Plant Soil
DOI 10.1007/s11104-012-1536-5

REGULAR ARTICLE

C and N allocation in soil under ryegrass and alfalfa estimated by ^{13}C and ^{15}N labelling

Andreas Schmitt · Johanna Pausch · Yakov Kuzyakov

Received: 14 May 2012 / Accepted: 15 November 2012

Abstract
Background and Aims Below-ground translocated carbon (C) released as rhizodeposits is an important driver for microbial mobilization of nitrogen (N) for plants. We investigated how a limited substrate supply due to reduced photoassimilation alters the allocation of recently assimilated C in plant and soil pools under legume and non-legume species.
Methods A non-legume (*Lolium perenne*) and a legume (*Medicago sativa*) were labelled with ^{15}N before the plants were clipped or shaded, and labelled twice with $^{13}CO_2$ thereafter. Ten days after clipping and shading, the ^{15}N and ^{13}C in shoots, roots, soil, dissolved organic nitrogen (DON) and carbon (DOC) and in microbial biomass, as well as the ^{13}C in soil CO_2 were analyzed.
Results After clipping, about 50 % more ^{13}C was allocated to regrowing shoots, resulting in a lower translocation to roots compared to the unclipped control. Clipping also reduced the total soil CO_2 efflux under both species and the ^{13}C recovery of soil CO_2 under *L. perenne*. The ^{15}N recovery increased in the shoots of *M. sativa* after clipping, because storage compounds were remobilized from the roots and/or the N uptake from the soil increased. After shading, the assimilated ^{13}C was preferentially retained in the shoots of both species. This caused a decreased ^{13}C recovery in the roots of *M. sativa*. Similarly, the total soil CO_2 efflux under *M. sativa* decreased more than 50 % after shading. The ^{15}N recovery in plant and soil pools showed that shading has no effect on the N uptake and N remobilization for *L. perenne,* but, the ^{15}N recovery increased in the shoot of *M. sativa*.
Conclusions The experiment showed that the dominating effect on C and N allocation after clipping is the need of C and N for shoot regrowth, whereas the dominating effect after shading is the reduced substrate supply for growth and respiration. Only slight differences could be observed between *L. perenne* and *M. sativa* in the C and N distribution after clipping or shading.

Keywords Carbon allocation · N allocation · Isotope labeling · Grazing effects · N_2 fixation

Responsible Editor: Eric Paterson.

A. Schmitt · J. Pausch · Y. Kuzyakov
Department of Agroecosystem Research, BayCEER,
University of Bayreuth,
Universitätstr. 30,
95440 Bayreuth, Germany

J. Pausch (✉) · Y. Kuzyakov
Department of Soil Science of Temperate Ecosystems,
University of Göttingen,
Büsgenweg 2,
37077 Göttingen, Germany
e-mail: j.pausch@gmx.de

Introduction

Below-ground translocation of carbon (C) by plants and its turnover are important drivers for ecological processes and functions in soil. These include nutrient availability for plants, microbe activity and turnover,

Published online: 04 December 2012

or the turnover of soil organic matter (SOM) (Merbach et al. 1999; Blagodatskaya et al. 2010). The amount of C allocation by plants into the soil is affected by many factors such as plant development (Gregory and Atwell 1991; Meharg and Killham 1990), nutrient availability (Merckx et al. 1987) or plant species and plant functional groups (Warembourg et al. 2003). Since symbiotic N_2 fixation requires abundant energy, legumes have a higher demand for the assimilated C for rhizosphere respiration than grasses and non-legume forbs (Phillips 1980; Vance and Heichel 1991; Warembourg et al. 2003).

For grasses, rhizodeposition is an important process affecting N availability and N uptake (Frank and Groffman 2009). Rhizodeposits enhance N mobilization by stimulating microbial activity and SOM degradation; this is termed as the 'priming effect' (Kuzyakov 2002). Thus, we expect that alterations in the amount of C translocated below-ground will trigger different responses in the N uptake between legumes and non-legumes.

The fast translocation of assimilates below-ground indicates a strong connection between current photosynthesis and root exudation (Gregory and Atwell 1991; Cheng et al. 1993; Kuzyakov et al. 1999; Jones et al. 2004). Hence, any change in photosynthetic activity will affect the turnover processes in the rhizosphere and thus influence N availability for plants (Kuzyakov 2002).

In this study we manipulated the photosynthetic activity by clipping or shading. After clipping (simulated grazing), photosynthesis is reduced due to a smaller leaf area (Detling et al. 1979). Clipped plants can meet their C supply for regrowth by remobilizing stored C from roots or from remaining shoot parts (Avice et al. 1996; Johansson 1993). Despite the demand for C for regrowth, root exudation after clipping was higher in many studies (Paterson and Sim 1999; 2000), however, also a reduced root exudation was found (Augustine et al. 2011). Some authors suggest that, besides C reserves, the remobilization of organic N compounds stored in roots or stubbles—such as amino acids or vegetative storage proteins—is also important for regrowth after clipping (Volenec et al. 1996).

In contrast, shading reduced the photosynthesis rate only at a lower light availability, without the removal of shoots. Like after clipping, C is preferentially allocated in above-ground plant parts after shading, as indicated by a decrease of the R:S ratio in *Lolium perenne* (Lambers and Posthumus 1980). Consequently, shading leads to less rhizodeposition (Hill et al. 2007). Thus, based on the different effects of clipping and shading on rhizodeposition, and based on the high demand of N for regrowth after clipping, we hypothesize that clipping enhances N uptake by plants, whereas shading reduces it.

