Effect of shading and clipping on C allocation and fluxes under ryegrass and alfalfa as estimated by $^{14}$C labeling

Diploma thesis in Geoecology

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October, 2010
Acknowledgements

I would like to thank

Prof. Dr. Yakov Kuzyakov, for the opportunity to carry out this investigation at his department, for his guidance and support.

PD Dr. Werner Borken, who agreed to be the second reviewer of my thesis.

Johanna Pausch, for her ongoing assistance and many fruitful discussions concerning various aspects of this work.

All the people of the department of Agroecosystem Research helping me and giving advice during this work.

My parents and friends for their support and patience.
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Abstract

The important role of grasslands in the carbon (C) cycle and C sequestration may be affected by changing environmental factors and land use. The photosynthesis drives C allocation within various pools in the plant-soil-microorganisms system and control the substrate supply for plant and soil pools. Therefore, factors affecting the photosynthesis rate, like shading or clipping may reduce the assimilate supply and alter the distribution of reserve C in plants and soils. In this study the effect of clipping and shading on the C allocation for two grassland species, ryegrass (Lolium perenne, L.) and alfalfa (Medicago sativa, L.), were investigated.

The plants were grown under controlled conditions. To determine the redistribution of plant stored C in plant and soil pools, repeated \(^{14}\)CO\(_2\) pulse labeling was used. Five days after the last \(^{14}\)C pulse the plants were clipped or shaded and the total CO\(_2\) efflux and \(^{14}\)CO\(_2\) efflux from the soil was measured. The \(^{14}\)C content in above- and below-ground plant biomass, soil, rhizosphere and their microorganisms was determined 10 days after clipping or shading.

The total CO\(_2\) efflux from soil decreased after shading for both plant species whereas after clipping this was the case only for L. perenne. The \(^{14}\)CO\(_2\) efflux from soil decreased after clipping for L. perenne, whereas for M. sativa there was no alteration. After shading, for both plant species an increase in the \(^{14}\)CO\(_2\) efflux from soil was observed, indicating that due to a lower assimilation more reserve C is used for root respiration. \(^{14}\)C applied before clipping was detected in the newly grown shoots of clipped plants, tending to originate from roots rather than stubbles. No significant changes of the \(^{14}\)C content were observed in soil pools and microbial biomass after clipping and shading. However higher \(^{14}\)C recovery was observed in the rhizosphere of M. sativa after clipping because of accelerated root senescence.

The results show evidence that C stored in roots is an important factor for the recovery of plants after limiting assimilate supply caused by clipping or shading. However, whereas this C is important for shoot regrowth after clipping, after shading it is utilized mainly for maintenance respiration. The response of both plant species to clipping is different: Compared to Lolium perenne, the legume Medicago sativa has a higher C demand for root respiration probably due to the high energy costs of N\(_2\) fixation, resulting in different retranslocation patterns of reserve C.

Keywords: \(^{14}\)C, carbon distribution, carbon reserves, clipping, Lolium perenne, shading, Medicago sativa, regrowth, rhizosphere, grazing.


Der Gesamt CO\(_2\) Efflux aus dem Boden verringerte sich nach dem Schattieren für beide Pflanzenarten während dies nach dem Schneiden nur bei *L. perenne* der Fall war. Nach dem Schneiden verringerte sich der \(^{14}\)CO\(_2\) Efflux aus dem Boden bei *L. perenne*, veränderte sich aber bei *M. sativa* nicht. Während des Schattierens war bei beiden Pflanzen ein Anstieg erkennbar, was aufgrund der geringen Assimilation auf eine erhöhten Anteil an gespeicherten C in der Rhizosphärenatmung hindeutet. \(^{14}\)C das vor dem Schneiden aufgenommen wurde, konnte in den neugewachsenen Spross gefunden werden. Dieses stammt eher aus den Wurzeln als aus den Stoppen. In den Boden-Pools und der mikrobiellen Biomasse wurde keine signifikante Änderung nach dem Schneiden und Schattieren festgestellt. Allerdings wurde ein höherer Anteil an \(^{14}\)C in der Rhizosphäre von *M. sativa* nach dem Schneiden gefunden, was durch ein beschleunigtes Wurzelsterben zu erklären ist.

Die Ergebnisse zeigen, dass in Wurzeln gespeichertes C für die Pflanze ein bedeutender Faktor bei limitierter Substratverfügbarkeit durch Schneiden sein kann. Während jedoch dieses C nach dem Schneiden besonders bedeutend für das Erneuern oberirdischer
Zusammenfassung

Biomasse ist, ist es beim Schattieren hauptsächlich für die Erhaltungsatmung wichtig. Zwischen den Pflanzenarten konnten unterschiedliche Auswirkungen des Schneidens aufgezeigt werden. Die Leguminose *M. sativa* hat wahrscheinlich wegen den hohen Energiekosten für die N\(_2\) Fixierung einen höheren C Bedarf als *L. perenne* was zu einer unterschiedliche Retranslokation von gespeicherten C führt.
1. Introduction

Below-ground translocation of carbon (C) by plants and its turnover in soils are important factors controlling the global carbon cycle. Thus, it is not surprising that in the last decades many studies investigated the distribution and dynamics of assimilates in the plant-soil system. Kuzyakov and Domanski (2000) reviewed that in grasses on average 40% of assimilated C is translocated below-ground and 30% is allocated in shoots and 30% respired by shoots. 50% of the below-ground C is incorporated in root biomass, 12% remains in the soil and in the microbial biomass and 36% is respired by roots or microorganisms. The C translocated below-ground is an important driver for many processes in terrestrial ecosystems. Root exudates influence nutrient availability for plants, activity and turnover of microbes, turnover of soil organic matter, protection against pathogens or formation of microaggregates (Merbach et al., 1999). Moreover, the soil CO₂ efflux, to which roots contribute 30-70% (Schlesinger, 1977), is the second largest C-flux in terrestrial ecosystems and accounts for 60-90% of ecosystem respiration (Goulden et al., 1996; Longdoz et al., 2000). In agriculturally used areas changes in land use and management can alter the C sequestration. For grasslands, an alteration in the net CO₂ exchange after changing the management intensity was observed (Schmitt et al., 2010). Another manipulation of the C economy in grasslands occurs by grazing management. Grazing can affect the plant biomass, depending on the grazing intensity and history (Milchunas and Lauenroth, 1993), and soil respiration (Cao et al., 2004). Since temperate grasslands contain 12% of the earth’s detritus (Schlesinger 1977), and thus play an important role in the global C cycle, deep knowledge of factors affecting C distribution between plant and soil pools and the respective below-ground processes is important.

