems and all but two leaves	5–6%	
Legumes		Mainly roots			9/9.9	Median/average
Lolium perenne	^{14}C pulse for a single leaf	Stem bases (stubbles)	10	2 cm		Danckwerts and Gordon (1987)
	Repeated ^{14}C pulse	Predominantly stubbles	15	4 cm	2.4–4.7%	Kuzyakov et al. (2002)
	^{13}C continuous	Elongated leaf bases, sheaths of stubble	28	4 cm	1%	Morvan-Bertrand et al. (1999)
	Repeated ^{14}C pulse	Roots	10	4 cm	5%	This study
Panicum maximum	^{14}C continuous	Crowns and roots	19	8 cm		Bushby et al. (1992)
Festuca pratensis	^{14}C + ^{13}C continuous	Stubbles and root	15	1.5 cm	21%	Johansson (1993)
Agropyron–Koeleria association	^{14}C continuous	Roots (stubbles were not measured)	120		6%	Warembourg and Paul (1977)
Grasses		Mainly roots			5/6.8	Median/average

[a]np: data were not presented in the paper.

biomass production and ^{14}C specific activity were less affected by shading compared to *L. perenne*. This indicates a better strategy of *M. sativa* to cope with low light conditions.

4.3. Redistribution of stored C in soil and soil CO_2

Many studies investigated the effect of clipping on root exudation, however, with contradicting results. An increase (Hamilton and Frank, 2001; Paterson and Sim, 1999), no change (Kuzyakov et al., 2002; Murray et al., 2004; Todorovic et al., 1999) or decrease (Mikola and Kytöviita, 2002) of exudation after defoliation have been noted. These differences depend on plant species and methods used in the studies (Mikola and Kytöviita, 2002). Paterson and Sim (1999) measured the release of total organic C and hypothesized that an increase in exudation after defoliation was a consequence of the remobilization of storage compounds in roots, increasing the concentration of diffusible exudates in the root system. In our study, an increased ^{14}C recovery rate, indicating a remobilization of stored C, was only found in the rhizosphere soil under *M. sativa*. This is caused by a higher exudation and/or an increased root senescence. However, this was not found in any of the other investigated soil pools (bulk and rhizosphere soil) under both plants (Fig. 4). The increase in total root exudation lasts only 2 days after defoliation (Paterson et al., 2005), which may explain that no effects were detected 10 days after clipping.

Many authors observed an increase in soil microbial biomass after defoliation (Butenschoen et al., 2008; Guitian and Bardgett, 2000). It is assumed that plants are able to stimulate rhizodeposition to enhance nutrient availability by promoting the activity of microbial populations (Blagodatskaya et al., 2010; Hamilton and Frank, 2001; Lambers et al., 2009). In our study the results of the ^{14}C recovery and the ^{14}C specific activity (data not shown) indicate that there is no effect of clipping on the availability and uptake of plant-stored C by microorganisms (Fig. 4b).

Rhizodeposits are an important driver for soil CO_2 efflux, as their microbial decomposition is an important source for soil CO_2 (Kuzyakov, 2006). After clipping, a decrease in total CO_2 efflux was observed for *L. perenne*, confirming the results from previous studies (Craine et al., 1999; Detling et al., 1979; Kuzyakov et al., 2002). This decrease is caused by reduced root respiration and microbial respiration after clipping (Gavrichkova et al., 2010) and indicates a strong connection between photosynthesis and soil respiration (Kuzyakov and Gavrichkova, 2010). Lower assimilation after clipping leads to less available C for below-ground translocation and thus, reduces soil CO_2 efflux. The unaltered CO_2 efflux under *M. sativa* (Fig. 5) by clipping was unexpected. Like *L. perenne*, a lower CO_2 efflux from soil was assumed due to a lower photosynthesis after clipping. A high energy demand for N_2 fixation by legumes may lead to an increase in root and rhizomicrobial respiration after clipping, diminishing the effect of limited photosynthesis. The ^{14}C specific activity of soil CO_2 increased after clipping of *M. sativa* (Fig. 6). C stored in nodules plays an important role in supporting N_2 fixation after defoliation of *M. sativa* (Ta et al., 1990). Thus, in contrast to *L. perenne*, *M. sativa* showed increased ^{14}C specific activity of the CO_2 efflux after clipping.

In former studies a limited photosynthesis after reduced light intensity decreases root exudation (Hill et al., 2007). This leads to a reduced incorporation of exuded C into microorganisms and decreased microbial growth (Zagal, 1994). In the present study, no change in the ^{14}C specific activities (data not shown) and ^{14}C recoveries of the soil and microbial biomass were observed (Fig. 4).

Root respiration and rhizomicrobial respiration are very closely linked to the supply of assimilates (Kuzyakov and Gavrichkova, 2010). In grassland, shading reduces the soil CO_2 flux by 40% (Craine et al., 1999). Our results also showed a decrease in the CO_2 efflux after shading (Fig. 5). The higher $^{14}CO_2$ efflux (Fig. 6) seems to contradict the decreasing total CO_2 efflux from soil for *L. perenne* and for *M. sativa*. This effect of low light conditions was also observed for wheat and maize (Kuzyakov and Cheng, 2001, 2004). The authors of

these studies explained the effects on the need for recently assimilated C for maintaining respiration, increasing ^{14}C efflux, and also because of the reduced photosynthesis, decreasing the total CO_2 efflux from soil. Our results demonstrate that the respired CO_2 was not only composed of recently assimilated C but also of translocated reserve C (^{14}C). Indeed, respiration of old C was closely related to maintenance, which dominated the respiratory costs when relative growth rate was low, e.g., after shading (Lötscher et al., 2004). Changing respiration regimes (increased maintenance respiration after shading and increased growth respiration after clipping) with their different demands on stored C and newly assimilated C influence the relative amount of reserve C in the root respiration.

5. Conclusions

Limited photosynthesis after clipping or shading alters C allocation in grassland plants. Shading reduced the total biomass of both *L. perenne* and *M. sativa*, whereas the response to clipping was different between the two species. While the biomass of *L. perenne* decreased, the biomass of *M. sativa* increased by regrowth after clipping. The redistribution of reserve C after clipping was governed not only by the lower photosynthesis but also by the C demand for the regrowth of new shoots. In particular, clipping induced a higher demand of reserve C for newly growing shoots. In contrast, only the lower photosynthesis, without the regrowth of shoots, determined the redistribution of reserve C after shading. The main effect after shading was a higher utilization of stored C for maintaining respiration. These differences indicate that the removal of biomass after clipping is more important for the translocation of stored C than limited photosynthesis.

The CO_2 efflux from soil declined by *L. perenne* after shading. The decrease of the CO_2 efflux is more pronounced after clipping compared to shading because of a higher C demand for the newly growing shoots. For *M. sativa*, a decrease in soil CO_2 efflux was observed only after shading but not after clipping. This indicates that the non-legume *L. perenne* and the legume *M. sativa* have different mechanisms to cope with clipping. While *L. perenne* uses stored C mainly for shoot regrowth, *M. sativa* has also a high C demand for N_2 fixation compared to the nutrient uptake of non-legumes.

The results show that C storage by plants is a very important mechanism to overcome stress periods like grazing or limited light availability. This C can be useful to recover from the removal of biomass by supporting the regrowth of new shoots or to obtain vital functions like respiration or the N_2 fixation of legumes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2012.12.015.

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