Using repeated $^{13}CO_2$ labelling of two plant species, a legume (*Medicago sativa*) and a non-legume plant (*Lolium perenne*), we investigated how a limited substrate supply after clipping and shading affected the C allocation within the plant and the below-ground C translocation. Labelling with $^{15}NO_3^-$ was carried out to investigate how the altered C allocation after limited assimilate supply affects N remobilization and N uptake by both plant species. The specific questions were:

(1) How does a limited substrate supply affect plant biomass production and alter the distribution of C in plant, soil, microorganisms and CO_2 efflux from soil?
(2) How does a limited assimilate supply affect the remobilization of plant-stored N?
(3) How does the effect of a limited substrate supply affect the N uptake by plants from soil?
(4) Do shading and clipping induce different responses with respect to the distribution of C and N in the plant and soil pools?

Materials and methods

Soil properties and plant growing conditions

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

The seedlings of ryegrass (*Lolium perenne* L.) and alfalfa (*Medicago sativa* L.) were first germinated on wet filter paper for 5 (*M. sativa)* and 8 days (*L. perenne*) and thereafter transferred to the plant pots (inner diameter 7 cm, height 20 cm), each of them filled with 700 g of air-dried, sieved (≤ 2 mm) soil. In each pot, 3 seedlings of *M. sativa* or 5 seedlings of *L. perenne* were transferred to achieve a similar biomass for both plant species. The pots were closed with a

Table 1 Basic characteristics of the soil sampled from the A_p horizon of a haplic Luvisol originated from loess near Göttingen (Germany). CEC: Cation Exchange Capacity; BS: Base saturation. [1]Texture according to the German classification system (KA5 2005; 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[1] clay/silt/sand (% (w/w))	7.0/87.2/5.8
pH (H_2O)	6.6
pH ($CaCl_2$)	6.0

plastic lid with holes for shoots. The plants were grown at 26 to 28 °C day temperature and at 22 to 23 °C night temperature. At a day length of 14 h the light intensity was approximately 210 $\mu mol\,m^{-2}s^{-1}$, approximately corresponding to a cumulative daily radiation in the range of field conditions. The soil moisture was maintained at 70 % of the available field capacity by daily watering with distilled water.

^{13}C and ^{15}N labelling

To label the soil of all pots with ^{15}N, 16 mg of $K^{15}NO_3$ (enrichment: 52.7 at.%) were dissolved in water and added to the pots with the watering (28 days after planting).

The ^{13}C labelling was conducted for the first time 50 days after planting (the day of clipping or beginning of shading). One day before ^{13}C labelling, all pots were sealed with silicone paste (NG 3170, Thauer & Co., Dresden). All plants were labelled in a Plexiglas chamber as described by Werth and Kuzyakov (2008). Briefly, $^{13}CO_2$ was introduced to the chamber by circulating air through a flask containing 150 mg of $Na_2{}^{13}CO_3$ (^{13}C enrichment: 99.9 atm. %) for labelling of *L. perenne* or 15 mg of the same $Na_2{}^{13}CO_3$ for *M. sativa* solved in 10 ml deionized water. To produce $^{13}CO_2$, an excess of 5*M* H_2SO_4 was added to the $Na_2{}^{13}CO_3$ solution. The plants were labelled in the $^{13}CO_2$ enriched atmosphere for 3 h. Before opening the labelling chamber, the chamber air was pumped through 1*M* NaOH solution to remove unassimilated $^{13}CO_2$. Since the amount of ^{13}C found in the NaOH solution was negligible, it can be assumed, that all $^{13}CO_2$ was assimilated. Then the chamber was opened and the trapping of CO_2 evolved from the soil started. ^{13}C labelling was repeated on day 55 after planting.

Clipping and shading

Three pots of each plant species were used for the clipping procedure or exposed to shading. Additionally, three pots of each plant species were grown under normal conditions as a control treatment. The plants were clipped or shaded 2 h before the first $^{13}CO_2$ pulse. *Lolium perenne* shoots were clipped 4 cm above the soil surface, those of *M. sativa* 8 cm above the surface. Due to the different clipping heights, both plant species achieve similar stubble biomass. The clipped plants continued growth under the conditions described above. For shading, the light intensity was reduced to about 17 $\mu mol\,m^{-2}s^{-1}$ for 10 days.

Sampling and analysis

Starting after the first labelling, the CO_2 evolved from soil was trapped using a closed-circulating system. The air was pumped through tubes containing 15 ml of 1*M* NaOH solution. Because of the circulation there were no losses of CO_2 due to incomplete absorption by NaOH solution. The NaOH solution was changed 1, 3 and 5 days after each labelling. The pots were destructively harvested at day 60 after planting. Roots were separated from soil by handpicking. Plant and soil material was dried at 65 °C for 3 days.

To estimate total CO_2 efflux, the C content of the NaOH solution was determined by titration with 0.01 *M* HCl against phenolphthalein after adding 1.5*M* $BaCl_2$ solution. For ^{13}C measurements the CO_2 trapped in NaOH was precipitated as $SrCO_3$ with an excess of 0.5*M* $SrCl_2$ solution. The precipitants were centrifuged at 3800 g, washed with deionized water until the pH reached neutral conditions and dried at 65 °C.