The importance of the below-ground C fluxes induced many studies investigating the effect of environmental conditions on the C distribution in plant and soil pools. It was reported that the CO₂ concentration (Hill et al., 2007b), nutrient availability (Merckx et al., 1987; Liljeroth et al., 1990; Warembourg and Estelrich, 2001) or drought (Borken et al., 2006) can have an effect on the C partitioning in plant and soil. The C allocation in plant and soil can also be affected by plant properties. During plant development the portion of C stored in shoots increase, leading to a decreased below-ground translocation (Keith and Oades, 1986; Gregory and Atwell, 1991; Meharg and Killham, 1999). Furthermore, different C allocation patterns between plant species were observed. Perennial plants translocate more C below-ground than annual plants (Kuzyakov and Domanski, 2000). This indicates a higher C storage in roots of perennial plants, whereas annual plants allocate more C in above-ground
parts, especially grains. Warembourg et al. (2003) investigated the C input in the rhizosphere of 12 Mediterranean plant species. They found significant differences in the C below-ground allocation of assimilated C between the plant species, ranging from 41% to 76% of assimilated C. They separated the plants in different functional groups and found the highest portion of rhizosphere respiration on below-ground C for legumes compared to grasses. The lowest portion was found for non-legume forbs. Legumes have a higher C demand compared to non-legumes because of the high energy requirement for N_2 fixation (Philips, 1980). Vance and Heichel (1991) estimated that about 6 mg C is necessary to fix 1 mg N. After Witty et al. (1983) the respiration losses tied to N_2 fixation can account for up to 70% of total root respiration. Thus, because of the high C costs for N_2 fixation, it is assumed that a changing photosynthetic rate provokes different effects between legumes and non-legumes concerning the distribution of assimilates in the plant.

The carbon allocated below-ground by plants is fixed by photosynthesis. Dilkes et al. (2004) found maximal exudation from wheat roots of recent photosynthates 2 to 3 hours after fixation, declining to one-third of the maximum after 5 hours. In a tree girdling experiment Högberg et al. (2001) showed a strong decrease of soil respiration after pulling up the assimilate transport in roots. Both results indicate that current photosynthesis and the supply of recent assimilates to roots are key drivers for rhizodeposition and soil respiration (Kuzyakov and Gavrichkova, 2010). Thus, any alteration in environmental factors affecting photosynthesis activity, and thereby influencing availability of recent assimilates, is assumed to provoke effects on C pools, like soil organic matter, soil CO_2 efflux or microbial biomass turnover, replenished by plant-originated C.

Defoliation by grazing (Detling et al., 1979) and shading are such factors reducing the photosynthetic rate due to smaller leaf areas and less light availability, respectively. Thus, it is assumed that clipping and shading change allocation patterns of assimilates in the plant-soil-microorganism system, due to a limited C availability. It was shown that defoliation increases sink strength for regrowing leaves and reduces below-ground C-allocation (Detling et al. 1979; Mackie-Dawson 1999). On the other hand, Holland et al. (1996) found a positive relationship between herbivory and below-ground allocation of C. They hypothesized that C allocation in roots after grazing makes assimilated C less accessible to herbivores compared to allocation in shoots. Low light intensity decreased the R:S ratio in Zea mays (Lambers and Posthumus, 1980), whereas for Lolium perenne an increase was observed (Hodge et al., 1997). The inconsistent results for the C distribution in plant and soil of clipped and non-clipped plants as well as for plants exposed to different light conditions makes it difficult to interpret these findings. It is assumed that these differences could be plant species specific.
Therefore, in this study the C distribution of two plant species after clipping and shading was investigated, to find out if they differ in their response to limited substrate supply.

Carbon storage is an important mechanism in plants to overcome tremendous interferences like clipping or unfavorable growth conditions like low light intensity. Admittedly, only a small portion of current assimilated C is invested in reserves, but over an extended period this forms a considerable reserve pool (Danckwerts and Gordon, 1987). Especially after clipping these reserves play an important role for plant recovery and especially for shoot regrowth. Grasses store assimilated C in roots, hypocotyle and stubble and utilize this C for regrowth, respiration or exudation (Davidson and Milthorpe, 1966; Bokhari, 1977). Davies (1965) found that the yield of *Lolium perenne* after cutting was associated with the level of carbohydrates in the stubble. Reserve C also plays an important role for *Medicago sativa* for N₂ fixation after defoliation (Ta et al., 1990). However, less is known about the effect of shading on the redistribution of C reserves. Merlo et al. (1994) showed a rapid reduction of C reserves for low light conditions due to limited C supply. One aim of this study is to get an insight in the reuse of stored C after clipping and shading.

Although both, clipping and shading provoke a reduced assimilation, it is assumed that these treatments have different impacts on the redistribution of stored C. Whereas, after shading the reuse of stored C is only governed by the reduced assimilation, after clipping the removal of plant biomass will also have an effect on the relocation of C and may be a more important factor than shading. Thus, it is expected that both treatments initiate specific mechanisms of redistribution of reserve C. The implementation of both treatments in one study makes it possible to evaluate the response to the different treatments with respect to the C allocation.

In the present study the differences in the C redistribution after clipping and shading of *Lolium perenne* and *Medicago sativa* were investigated. Physiological and morphological differences between these two plant species are expected to cause different responses in the C allocation after clipping and shading. *Lolium perenne* is a plant species tolerant for herbivory, whereas *Medicago sativa* has a lower persistence under grazing (Counce et al., 1984). It is assumed that this can lead to a different capability of plant species in the reuse of stored C. Further, the comparison of these two plant species allows discerning the effect of clipping and shading between a legume and a non-legume. Because of their specific C distribution, it is assumed that both differ in their retranslocation patterns of stored C after limiting the substrate supply. Also both investigated plant species differ in their root architecture. *Lolium perenne* is a monocotyledous plant species and thus has an adventitious root system with nodal roots. Contrary, *Medicago sativa* is a dicotyledous plant species with a root system deriving from primary roots and lateral branching (primary
Introduction

root system) and can exhibit radial growth (Hodge et al., 2009). Different root systems may have an effect on the amount of rhizodeposition. This was suggested by Whipps (1987) who assumed that dicotyledonous plants exude more C from roots than monocotyledonous. Bekku et al. (1997) came to the same conclusion after observing a higher exudation from the dicotyledonous Ambrosia gartemisiifalia compared to the monocotyledonous Digitaria adscendem and Hordeum vulgare.

Therefore, the aims of this study were to determine (1) the change of root-derived CO₂ efflux of soils after clipping or shading and the possible impact on the contribution of stored C to the CO₂ efflux, (2) the redistribution of stored C in plant, soil and microorganisms after limiting the substrate supply, (3) the difference in the redistribution of stored C after clipping and shading, and (4) the different responses of a legume and a non-legume to clipping or shading. To address these questions an experiment was conducted in which a legume (Medicago sativa L.) and a non-legume (Lolium perenne L.) were labeled with repeated ¹⁴C pulses before they were clipped or shaded.
2. Materials and Methods

2.1 Soil and growing conditions

The soil used in this study, a loamy haplic Luvisol originated from loess, was collected from the upper 10 cm of arable land near Göttingen (Germany, 51°33’36.8´´N, 9°53’46.9´´E) and was passed through a 2 mm sieve. The soil can be characterized as follows: 1.2 mg g⁻¹ total N, 11.7 mg g⁻¹ total C, 9.76 C/N, 0.083 mg g⁻¹ NO₃⁻, 0.160 mg g⁻¹ P, 0.009 mg g⁻¹ S, 108 mmol kg⁻¹, 99.7% BS.

Seeds of ryegrass (Lolium perenne, L.) and alfalfa (Medicago sativa, L.) were germinated on wet filter paper in Petri dishes. After 5 days 3 seedlings of Medicago sativa and after 8 days 5 seedlings of Lolium perenne were planted per pot (inner diameter 7 cm, height 20 cm) containing 665 g dry soil each. The pots were closed with a plastic lid with drilled holes for plants. The plants were grown at 26-28 °C day and at 22-23 °C night temperature with a day-length of 14 h and light intensity of approximately 175 μmol m⁻² s⁻¹. The soil water content was measured gravimetrically and adjusted daily to 70% of the available field capacity.

2.2 Labeling procedure

Repeated ¹⁴C pulse labeling was used. All plants of one species were labeled simultaneously in a ¹⁴CO₂ atmosphere on days 35, 40 and 45 after germination. One day before the first labeling the holes in the lids were sealed around the stem of plants using silicone paste (NG 3170, Thauer & Co., Dresden) and checked for air tightness. For labeling, the plants were placed in a Plexiglas chamber. The Plexiglas chamber and labeling technique were described in detail elsewhere (Kuzyakov et al., 2006). Briefly, in a test tube, containing 10 ml deionised water and 0.1 ml 1 M NaOH, 45 μl Na₂¹⁴CO₃ (1 mCi ml⁻¹) solution was added, what equals to a ¹⁴C activity of 139 kBq per pot. Three ml of 5 M H₂SO₄ were added to the solution in the test tube leading to complete generation of ¹⁴CO₂ into the chamber. The plants were labeled for 3 hours in the ¹⁴CO₂ atmosphere. Thereafter, for 2 hours the chamber air was pumped through two test tubes containing 15 ml 1 M NaOH to trap unassimilated ¹⁴CO₂. Finally, the top of the chamber was removed and the plants were grown under normal conditions until the next ¹⁴CO₂ pulse was applied (after 5 days).
2.3 Clipping and shading

For each plant the following treatments were applied with four replicates: normal light conditions, shading and clipping. Five days after the last $^{14}$C pulse 4 pots of each species were cut at 4 cm above the soil surface for *Lolium perenne* and 8 cm for *Medicago sativa* (Kuzyakov et al., 2002; Katepa-Mupondwa et al., 2002). Different clipping heights for the plant species were used to achieve similar stubble biomasses for both plant species. The plants were allowed to regrow for 10 days under the conditions described above. Another 4 pots of each species were shaded with a light intensity of about 4 μmol m$^{-2}$ s$^{-1}$. They also grew under these conditions for 10 days until harvest. The remaining control pots were kept at normal conditions as described above until harvest.

2.4 Sample analysis

Starting after the first labeling, the CO$_2$ evolved from soil was pumped by membrane pumps and trapped in tubes containing 15 ml 1 M NaOH solution under formation of Na$_2$CO$_3$. The NaOH solution was changed 1, 3 and 5 days after each labeling and on 6 days after clipping or beginning of shading (on days 1, 3, 5, 6, 8 and 10 after the beginning of the treatments). The total content of CO$_2$ collected in NaOH solution was measured by titration with 0.01 M HCl against Phenolphthalein after addition of 1.5 M BaCl$_2$ solution.

To measure the $^{14}$C activity of the CO$_2$ trapped in the NaOH solution 2 ml of scintillation cocktail Rotiszint EcoPlus (Carl Roth, Germany) were added to 1 ml of a sample. The $^{14}$C measurements were carried out by means of a liquid scintillation counter (MicroBeta TriLux, 205 Perkin Elmer Inc., USA).

The plants were harvested 10 days after clipping or beginning of shading. The harvested plants were divided into shoot (biomass above the cutting height of 4 or 8 cm), stubble (biomass below the cutting height) and the cut shoot parts (shoots removed at clipping). Roots were separated from soil by handpicking. The soil was separated in rhizosphere soil and bulk soil. For this, the soil was slightly shaken and the remaining soil attached to the roots was accepted as rhizosphere soil.

For the determination of microbial-C and $^{14}$C incorporated in the microbial biomass the chloroform fumigation-extraction-method (CFE) was used (modified after Vance et al. 1987).
5 g of soil were extracted with 20 ml of 0.05 M K$_2$SO$_4$. Another 5 g of soil were firstly fumigated with chloroform for 24 hours and then extracted as described for unfumigated samples. Both samples were shaken for 1 h and thereafter centrifuged for 10 min at 3070 rev min$^{-1}$. The extracts were frozen till analysis. Total C content of fumigated and non-fumigated soil samples was measured using an N/C analyzer (Multi N/C 2100, AnalytikJena, Germany). The microbial biomass C was calculated by dividing the microbial C flush (difference between extractable C from fumigated and unfumigated soil samples) with a $k_{EC}$ value of 0.45 (Wu et al., 1990). In the same way and with the same $k_{EC}$ factor the $^{14}$C content of microbial biomass was calculated.

To analyze the $^{14}$C incorporated in plant and soil, roots, shoots, bulk soil and rhizosphere soil were oven-dried at 65 °C for 3 days and then ground. For $^{14}$C measurements 50 mg of plant material or 500 mg of soil material were combusted in an oven (Feststoffmodul 1300, AnalytikJena, Germany) at 900 °C. The CO$_2$ released by combustion was trapped in 10 ml 1 M NaOH. To measure the $^{14}$C activity of these samples and also the $^{14}$C activity of the extracts of the CFE 2 ml of the sample was added with 4 ml of the same scintillation cocktail as used for CO$_2$ measurements. The $^{14}$C activity was measured on a liquid scintillation counter (LS 6500 Multi-Purpose Scintillation Counter, 217 Beckman, USA).

2.5 Calculations and statistics

The $^{14}$C data for shoots, roots, soil and microbial biomass and the soil CO$_2$ efflux are presented as percentage of $^{14}$C recovered in all examined pools and as the $^{14}$C specific activity in kBq g$^{-1}$ sample. The experiment was conducted with four replicates for all treatments. All displayed results are presented as means with standard errors. To examine differences between plants and treatments a two-way ANOVA ($\alpha < 5\%$) was used. A Fisher-LSD test ($p < 0.05$) was conducted as post-hoc test. The significances of differences between individual means was determined by a Newman-Keuls-test as least significant differences (LSD; $p < 0.05$). These tests were performed using STATISTICA statistical software (version 7.0, StatSoft, inc., OK; USA).
3. Results

3.1 Plant and microbial biomass

The amounts of the above-ground biomass showed that *Medicago sativa* produced significantly more shoot biomass ($p < 0.001$) as well as stubble biomass ($p < 0.05$) compared to *Lolium perenne* (Fig. 1). Only for the stubbles grown under normal conditions there was no difference between both plant species. Besides the plant species, also the treatments provoked effects on the above-ground biomass. Generally, clipping showed no significant effect on the shoot biomass (including the removed shoots at clipping) after 10 days of regrowth. However, after clipping, for the shoot biomass a trend for oppositional effects of both plant species was observed. For *Lolium perenne* the shoot biomass tends to decrease after clipping compared to the control plants, whereas, it increased for *Medicago sativa* indicating a faster regrowth of *Medicago sativa*. The biomass of the stubble decreased significantly after clipping compared to the control ($p < 0.05$). However, considering the single plant species, the decrease was only observed for *Lolium perenne* (Fig. 1). Shading reduced the growth of the above-ground biomass during 10 days. This could be observed by the significantly decreased biomass of the stubbles ($p < 0.05$) and the shoots ($p < 0.001$) after shading (Fig. 1). After 10 days of shading, the biomass of the shoots and the stubbles was significantly smaller compared to the biomass after clipping for both plant species. Thus, after shading the shoot biomass was the lowest for both plant species, whereas, after clipping the above-ground biomass was different between the plant species.