Microbial biomass C and N was determined by the chloroform fumigation-extraction-method (CFE) (modified after Vance et al. 1987). For this, the soil was separated into two samples with 5 g each. One of these samples was firstly fumigated with chloroform for 24 h. Both samples were extracted with 20 ml of

0.05 *M* K_2SO_4, shaken for 1 h and, thereafter, centrifuged for 10 min at 3800 g. Total C and N contents of fumigated and non-fumigated soil extracts were measured using a N/C analyzer (Multi N/C 2100, AnalytikJena, Germany). The extracts of the non-fumigated samples were used to measure dissolved organic carbon (DOC) and dissolved organic nitrogen (DON). For the determination of ^{13}C and ^{15}N in the microbial biomass, DOC and DON the extracts were oven-dried at 60 °C and measured as described below.

The ground plant and soil material (ball mill), the $SrCO_3$ and the dried extracts of the CFE were analyzed for their ^{13}C and ^{15}N isotope ratios. This was done using an elemental analyzer NC 2500 (CE Instruments, Milano, Italy) linked to a delta plus gas-isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) via a ConFlo III (Thermo Fisher Scientific, Bremen, Germany) interface.

Calculations and statistics

The ^{13}C enrichment of a particular C pool ($^{13}C_{excess;p}$; $\mu g\,g^{-1}$) was calculated as follows:

$$^{13}C_{excess;p} = \left(^{13}C_p - ^{13}C_{NA;p}\right) \cdot C_p \quad (1)$$

where $^{13}C_{NA;p}$ is the ^{13}C natural abunxdance of the respective pool (atom%), $^{13}C_p$ is the amount of ^{13}C of the pool after labelling (atom%), and C_p is the total amount of C in this pool ($\mu g\,g^{-1}$).

The ^{13}C recovery in a particular C pool ($^{13}C_{rec;p}$; %) was calculated by dividing the amount of ^{13}C (mg) of that particular pool (^{13}C enrichment multiplied by the pool mass (mg)) by the sum of the ^{13}C amount (mg) of all pools (shoot, root, soil, DOC. soil microbial biomass and soil CO_2):

$$^{13}C_{rec;p} = \frac{^{13}C_{excess;p} \times mass_p}{\sum {}^{13}C_{excess;p} \times mass_p} \times 100 \quad (2)$$

To determine the $\delta^{13}C$ value of microbial biomass ($\delta^{13}C_{MB}$; ‰) a mass balance equation was used:

$$\delta^{13}C_{MB} = \frac{\delta^{13}C_{fum} \cdot C_{fum} - \delta^{13}C_{nf} \cdot C_{nf}}{C_{fum} - C_{nf}} \quad (3)$$

where $\delta^{13}C_{fum}$(‰) and $\delta^{13}C_{nf}$ (‰) are the $\delta^{13}C$ values of the fumigated and non-fumigated samples, respectively, and C_{fum} (mg) and C_{nf} (mg) are the amounts of C in the fumigated and non-fumigated samples, respectively.

The calculations for ^{15}N correspond to those for ^{13}C.

The experiment was conducted with 3 replicates for all treatments. The values presented in the figures and tables are given as means±standard errors of the means (±SEM). Significant differences between the treatment and the plant species were obtained by a two-factor analysis of variance (ANOVA) in combination with a post hoc Fisher LSD test.

Results

Plant biomass production

M. sativa produced significantly more shoot biomass per plant than *L. perenne* during 60 days (Tab. 2). Clipping has no effects on the shoot and root biomass of *M. sativa* and *L. perenne* when measured after 10 days of regrowth (Tab. 2). Ten days of shading were also not sufficient to decrease the shoot or root biomass of both species. The R:S ratio decreased after clipping and shading of *L. perenne*, whereas it increased for *M. sativa* after clipping and slightly after shading (Tab. 2).

Effect of clipping and shading on ^{13}C distribution in plant and soil

In the control treatments of *L. perenne* and *M. sativa*, about 50 % of ^{13}C were recovered in shoots; 30 % and 20 % were found in the roots of *L. perenne* and *M. sativa*, respectively (Fig. 1). The ^{13}C recovery in CO_2 efflux, the soil, microbial biomass and DOC did not differ between both plant species (Fig. 2).

Clipping increased the ^{13}C recovery in the shoot by about 30 % and 20 % for *L. perenne* and *M. sativa*, respectively. The retention of newly assimilated C (^{13}C) in the shoots resulted in a lower translocation to the roots, and thus, the ^{13}C recovery of the roots of both plant species was lower compared to the respective control (Fig. 1). However, the retention of ^{13}C in the shoots after clipping had no effects on the ^{13}C recovery in the soil (Fig. 2). Also, all other belowground C pools of both plant species were not affected by clipping (Fig. 2).

Shading increased the ^{13}C recovery in the shoots of *L. perenne* and *M. sativa* (Fig. 1). The ^{13}C recovery was reduced only in the roots of *M. sativa* (Fig. 1).

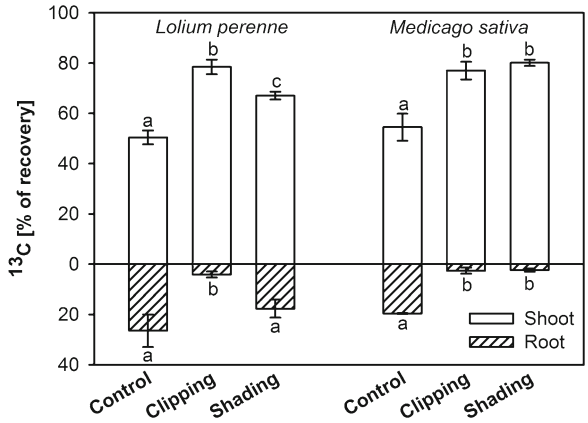

Fig. 1 ^{13}C recovery (± SEM) in shoots and roots 10 days after clipping or beginning of shading of 60-day-old *L. perenne* and *M. sativa.* Significant differences are marked by different letters ($p<0.05$)

Like after clipping, the ^{13}C recovery in the soil, microbial biomass and DOC was not affected by shading (Fig. 2).