The root biomass showed no significant differences between the control plants, the clipped and the shaded plants (Fig. 1). Also between *Lolium perenne* and *Medicago sativa* there was no significant difference in root biomass.
Figure 1: Above-ground and below-ground plant dry mass of *Lolium perenne* and *Medicago sativa* 10 days after clipping and shading. LSD values (p < 0.05) are presented as whisked segment.
For the R:S ratio no significant differences after the treatments were found. The lowest R:S ratio was observed for the clipped *Lolium perenne* and *Medicago sativa* as a trend compared to shading and the control (Fig. 2). For *Lolium perenne* it tended to be highest after shading, whereas, for *Medicago sativa* the R:S ratio was the same for normal conditions and shading. Comparing the two plant species, *Lolium perenne* has a higher R:S ratio than *Medicago sativa*.

Figure 2: Root-to-shoot ratio of *Lolium perenne* and *Medicago sativa* 10 days after clipping and shading. LSD value (p < 0.05) is presented as whisked segment.
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The microbial biomass in the bulk soil was not influenced by clipping and shading (Fig. 3). The plant species had also no effect on the microbial biomass in the bulk soil. However, clipping ($p < 0.001$) and shading ($p < 0.01$) decreased the microbial biomass in the rhizosphere, indicating a changed C availability for microorganisms after clipping and shading. However, regarding each plant species on its own the decrease of the microbial biomass in the rhizosphere after shading was only observed for *Medicago sativa*. Like in the bulk soil, also in the rhizosphere soil was no difference between both plant species.

![Graph showing microbial biomass C in soil under Lolium perenne and Medicago sativa in 10 days after clipping or beginning of shading. LSD values ($p < 0.05$) are presented as whisks.](image)

Figure 3: Microbial biomass C in soil under *Lolium perenne* and *Medicago sativa* in 10 days after clipping or beginning of shading. LSD values ($p < 0.05$) are presented as whisks.
3.2 Distribution of $^{14}$C in plant and soil after clipping and shading

The amount of stored C in the investigated plant and soil pools was determined as the $^{14}$C specific activity and as percentage of $^{14}$C recovery. The specific activity allows to compare the amount of $^{14}$C in the different pools after the treatments, whereas, with the percentage of $^{14}$C recovered it is possible to compare the effect of clipping and shading on the relative distribution between different pools in the plant and soil. The $^{14}$C recovery in the root was affected by the plant species ($p < 0.01$) with higher values for *Medicago sativa*. In the shoot the $^{14}$C recovery was higher for *Medicago sativa* compared to *Lolium perenne* only under normal conditions in the control. For both plant species the highest percentage of $^{14}$C recovery was found in the shoots including the cut shoots removed at clipping (Fig. 4). For *Medicago sativa* the lowest $^{14}$C recovery was found in the stubble, whereas for *Lolium perenne* the $^{14}$C recovery was nearly the same in stubble and roots. The most interesting result of the clipping treatment is the detection of $^{14}$C in new grown shoots of both investigated plant species, indicating a translocation of reserve C in these plant parts. After clipping there was no significant change of the $^{14}$C recovery and the $^{14}$C specific activity in the stubbles and the roots (Fig. 4 and 5). Only a tendency for a decrease could be observed in the roots of *Lolium perenne*, indicating that roots could be a source of reused C reserves after clipping. Shading did not change the redistribution of stored C as shown by the constant $^{14}$C recovery of *Lolium perenne* and *Medicago sativa* in stubbles, shoots and roots compared with control pots (Fig. 4). However, due to the less above-ground biomass (Fig. 1) and a lower assimilation of new C, the $^{14}$C specific activity was highest for shoot ($p < 0.001$) and stubble ($p < 0.01$) after shading. Regarding each plant species on its own this is only valid for *Lolium perenne* (Fig. 5).
Results

Figure 4: Percentage of $^{14}$C recovery in above-ground and below-ground plant parts for different treatments 10 days after clipping or beginning of shading. LSD values ($p < 0.05$) are presented as whisked segment.

Figure 5: $^{14}$C specific activity of above-ground and below-ground plant parts for different treatments 10 days after clipping or beginning of shading. LSD values ($p < 0.05$) are presented as whisked segment.
Because of the higher root growth of unclipped plants some pots of control and shaded treatments of *Lolium perenne* were completely penetrated with roots. The whole soil in these pots was considered as rhizosphere soil. Therefore, for *Lolium perenne* there is only one value for bulk soil for control treatments and no value for shaded treatments. Accordingly, this is also valid for the $^{14}$C incorporated in the microbial biomass.

The $^{14}$C recovery and the specific activity in the bulk soil showed no significant effect of clipping or shading for both plant species (Fig. 6). Also the plant species has no influence on the $^{14}$C amount in the bulk soil. However, the plant species had a significant effect on the $^{14}$C recovery in the rhizosphere soil ($p < 0.05$) with a higher proportion of exuded C for *Lolium perenne* compared to *Medicago sativa*. In the rhizosphere of *Lolium perenne* no change of the $^{14}$C recovery and specific activity was observed after clipping. However, clipping can alter the relative amount of stored C in the rhizosphere soil of *Medicago sativa*, as shown by a higher $^{14}$C recovery. However, the unaltered $^{14}$C specific activity indicates no change in the total amount of exuded $^{14}$C (Fig. 7). Shading did not induce any alterations in the $^{14}$C recovery rate and specific activity of rhizosphere soil in both plant species. After shading and under normal conditions, in *Medicago sativa* pots a higher $^{14}$C recovery rate was observed in bulk soil than in rhizosphere soil, whereas after clipping more $^{14}$C was found in rhizosphere soil (Fig. 6).

The $^{14}$C recovery in the microbial biomass was the same for both plant species (Fig. 6) and was uninfluenced by clipping and shading. Also the $^{14}$C specific activity in microbial biomass showed no significant effect after clipping or shading (Fig. 7). However, because of the high variations, these results are difficult to interpret.
Figure 6: Percentage of $^{14}$C recovery in soil and microbial biomass under *Lolium perenne* and *Medicago sativa* 10 days after clipping and beginning of shading. LSD values ($p < 0.05$) are presented as whisked segment.
Figure 7: $^{14}$C specific activity of soil and microbial biomass under *Lolium perenne* and *Medicago sativa* 10 days after clipping and beginning of shading. LSD values ($p < 0.05$) are presented as whisked segment.
3.3 Total CO₂ and ¹⁴C efflux from soil

The cumulative CO₂ efflux from soil under *Lolium perenne* was highest in control pots (Fig. 8). The reduced availability of assimilates after clipping or shading induce a decrease of CO₂ efflux, whereas, this decrease was stronger after clipping compared to shading. For *Medicago sativa* the soil CO₂ efflux was also reduced after clipping and shading (Fig. 8). However, the reduction caused by clipping was only observed until day 5 after clipping and thereafter it increased and reached after 10 days of regrowth the same level as under control plants. The lowest amounts of the soil CO₂ efflux for *Medicago sativa* were observed after shading. Comparing the two plant species, the total soil CO₂ efflux was higher for *Medicago sativa* than for *Lolium perenne*. 
Figure 8: Cumulative C-CO₂ efflux from soil under *Lolium perenne* and *Medicago sativa* beginning on the day of first $^{14}$C labeling for control, clipping and shading treatments.
Regarding the $^{14}\text{C}$ efflux from soil, *Lolium perenne* and *Medicago sativa* showed different responses in respect of clipping (Fig. 9). The percentage of the $^{14}\text{C}$ recovery in the CO$_2$ efflux increased under *Lolium perenne*, whereas, for *Medicago sativa* no significant change was observed. After shading the $^{14}\text{C}$ recovery showed no significant change (Fig. 9). The $^{14}\text{C}$ specific activity of the CO$_2$ efflux is presented as mean value of the specific activities measured between clipping or shading and the harvest, since they kept nearly constant during this time period (Fig. 10). The $^{14}\text{C}$ specific activity was significantly higher under *Medicago sativa* than under *Lolium perenne* ($p < 0.001$). Only for the control no difference between both plant species was observed. For *Lolium perenne* the $^{14}\text{C}$ specific activity of the soil CO$_2$ after clipping was lower compared to control plants (Figure 10). In contrast to *Lolium perenne*, the specific $^{14}\text{CO}_2$ efflux from soil under *Medicago sativa* showed no significant effect after clipping. While after clipping under *Lolium perenne* and *Medicago sativa* the specific $^{14}\text{CO}_2$ efflux from soil showed different reactions, shading provoked an increase ($p < 0.001$). Differently from clipping, after shading the remobilization of reserve C play a more important role to maintain respiration. This was indicated by the higher $^{14}\text{CO}_2$ efflux from soil of both plant species after shading, which was the highest in comparison with clipped and control treatments (Figure 10). Comparing the two plant species, a higher $^{14}\text{CO}_2$ efflux was observed for *Medicago sativa*. 
Figure 9: Percentage of $^{14}$C recovery in the soil CO$_2$ efflux under *Lolium perenne* and *Medicago sativa*, calculated from the cumulated $^{14}$C efflux 10 days after clipping and beginning of shading. LSD value ($p < 0.05$) is presented as whisked segment.

Figure 10: Mean value of $^{14}$C specific activity of the soil CO$_2$ under *Lolium perenne* and *Medicago sativa* measured from clipping and beginning of shading until harvest. LSD value ($p < 0.05$) is presented as whisked segment.
4. Discussion

To determine the retranslocation of $^{14}$C a repeated pulse labeling was implemented because information about the allocation of assimilates than continuous labeling or single pulse labeling (Warembourg and Elsterlich, 2000; Werth and Kuzyakov, 2008). Also the distribution of the label is more homogeneous compared to single pulse labeling, what is especially important for estimation of reserve utilization. The last $^{14}$C labeling was implemented 5 days before shading, because it is assumed that after this time period the distribution of assimilated C between above-ground and below-ground pools is mostly completed (Domanski et al., 2001). Consequently, the retranslocated $^{14}$C found in the different pools is considered as remobilized reserve C. This is accordance with Danckwerts and Gordon (1987) who found that $^{14}$C reached its final destination within 4-6 days and terminated this $^{14}$C as reserve C.

4.1 Partitioning of reserve C in *Lolium perenne* and *Medicago sativa* under normal conditions

The results show that the non-legume *Lolium perenne* and the legume *Medicago sativa* show different C allocation patterns, resulting in a variable biomass production and $^{14}$C distribution in the shoots, roots and various soil pools. At the end of the experiment the root biomass was similar between the both plant species, whereas, for the above-ground plant parts a higher biomass production was observed by *Medicago sativa* than by *Lolium perenne* (Fig. 1). These results are in accordance with the higher $^{14}$C recovery and coincident with the equal $^{14}$C specific activity in the shoot of the control plants of *Medicago sativa* compared to *Lolium perenne* (Fig. 4). However, in the roots of *Lolium perenne* there is a tendency for a lower $^{14}$C allocation compared to *Medicago sativa*, although the root dry weight was the same for both plant species. This can be explained by a higher amount of $^{14}$C in the root exudation of *Lolium perenne*, what is supported by the higher $^{14}$C recovery found in its rhizosphere compared to *Medicago sativa* (Fig. 6). Neergaard and Gorissen (2004) found also a higher below-ground allocation of $^{14}$C for *Lolium perenne* compared to the legume *Trifolium repens*. They also found that exudates released by *Trifolium repens* are faster decomposed by microorganisms. Assuming this is valid for all legumes, this could explain the higher $^{14}$C recovery found in the soil of *Lolium perenne* (Fig. 6). Another explanation for the high $^{14}$C amount in the soil of *Lolium perenne* could be an enhanced rhizodeposition leading to an
increased nutrient availability for plant roots, what is of major importance for non-legumes rather than legumes. On the other hand legumes has higher C costs of $\text{N}_2$ fixation estimated as 4-12% of photosynthesis (Lambers, 1987), resulting in a higher root respiration. Thus, it can be concluded, that the non-legume *Lolium perenne* has a higher root exudation whereas the legume *Medicago sativa* has a higher root respiration. Assuming, the fast microbial decomposition of root exudates found for *Trifolium repens*, as mentioned above, is also valid for *Medicago sativa*, since both are legumes, this and the higher root respiration because of the $\text{N}_2$ fixation could explain the observed higher soil CO$_2$ efflux of *Medicago sativa* compared to the non-legume *Lolium perenne* (Fig. 8). However, since this study was not designed to distinguish between root and microbial respiration, a closer insight in the composition of the soil CO$_2$ and the contribution of its sources was not possible.