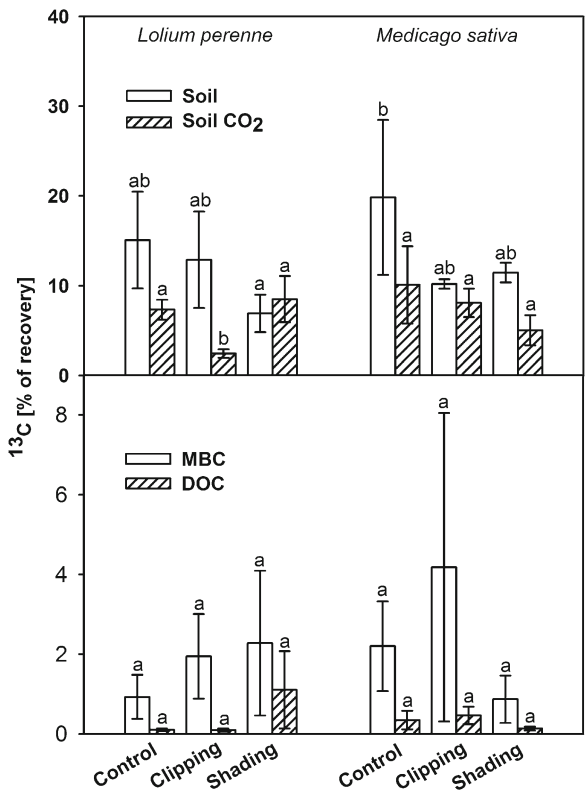

Fig. 2 ^{13}C recovery (± SEM) in the soil and in soil CO_2 (*top*), and in DOC and microbial biomass (*bottom*) under *L. perenne* and *M. sativa* 10 days after clipping and beginning of shading. Significant differences are marked by different letters ($p<0.05$)

Effect of clipping and shading on total CO_2 and ^{13}C efflux from soil

The total CO_2 efflux from soil was significantly higher under *M. sativa* than under *L. perenne* (Fig. 3); this indicates the higher C demand in legume roots. Both treatments for reduced C assimilation decreased the CO_2 efflux from soil under *L. perenne*. This reflects the limited substrate availability, whereby the CO_2 reduction was significant only after clipping at the end of the experiment (Fig. 3). Under *M. sativa*, clipping and shading significantly decreased the soil CO_2 efflux (Fig. 3). After clipping, however, this reduced CO_2 efflux from soil lasted only until day 5. Contrary to *L. perenne*, the soil CO_2 efflux under *M. sativa* was lowest after shading (Fig. 3).

Clipping also significantly reduced the ^{13}C recovery of the soil CO_2 efflux under *L. perenne*, because ^{13}C was used for shoot regrowth (Fig. 2). Shading had no effect on the ^{13}C recovery in CO_2 under *L. perenne*.

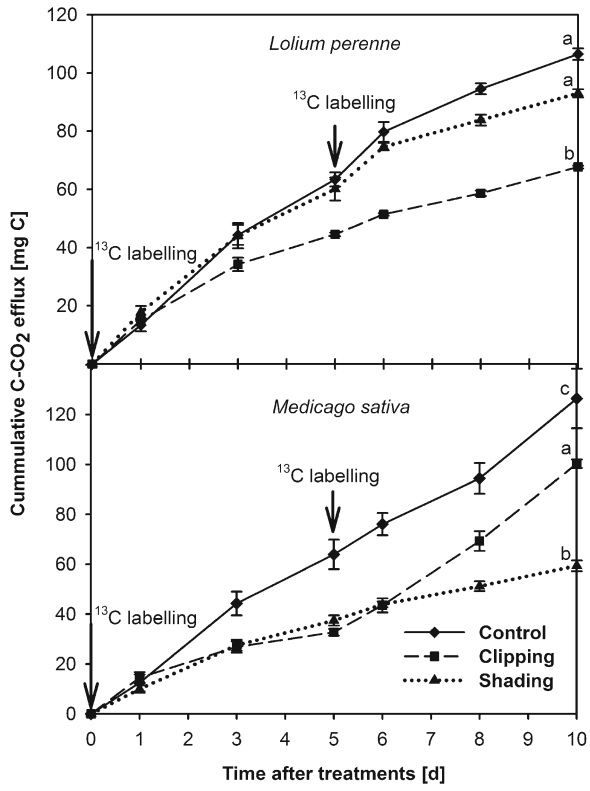

Fig. 3 Cumulative CO_2 efflux from soil (± SEM) under *L. perenne* (*top*) and *M. sativa* (*bottom*) beginning at clipping or start of shading and the effect of clipping and shading on the CO_2 efflux. Significant differences at the end of the experiment are marked by different letters ($p<0.05$)

The ^{13}C recovery of the soil CO_2 efflux under *M. sativa* was not affected by clipping or shading (Fig. 2).