### 4.2 Redistribution of reserve C in the plant biomass

**Effect of clipping**

Clipping induced different responses of *Lolium perenne* and *Medicago sativa* in respect to plant biomass. Ferraro and Oesterheld (2002) reviewed 28 studies investigating the effect of clipping on plant growth. They found out that most plant species decrease their biomass production after defoliation, depending on a) the time of recovery from the last defoliation b) the period of time of between clipping events and c) the nitrogen (N) availability, as plants grown at high N levels were more negatively affected by clipping. Different responses to defoliation between plant species was also observed by Guitian and Bardgett (2000). They found for herbivory tolerant grass species a defoliation-induced reduction of root growth as a consequence of allocation of assimilates to support shoot regrowth. Consequently the R:S ratio of the biomass decreased for herbivory tolerant plants. In the present study, after clipping of *Lolium perenne* the above-ground biomass was reduced, whereas for *Medicago sativa* it was enhanced (Fig. 1). The root production of both plant species was not influenced by clipping, however a trend for a higher reduction of root biomass was observed for *Lolium perenne*, because it is more tolerant to herbivory compared to *Medicago sativa*. The R:S ratio showed no significant effect in response to clipping (Fig. 2). However, there was a trend for a decreased R:S ratio of both plant species, indicating a translocation of assimilates from roots to shoots after clipping (Fig. 2).
The percentage of $^{14}$C recovery in all plant parts showed no significant change after clipping compared to the non-clipped control for *Lolium perenne* and for *Medicago sativa* (Fig. 4), implying that retranslocation of stored C is not influenced by the treatments. However, $^{14}$C was found in the newly grown shoots of both species. This is supported by many other studies (Kuzyakov et al. 2002; Morvan-Bertrand et al., 1999, Johansson 1993). The $^{14}$C in the shoot must have been retranslocated from stubbles or roots left after clipping since both are the only possible source for reserve C. The importance of water soluble carbohydrates stored in roots and shoots for supporting regrowth is reported in many studies (Morvan-Bertrand et al., 1999, Lee et al., 2009). However, this seems to contradict the results of this study mentioned above, which show no decrease of the $^{14}$C content in the stubbles and the root after clipping. It is assumed, that the $^{14}$C content in the stubble and the root of control plants at the end of the experiment is the maximal amount of $^{14}$C possible under the given conditions. From this assumption it was calculated that 5% and 8% of $^{14}$C in *Lolium perenne* and *Medicago sativa*, respectively, were retranslocated from roots, hypocotyle and stubble in new grown shoots. The remobilization was too low to cause significant changes in the stubble or roots. These results are in accordance with Kuzyakov et al. (2002) who found that 4.8% and 2.7% of unfertilized or fertilized *Lolium perenne*, respectively, were retranslocated from reserves and Waremoung and Paul (1977), who found 6% of root reserves in new grown shoots. A lower proportion of reserve carbon of 1% was found by Morvan-Bertrand (1999) for *Lolium perenne* whereas Johansson (1993) found 21% retranslocated in the new shoot parts of *Festuca pratensis* (outlined in Tab. 1). The various results between these studies can be explained by the various methodological approaches and calculations used and the length of the growth period after clipping. The relative amount of retranslocated reserve C in new grown shoot parts is depending on the time passed after defoliation (Briske et al. 1996). After Schnyder and de Visser (1999) until three days after defoliation stored C is the most important C source for the elongation and the maturation zone and after this period photosynthesis meets the demand of assimilates. However, reserve mobilization still occurs for about one week (Morvan-Bertrand 1999). Also specific mechanisms of various plant species to tackle with the removal of shoot biomass can explain the different amounts of retranslocated C in new grown shoots. The difference between *Medicago sativa* and *Lolium perenne* observed in this study can be explained by a higher use of stored C for *Medicago sativa* because of a higher growth of new shoots. However the $^{14}$C specific activity is higher in new grown shoots of *Lolium perenne* indicating a proportional higher use of stored C to support regrowth compared to *Medicago sativa*. Since *Lolium 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 *Medicago sativa*. 
There are different assumptions with regard to the origin of this retranslocated reserve C. While it is clear that the only sources of these C can be the stubble, the hypocotyle and/or the root, the dimensions of the contribution of each source pool in this retranslocation is still unclear. Whereas for *Lolium perenne* Kuzyakov et al. (2002) and Danckwerts and Gordon (1987) supposed a higher proportion of C stored in the crown compared with roots is retranslocated in newly grown shoots, a higher proportion of root reserves were reported for *Festuca pratensis* (Johansson, 1993). For *Panicum maximum* Bushby et al. (1992) observed that the demand of stored C in new growing shoots is only supplied by root reserves whereas crown reserves are not involved in the retranslocation (Tab. 1). It can be assumed that different plant species use various C storage pools to gain reserves for the regrowth of shoots. This is supported by the varying results between *Lolium perenne* and *Medicago sativa* in this study. In *Lolium perenne* a trend for reduced $^{14}$C recovery rate was determined in roots but not in stubble, indicating a remobilization of stored C from roots rather than from stubble. In contrast, in *Medicago sativa* plants no difference in the amount of $^{14}$C was observed between clipped and control treatment neither in roots nor in stubbles (Fig. 4 and 5). Different results were observed by Ta et al. (1990) who found that 12% of C reserves in roots were used for shoot growth after defoliation of *Medicago sativa*. Crawford et al. (2000) found no net translocation of C from roots to shoots in the annual legume *Medicago truncatula*. Nonetheless the results of the present study for *Medicago sativa* were surprising since no source for the $^{14}$C in the new shoot could be found. However, a decrease of reserve C in root by translocation in shoots could be counterbalanced by a reduced proportion of this C in root respiration (discussed below).
Table 1: Sources and amounts of C relocated in the newly grown shoots after clipping of different grassland species from various studies.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Approach</th>
<th>Source</th>
<th>Duration after cutting</th>
<th>Amount retranslocated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Medicago sativa</em></td>
<td>$^{13}$C pulse labeling</td>
<td>Roots (taproots, lateral roots), Stubble stem</td>
<td>30 days</td>
<td>5%</td>
<td>Avice et al. 1996</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C pulse labeling</td>
<td>Roots (Stubbles were not investigated)</td>
<td>28 days</td>
<td>12%</td>
<td>Ta et al., 1990</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C pulse labeling</td>
<td>28 days</td>
<td>19%</td>
<td>Pearce et al., 1969</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{14}$C pulse labeling</td>
<td>Stubbles and Roots</td>
<td>9%</td>
<td>Smith and Marten, 1969</td>
<td></td>
</tr>
<tr>
<td></td>
<td>repeated $^{14}$C pulse labeling</td>
<td>Roots</td>
<td>10 days</td>
<td>8%</td>
<td>This study</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>$^{14}$C continuous labeling</td>
<td>Roots</td>
<td>19 days</td>
<td></td>
<td>Bushby et al., 1992</td>
</tr>
<tr>
<td><em>Medicago truncatula</em></td>
<td>$^{14}$C + $^{13}$C continuous labeling</td>
<td>Stubbles</td>
<td>23 days</td>
<td></td>
<td>Crawford et al., 2000</td>
</tr>
<tr>
<td><em>Lolium perenne</em></td>
<td>$^{14}$C pulse labeling of a single leaf</td>
<td>Stubbles</td>
<td>10 days</td>
<td></td>
<td>Danckwerts and Gordon, 1987</td>
</tr>
<tr>
<td></td>
<td>Repeated $^{14}$C pulse labeling</td>
<td>Predominantly stubbles</td>
<td>15 days</td>
<td>4.7% (fertilized)</td>
<td>Kuzyakov et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.4% (unfertilized)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{13}$C continuous labeling</td>
<td>Elongated leaf bases, sheaths of stubble</td>
<td>28 days</td>
<td>1%</td>
<td>Morvan-Bertrand et al., 1999</td>
</tr>
<tr>
<td></td>
<td>repeated $^{14}$C pulse labeling</td>
<td>Roots</td>
<td>10 days</td>
<td>5%</td>
<td>This study</td>
</tr>
<tr>
<td><em>Festuca pratensis</em></td>
<td>$^{14}$C + $^{13}$C continuous labeling</td>
<td>Stubbles and Root</td>
<td>15 days</td>
<td>21%</td>
<td>Johansson, 1993</td>
</tr>
<tr>
<td><em>Agropyron-Koeleria association</em></td>
<td>$^{14}$C labeling</td>
<td>Roots (Stubbles were not investigated)</td>
<td>4 months</td>
<td>6%</td>
<td>Warembourg and Paul, 1977</td>
</tr>
</tbody>
</table>
Discussion