Distribution of ^{15}N in plant and soil

Under normal light conditions a higher ^{15}N recovery was detected for the shoots of *L. perenne* compared to *M. sativa* (Fig. 4). In the roots, the ^{15}N recovery showed no significant differences between *M. sativa* and *L. perenne* (Fig. 4).

Clipping increased the ^{15}N recovery only in the shoots of *M. sativa*, but had no effect on the ^{15}N recovery in the roots of both plant species (Fig. 4). Also the ^{15}N recovery in the soil, DON and microbial biomass N was unaffected by clipping (Fig. 5).

The ^{15}N recovery in the shoots and roots of *L. perenne* was not affected by shading, however, it increased in the shoots of *M. sativa* (Fig. 4). In the soil, the DON and the microbial biomass under both plant species, shading showed no influence on the ^{15}N recovery (Fig. 5).

Discussion

Effect of plant species

The distribution of ^{13}C between above- and below-ground pools in the control treatment was similar for *L. perenne* and *M. sativa*, with about one half of the labelled assimilates being incorporated in the shoots (Fig. 1). This is in the range of earlier studies, reviewed

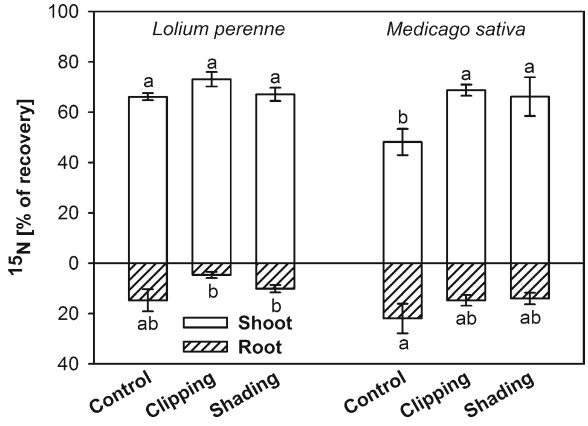

Fig. 4 ^{15}N recovery (± SEM) in shoots and roots 10 days after clipping or beginning of shading of 60-day-old *L. perenne* and *M. sativa*. Significant differences are marked by different letters ($p<0.05$)

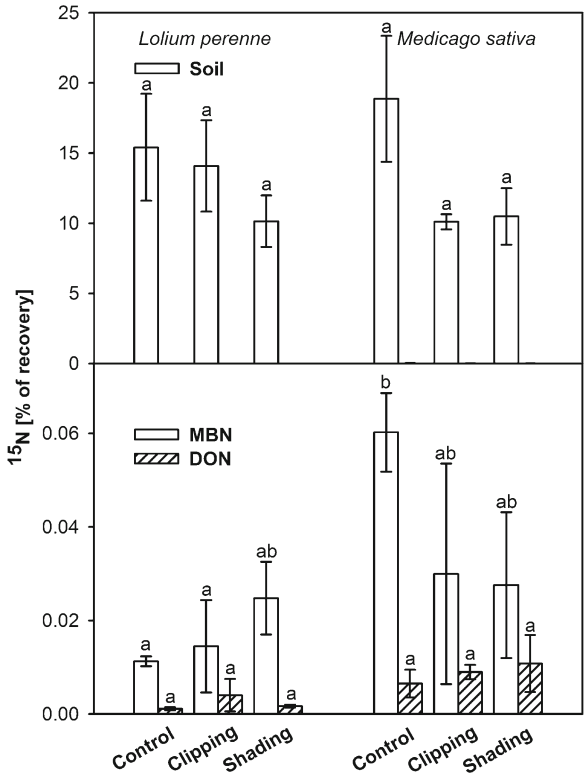

Fig. 5 ^{15}N recovery (± SEM) in soil (*top*), and in DON and microbial biomass (*bottom*) of *L. perenne* and *M. sativa* 10 days after clipping or beginning of shading. Significant differences are marked by different letters ($p<0.05$)

by Kuzyakov and Domanski (2000). The roots of *L. perenne* recovered more ^{13}C than *M. sativa*, whereas the portion of ^{13}C found in the soil CO_2 was higher under *M. sativa* (Figs. 1 and 2). A higher incorporation of assimilated C was found in the roots of the legume *Trifolium repens* compared to the roots of *L. perenne* (de Neergaard and Gorissen 2004), however, in our study there was no difference between the legume species and *L. perenne*. A higher total CO_2 efflux from the soil was found under *M. sativa* compared to *L. perenne*, indicating a high energy need for N_2 fixation.

Effect of clipping

After clipping, both species preferentially allocated ^{13}C in the above-ground biomass as shown by an increased ^{13}C recovery in shoots (Fig. 1). Recent studies observed an increased above-ground C allocation after clipping (Kuzyakov et al. 2002; Detling et al. 1979; Mackie-Dawson 1999). The assumption is that regrowing shoots retain photosynthates and prevent a

translocation below-ground (Mackie-Dawson 1999). This agrees with our results of less ^{13}C recovery in the roots of both plants after clipping (Fig. 1).

Especially on the first days after clipping, the remobilization of storage compounds is the major substrate supply for the regrowing shoots, including N compounds (Morvan-Bertrand et al. 1999; Ourry et al. 1988). This is confirmed by the higher post-clipping ^{15}N recovery in the shoots of *M. sativa* in our study (Fig. 4). The re-translocation of root N contributes substantially to the synthesis of amino acids and proteins in the regrowing tissue of *M. sativa* (Avice et al. 1996). In our study there were no indications for a retranslocation of N compounds from roots to shoots of *M. sativa*, since there was no significant decrease of the ^{15}N recovery in the roots. However, the design of our experiment does not allow us to make any predictions about a possible retranslocation of N which is taken up by N_2 -Fixation.

It is likely that the reduced C translocation to roots has implications for root respiration and rhizodeposition, as well as for ^{13}C incorporation in soil and availability for soil microorganisms. However, the unaffected ^{13}C recovery in the soil shows that exudation of newly assimilated C did not change after clipping because of assimilate retention in the shoots. The increased rhizodeposition found in earlier studies (e.g. Bardgett et al. 1998) may reflect remobilization of storage compounds in roots, which would increase the release of stored C in the soil (Paterson and Sim 1999). Our ^{13}C results, however, provide no information about the total rhizodeposition and the release of stored C. Former studies showed that an increased rhizodeposition has a positive effect on microbial activity, stimulates N cycling and thus enhances N availability for plant roots after defoliation (Guitian and Bardgett 2000; Hamilton and Frank 2001). It can be expected that this would lead to a reduced ^{15}N recovery in the soil, however, the high variability of the results of our results makes it impossible to see these effect.

The assimilate supply is a major factor affecting root respiration (Gavrichkova et al. 2010). A reduced soil CO_2 efflux after clipping, as observed for *L. perenne* (Fig. 3), was also found in many other studies (Detling et al. 1979; Craine et al. 1999; Kuzyakov et al. 2002). Since the ^{13}C recovery in microbial biomass and DOC under *L. perenne* did not change after clipping (Fig. 2), it can be concluded that these pools were not affected by clipping. Thus, the decrease in soil CO_2 can be ascribed to a reduced root respiration of current assimilates rather than reduced microbial respiration.

The soil processes under the legume *M. sativa* differed from those under *L. perenne*. The total CO_2 efflux under *M. sativa* decreased until day 5 after clipping and, thereafter, recovered and was approximately at the same level as observed in the control pots (Fig. 3). In the same time the ^{13}C recovery of the CO_2 efflux remained. Thus, the portion of newly assimilated C in the soil CO_2 is increasing after clipping. This corresponds with findings that newly assimilated C is closely related to growth respiration (Lötscher et al. 2004), which is important after clipping for the biomass production. The increasing CO_2 efflux after 5 days may point to enhanced nodule respiration to restore the N_2 fixation.

We conclude that high C and N demands of regrowing shoots after clipping led to a remobilization of N to the shoots and additionally, recently assimilated C was retained in the regrowing shoots.

Effect of shading

We implemented shading (besides clipping) to evaluate the effect of a limited substrate supply on the distribution of recently assimilated C and the impacts of such a limited supply on the N budget in plant and soil. In contrast to clipping, however, the effect of shading in limiting the substrate supply is not connected with the high demand for reserve C and N for shoot regrowth. The R:S ratio of *L. perenne* was reduced after shading (Table 2). The increased preference for shoot versus root growth is also reflected by the higher recovery of currently assimilated C (^{13}C) in the shoots. After shading, more assimilates are allocated into the terminal meristems to compensate for the reduced photosynthesis rate (Ryle and Powell 1976). For *M. sativa* the ^{13}C recovery in the shoots was very high after shading and was in the range of the clipped plants. Like after clipping, this took place at the expense of the ^{13}C translocation into the roots, however, this is significant only for *M. sativa*.

Below-ground translocation of C is very closely linked to the assimilate supply (Kuzyakov and Gavrichkova 2010). Reduced soil CO_2 efflux and rhizodeposition have been observed after shading (Craine et al. 1999; Hill et al. 2007). The present study indicates

Table 2 Plant biomass (± SEM) and root-to-shoot ratio (R:S) (± SEM) of *L. perenne* and *M. sativa* 10 days after clipping or shading. Significant differences are marked by different letters ($p<0.05$)

		Biomass [g plant^{-1}]				R:S
		Shoot	Clipped Shoot	Total Above-ground	Root	
Lolium perenne	Control	0.36±0.02^{ac}		0.36±0.02^{ad}	0.38±0.02^{ab}	1.08±0.09
	Clipped	0.12±0.01^{a}	0.13±0.02	0.25±0.03^{a}	0.23±0.16^{a}	1.04±0.77
	Shaded	0.24±0.01^{a}		0.24±0.01^{a}	0.21±0.07^{a}	0.88±0.26
Medicago sativa	Control	0.67±0.10^{b}		0.67±0.10^{bc}	0.59±0.25^{ab}	0.82±0.30
	Clipped	0.43±0.15^{b}	0.45±0.06	0.88±0.21^{b}	0.78±0.18^{b}	1.09±0.46
	Shaded	0.52±0.03^{abc}		0.52±0.03^{ac}	0.44 ±0.07^{ab}	0.85±0.17

that the shading effect on the CO_2 efflux from soil of currently assimilated C depends on the plant species.

For *M. sativa* the total soil CO_2 efflux decreased, whereas the portion of ^{13}C in CO_2 was not influenced by shading (Figs. 2 and 3). These apparently contradictory results can be explained by the need for recently assimilated C to maintain respiration (shown by the unchanged ^{13}C efflux) and by the reduced substrate supply (decreasing the total CO_2 efflux from soil) (Kuzyakov and Cheng 2001; 2004). Contrary, for *L. perenne*, the total CO_2 efflux and the ^{13}C recovery in the CO_2 did not change after shading.