Effect of shading

The effect of limited substrate supply on the retranslocation of reserve C by clipping could be overlapped by the demand of reserve C for the regrowth of new shoots. Contrary, after the shading of plants the limited substrate supply is the solely impact alter the redistribution of reserve C what allows to investigate the influence of C availability on the retranslocation of reserve C undisturbed by other factors. The results of this study show that shading reduces the accumulation of dry matter in above-ground biomass and roots but has no effect on the R:S ratio of Medicago sativa and Lolium perenne (Fig. 1 and 2). A positive relationship between plant biomass and light intensity was also observed by many other studies (Zagal, 1994; Lambers and Posthumus, 1980). However, former studies differ in their results of the response to the R:S ratio of shaded plants. Like in this study, Zagal (1994) found no changes in the R:S ratio after shading. An increasing R:S ratio at low light conditions was found by Todorovic et al. (1999) for Trifolium repens. A decrease of the R:S ratio for Lolium perenne, indicating a higher accumulation of assimilates in shoot was reported by Lambers and Posthumus (1980). Hodge et al. (1997) found a decrease and an increase of R:S ratio after shading and assumed that a reduced nutrient availability can alter the biomass accumulation. A decrease is in accordance with Thornley and Johnson (1990) who found in a modeling approach increased photosynthesis at high light conditions would increase partitioning to the root to maintain a balanced growth. Besides the plant species used, the different results in these studies can be explained by approaches used to maintain low light conditions such as shading or a reduced photoperiod. Ryle and Powell (1976) assumed that terminal meristems allocate more assimilates to compensate the reduced rate of photosynthesis and thereby limit the C supply for roots. It would last for about one week until the pattern of assimilate distribution is adapted to the new light regime. This may explain the absence of differences in the R:S ratio between the shaded plants and the control plants 10 days after the beginning of shading (Fig. 2). In the present study, also the percentage of $^{14}$C recovery in the plant biomass did not change after shading (Fig. 4), indicating that limited substrate supply do not alter the exchange of reserve C between different plant parts, what is in accordance with the unchanging R:S ratio. Thus, the plants do not counterbalance a limited C availability to support growth by a retranslocation of reserve C, instead they reduced the growth. The results of the shaded plants are contrary to that of the clipped plants. Because they have not only to cope with a limited substrate supply but also with the removal of shoot biomass, a retranslocation of reserve C is necessary to support the regrowth of the shoots. The $^{14}$C specific activity in the above-ground biomass of Lolium perenne was higher after shading than control plants and clipped plants (for the stubbles) (Fig. 5) what can be explained by the reduced biomass and a lower assimilation of unlabeled C due to lower photosynthesis.
4.3 Redistribution of reserve C in soil and soil CO₂

**Effect of clipping**

Clipping induces no significant effect on the distribution of plant stored C in the rhizosphere and bulk soil except an increase of the $^{14}$C recovery in the rhizosphere soil of *Medicago sativa*. Also the $^{14}$C specific activity in bulk soil and rhizosphere soil show no alterations after clipping. Many studies were conducted to investigate the effect of clipping on root exudation. However, the results were contradicting. Some investigators found an increase (Hamilton et al., 2001; Paterson and Sim, 1999), whereas others found no changes (Murray et al., 2004; Todorovic et al., 1999, Kuzyakov et al., 2002) or a decrease (Mikola and Kytöviita, 2002) of exudation after defoliation. These differences could be the result of different plant species and methods used in these studies (Mikola and Kytöviita, 2002). Most of these studies investigated the release of recently fixed C from roots by isotope labeling, whereas Peterson and Sim (1999) measured the release of total organic C. They hypothesized, that an increase of root exudation after defoliation was a consequence of remobilization of storage compounds in roots, which would increase the concentration of diffusible exudates in the root system. This is in accordance with the results of the present study for the rhizosphere of *Medicago sativa* but not in any of the other investigated soil pools of both plant species, where no indication of an enhanced exudation was found in the $^{14}$C recovery rate. Most of the mentioned studies investigated the effect of substrate supply on the distribution of recently fixed C or total organic C in soil or microorganisms. Studies investigating with stored C are rare. Crawford et al. (2000) and Johansson (1993) observed an ongoing release of old C from roots after defoliation. But both authors measure the root release of old C only on defoliated plants and did not compare these results with non-defoliated plants. Murray et al. (2004) found that defoliation increases the exudation of recently fixed C but has no effect on the total exudation. In contrast Paterson et al. (2005) found increased total root exudation for *Festuca pratensis* and determined the source in a higher remobilization of stored C up to two days after defoliation. The incorporation of retranslocated plant-stored C in microbial biomass is not well investigated. The rhizodeposition is of enormous importance for soil microorganism as an energy source and as a limiting factor for microbial growth. Thus, it is assumed that factors effecting root exudation also have an influence on rhizosphere microorganisms. Guitian and Bardgett (2000) and Butenschoen et al. (2008) found an increase of the microbial biomass after defoliation and explained this by a higher C availability for microorganisms. Hamilton et al. (2001) presume that plants are able to increase rhizodeposition to enhance the nutrient availability by promoting microbial populations. In contrast Bazot et al. (2005) found no change in the soluble C content in soil.
and thus no change in microbial biomass and C availability. In the present study for the soil and the microbial biomass in *Lolium perenne* pots there was no alteration in the distribution of plant stored C after clipping (Fig. 6). However, the microbial biomass decreased (Fig. 3). This could be caused by a reduced availability of root exudates for microorganism because of a reduced exudation. Since this would lead to less $^{14}$C in the soil a reduced C availability must be counterbalanced by root senescence. For *Medicago sativa* the results were the same besides an increased $^{14}$C recovery in the rhizosphere soil in response to clipping because of higher exudation and/or increased root senescence. The reduced microbial biomass can be an evidence for a higher amount of dead root material, since they are less available for the microorganism compared to exudates.

Root exudations are important drivers for the soil CO$_2$ efflux, as their decomposition by microorganisms is an important source for soil CO$_2$ besides root respiration. After clipping, a decrease of total CO$_2$ efflux was observed for *Lolium perenne*. This was also found by many other studies (Detling et al., 1979; Craine et al., 1999; Kuzyakov et al., 2002). Gavrichkova et al. (2010) separated root respiration and microbial respiration and observed for both a decrease after clipping. These results indicate a strong connection of photosynthesis activity to soil respiration. Lower assimilation after clipping leads to less available C for below-ground translocation and thus CO$_2$ efflux. However, Fu and Cheng (2004) showed an increase of root derived CO$_2$ until 4 hours after defoliation and thereafter a decrease. Besides total CO$_2$ efflux, also a decrease in the $^{14}$CO$_2$ efflux from soil after clipping was observed in *Lolium perenne* pots in this study (Fig. 8 and 10), probably because of a higher need of reserve C for shoot regrowth. The CO$_2$ efflux from soil after clipping was less decreased than after shading. A reason for this could be that clipped plants have to invest more energy for regrowth, resulting in an increased growth respiration. However, this mechanism could not offset the effect of limited substrate supply with a reduction of the total soil CO$_2$. The reduced $^{14}$C efflux from soil found in this study shows that this increase in soil CO$_2$ efflux compared to shaded pots is not driven by reserve C but newly assimilated C. This is accordance with Lötscher et al. (2004) who found that growth respiration is mainly associated with currently assimilated C.