Plants grown under normal light conditions have a higher N demand compared to shaded plants, which can be met by a higher rhizodeposition and the resulting SOM decomposition (Frank and Groffman 2009). The growth after shading is restricted by low assimilation rates (Shipley 2002), which also reduces the demand for N in the shoots. Moreover, under shaded conditions a reduced rhizodeposition causes a decreased turnover of the microbial biomass and SOM and, thus, a lower N mineralization (Zagal 1994). In our study no change of the ^{13}C recovery in the soil of both plants and no change of the ^{15}N recovery in the shoots of *L. perenne* was observed after shading. Thus, our results show no effect of shading on the rhizodeposition or the N uptake by this species. The unchanging ^{13}C recovery at a concurrent decreasing of the total CO_2 efflux underlines the importance of recently fixed C for the legume *M. sativa*. *M. sativa* uses recently fixed C for nodule respiration and stored C for root respiration (Avice et al. 1996). The decreased CO_2 efflux, however, indicates overall that the nodule respiration and the root respiration were reduced. It was expected that *M. sativa* would remobilize storage N from roots to overcome this limitation of the N supply to shoots, since remobilization requires less energy than N fixation and can thus be an adequate mechanism to meet the N demand in the shoots (Bakken et al. 1998). The increased ^{15}N recovery in the shoots of shaded *M. sativa* may be due to a reduced uptake of unlabelled N by the N_2 fixation after shading. However, our results cannot clarify if the origin of the increased recovery of ^{15}N in the shoots is the remobilization of N from roots or a higher ^{15}N uptake from soil. Both pools show a decrease of ^{15}N after shading, however for both this decrease was not significant.

We conclude that shading has a pronounced effect on the below-ground allocation of currently assimilated C for both plant species; on the other hand shading has effects on the N distribution only for *M. sativa* with a higher allocation of N in the shoots. However the origin of this N remains unclear.

Conclusion

After clipping, shoot regrowth is an important sink affecting the C distribution of newly assimilated C. To meet the demand of N for regrowth, the legume *M. sativa* increased the N allocation in the shoots. We assume that this is supported by a higher N uptake by the roots. The N pools in *L. perenne* were not affected by clipping. After shading, more C was allocated above-ground compared to normal light conditions leading to reduced translocation of assimilates in the roots of *M. sativa*. An increased need for N after shading was observed for the shoots of *M. sativa*, but the source of this N remains unclear. The results

indicates that the allocation of recently assimilated C in plants and its translocation below-ground is strongly influenced by the altered substrate supply after clipping and shading. However, the reduced assimilation is of minor importance for the N distribution.

Acknowledgments We are grateful to Dr. Martina Gocke for her comments on the earlier version of the manuscript. Financial support for this work was provided by the German Research Foundation (DFG).

References

Augustine DJ, Dijkstra FA, Hamilton EW III, Morgan JA (2011) Rhizosphere interactions, carbon allocation, and nitrogen acquisition of two perennial North American grasses in response to defoliation and elevated atmospheric CO2. Oecologia 165:755–770

Avice JC, Ourry A, Lemaire G, Boucaud J (1996) Nitrogen and carbon flows estimated by ^{15}N and ^{13}C pulse-chase labeling during regrowth of alfalfa plant. Plant Physiol 112:281–290

Bakken AK, Macduff JH, Collison M (1998) Dynamics of nitrogen remobilization in defoliated *Phleum pratense* and *Festuca pratensis* under short and long photoperiods. Physiol Plant 103:426–436

Bardgett RD, Wardle DA, Yeates GW (1998) Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biol Biochem 30:1867–1878

Blagodatskaya E, Blagodatsky S, Dorodnikov M, Kuzyakov Y (2010) Elevated atmospheric CO_2 increases microbial growth rates in soil: results of three CO_2 enrichment experiments. Glob Chang Biol 16:836–848

Cheng W, Coleman DC, Carroll CR, Hoffman CA (1993) In situ measurement of root respiration and soluble C concentrations in the rhizosphere. Soil Biol Biochem 25:1189–1196

Craine FM, Wedin DA, Chapin FS III (1999) Predominance of ecophysiological controls on soil CO_2 flux in a Minnesota grassland. Plant Soil 207:77–86

de Neergaard A, Gorissen A (2004) Carbon allocation to roots, rhizodeposits and soil after pulse labelling: a comparison of white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.). Biol Fertil Soils 39:228–234

Detling JK, Dyer MI, Winn DT (1979) Net photosynthesis, root respiration, and regrowth of *Bouteloua gracilis* following simulated grazing. Oecologia 41:127–134

Frank DA, Groffman PM (2009) Plant rhizospheric N processes: what we don't know and why we should care. Ecology 90:1512–1519

Gavrichkova O, Moscatelli MC, Kuzyakov Y, Grego S, Valentini R (2010) Influence of defoliation on CO_2 efflux from soil and microbial activity in a Mediterranean grassland. Agr Ecosyst Environ 136:87–96

Gregory PJ, Atwell BJ (1991) The fate of carbon in pulse labelled crops of barley and wheat. Plant Soil 136:205–213

Guitian R, Bardgett RD (2000) Plant and soil microbial responses to defoliation in temperate semi-natural grassland. Plant Soil 220:271–277

Hamilton EW, Frank DA (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82:2397–2402

Hill P, Kuzyakov Y, Jones D, Farrar J (2007) Response of root respiration and root exudation to alterations in root C supply and demand in wheat. Plant Soil 291:131–141

Johansson G (1993) Carbon distribution in grass (*Festuca pratensis* L.) during regrowth after cutting-utilization of stored and newly assimilated carbon. Plant Soil 151:11–20

Jones DL, Hodge A, Kuzyakov K (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480

KA5 (2005) Bodenkundliche Kartieranleitung.Ad-Hoc-Arbeitsgruppe Boden:5. verbesserte und erweiterte Auflage, ISBN 978-3-510-95920-4, E. Schweizerbart Science Publishers

Kramer S, Marhan S, Ruess L, Armbruster W, Butenschoen O, Haslwimmer H, Kuzyakov Y, Pausch J, Schoene J, Scheunemann N, Schmalwasser A, Totsche KU, Walker F, Scheu S, Kandeler E (2012) Carbon flow into microbial and fungal biomass as a basis to understand the belowground food web in an agroecosystem. Pedobiologia, in press

Kuzyakov Y (2002) Review: factors affecting rhizosphere priming effects. J Plant Nutr Soil Sci 16:382–396

Kuzyakov Y, Cheng W (2001) Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biol Biochem 33:1915–1922

Kuzyakov Y, Cheng W (2004) Photosynthesis controls of CO_2 efflux from maize rhizosphere. Plant Soil 263:85–99

Kuzyakov Y, Domanski G (2000) Carbon inputs into soil. Review. J Plant Nutr Soil Sci 163:421–423

Kuzyakov Y, Gavrichkova O (2010) Time lag between photosynthesis and carbon dioxide efflux from soil: a review. Glob Chang Biol 16:3386–3406

Kuzyakov Y, Kretzschmar A, Stahr K (1999) Contribution of Lolium perenne rhizodeposition to carbon turnover of pasture soil. Plant Soil 213:127–136

Kuzyakov Y, Biryukova OV, Kuznetzova TV, Molter K, Kandeler E, Stahr K (2002) Carbon partitioning in plant and soil carbon dioxide fluxes and enzyme activities as affected by cutting ryegrass. Biol Fertil Soils 35:348–358

Lambers H, Posthumus L (1980) The effect of light intensity and relative humidity on growth rate and root respiration of *Plantago lanceolata* and *Zea mays*. J Exp Bot 31:1621–1630

Lötscher M, Klumpp K, Schnyder H (2004) Growth and maintenance respiration for individual plants in hierarchically structured canopies of *Medicago sativa* and *Helianthus annuus*: the contribution of current and old assimilates. New Phytol 164:305–316

Mackie-Dawson LA (1999) Nitrogen uptake and root morphological responses of defoliated *Lolium perenne* (L.) to a heterogeneous nitrogen supply. Plant Soil 209:111–118

Meharg AA, Killham K (1990) Carbon distribution within the plant and rhizosphere in laboratory and field grown *Lolium*

perenne at different stages of development. Soil Biol Biochem 22:471–477

Merbach W, Mirus E, Knof G, Remus R, Ruppel S, Russow R, Gransee A, Schulze J (1999) Release of carbon and nitrogen compounds and their possible ecological importance. J Plant Nutr Soil Sci 162:373–383

Merckx R, Dijkstra A, Hartog A, Veen JA (1987) Production of root-derived material and associated microbial growth in soil at different nutrient levels. Biol Fertil Soils 5:126–132

Morvan-Bertrand A, Pavis J, Boucaud J, Prud´homme MP (1999) Partitioning of reserve and newly assimilated carbon in roots and leaf tissues of *Lolium perenne* during regrowth after defoliation: assessment by ^{13}C steady-state labelling and carbohydrate analysis. Plant Cell Environ 22: 1097–1108

Ourry A, Boucaud J, Salette L (1988) Nitrogen mobilization from stubble and roots during re-growth of defoliated perennial ryegrass. J Exp Bot 39:803–809

Paterson E, Sim A (1999) Rhizodeposition and C partitioning of *Lolium perenne* in axenic culture affected by nitrogen supply and defoliation. Plant Soil 216:155–164

Paterson E, Sim A (2000) Effect of nitrogen supply an defoliation on loss of organic compounds from roots of *Festuca rubra*. J Exp Bot 51:1449–1457

Phillips DA (1980) Efficiency of symbiotic nitrogen fixation in legumes. Ann Rev Plant Physiol 31:29–49

Ryle GJA, Powell CE (1976) Effect of rate of photosynthesis on the pattern of assimilate distribution in the graminaceous plant. J Exp Bot 27:189–199

Shipley B (2002) Trade-offs between net assimilation rate and specific leaf area in determining relative growth rate: relationship with daily irradiance. Funct Ecol 16:682–689

Vance CP, Heichel GH (1991) Carbon in N_2 fixation: limitation or exquisite adaption. Ann Rev Plant Physiol 42:373–392

Vance E, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

Volenec JJ, Ourry A, Joern BC (1996) A role for nitrogen reserves in forage regrowth and stress tolerance. Physiol Plant 97:185–193

Warembourg FR, Roumet C, Lafont F (2003) Differences in rhizosphere carbon-partitioning among plant species of different plant families. Plant Soil 256:347–357

Werth M, Kuzyakov Y (2008) Determining root-derived carbon in soil respiration and microbial biomass using ^{14}C and ^{13}C. Soil Biol Biochem 40:625–637

Zagal E (1994) Influence of light intensity on the distribution of carbon and consequent effects on mineralization of soil nitrogen in a barley *(Hordeum vulgare* L.)-soil system. Plant Soil 160:21–31