The result that the total soil CO$_2$ efflux of *Medicago sativa* (Fig. 8) was not altered by clipping was unexpected. Like for *Lolium perenne*, a lower CO$_2$ efflux from soil was expected due to a lower substrate supply after clipping. One reason for this could be that the high energy demand of N$_2$ fixation leads to an increase of root respiration, diminishing the effect of limited photosynthesis. Like the total CO$_2$ efflux, also the $^{14}$C specific activity of soil CO$_2$ shows no change after clipping of *Medicago sativa* (Fig. 10) and thus also differs from the results found for *Lolium perenne*. C stored in nodules plays an important role in supporting N$_2$ fixation after
defoliation of *Medicago sativa* (Ta et al., 1990). Thus, the effect of lower substrate supply, what is expected to reduce the $^{14}$C efflux as shown for *Lolium perenne*, was counterbalanced by a higher remobilization of reserve C, leading to no reduction of the reserve C to total soil CO$_2$ efflux (Fig. 10).

**Effect of shading**

Previous labeling studies showed that a limited substrate supply caused by a reduced light intensity provoke a decrease in root exudation (Hill et al., 2007a). This leads to a lower incorporation of exuded C in microorganisms and a decreased growth of microbial biomass (Zagal, 1994). Hodge et al. (1997) found no effect of shading on the C partitioning in soil for *Lolium perenne* and the microbial biomass therein. In the present study, no change in the $^{14}$C specific activity and $^{14}$C recovery of the soil was observed for *Lolium perenne* and *Medicago sativa* (Fig. 10). This indicates that a possible change of root exudation after shading do not affect a change in the redistribution of reserve C. This is supported by the unchanging specific activity and $^{14}$C recovery in the microbial biomass after shading (Fig. 6 and 7), since it is expected, that an alteration in the $^{14}$C exudation from roots change the $^{14}$C incorporation in the microbial biomass. The slightly decrease of the recovered $^{14}$C in the microorganisms under *Medicago sativa* is probably because of the reduced microbial biomass after shading. However, from the here presented results on reserve C, no conclusions can be drawn in respect to the effect of a limited substrate supply on the total exudation, including recently assimilates. The decreasing microbial biomass after shading (Fig. 3) gives evidence that the total exudation is reduced, since the rhizodeposition is a limiting factor for microbial growth. However, the microbial biomass decrease could also be a result of a changing composition of the root exudates. Shading seems to affect the exudation of plant stored C less than clipping. Since root exudation is important for nutrient uptake by plants, the differences between shaded and clipped treatments can be explained by a different nutrient demand. Shaded plants may show a lower nutrient demand since for these plants the light is the limiting growth factor making a higher nutrient uptake insufficient. Whereas after clipping, plants may have a higher need for nutrients to support the regrowth of shoots.

The results of this study show that shading provokes alterations in the soil CO$_2$ flux (Fig. 8). Root respiration and rhizomicrobial respiration are very closely linked to the supply of assimilates (Kuzyakov and Gavrichkova, 2010). This is supported by my results which show a decrease of the CO$_2$ efflux after shading. The higher $^{14}$CO$_2$ efflux (Fig. 10) seems to
contradict to the decreasing total CO\(_2\) efflux from soil for *Lolium perenne* and for *Medicago sativa*. These results imply an alteration of the preferred fraction of C used for respiration after limiting the substrate supply. In grassland a reduction of soil CO\(_2\) by 40% was observed after shading by Craine et al. (1999), concluding an effect of substrate availability to belowground translocation and soil CO\(_2\) efflux. A reduction was also observed by Yang et al. (2007). Kuzyakov and Cheng (2001) showed a tight connection between rhizosphere respiration and photosynthetic activity by reducing the root-derived CO\(_2\) efflux after exposing wheat plants to a prolonged night period. Like in the present study, Kuzyakov and Cheng (2001) found a contrary effect of lower light conditions for labeled C efflux and total CO\(_2\) efflux from soil with an increased \(^{14}\)C efflux and a decreased total CO\(_2\) efflux. They explained these findings on the one hand by the need for recently assimilated C for maintenance respiration, increasing the \(^{14}\)C efflux and on the other hand by the reduced substrate supply, decreasing the total CO\(_2\) efflux from soil. The connection between a reduced substrate supply after shading and a reduced soil CO\(_2\) efflux matches with my results. Furthermore, my results show evidence that the respiration was not only composed of recently assimilated C but also of retranslocated reserve C. Indeed, respiration of old C was closely related to maintenance what dominates the respiratory costs when relative growth rate was low, like after shading of plants (Lötscher et al., 2004). When compared with the clipped plants, it can be assumed that a higher C demand of other plant parts, like new grown shoots, have a negative effect on the availability of reserve C for root respiration. However, also changing respiration regimes - increased maintenance respiration after shading and increased growth respiration after clipping - with their different demand on stored C and newly assimilated C influence the relative amount of reserve C on the root respiration. The proportions in which the C demand for new grown shoots and the respiration regime contribute to the amount of reserve C on the root respiration cannot be clarified in this study.
5. Restrictions

Some restrictions are to consider concerning the present results of $^{14}$C in the soil and microbial biomass after clipping and shading. The amount of $^{14}$C was investigated only on one date, 15 days after the last labeling, and 10 days after clipping and the shading start. The long time period can induce a reduction of $^{14}$C in soil pools because of an ongoing decomposition. Especially the turnover rate of microbial biomass is very rapid (Rattray, 1995). Thus the $^{14}$C activity could be too low to recognize differences between the treatments and the time when clipping or shading have their greatest impact on reserve C is missed. Also it could be that 10 days after beginning of the treatments the plants are adapted to the impacts and the effects on plant and soil pools are getting lower. Thus, it would be better to have more sampling dates to get a higher time resolution of the amounts of $^{14}$C in the different soil pools. Another restriction is that there is still ‘old’ $^{14}$C, released before clipping, in the soil what can be exchanged between different the pools and possible diminishing differences between the treatments. This causes also problems for the measurement of soil respiration since the $^{14}$C can originate from actual root respiration or degradation of ‘old’ organic substrates released before or ‘new’ organic substrates released after shading or clipping.
6. Conclusion

The results of this study show that limited substrate supply after clipping or shading can alter the C allocation in grassland plants. Shading reduced the plant biomass, whereas after clipping the response was different between the plant species. Whereas for *Lolium perenne* the biomass decreased, the biomass of *Medicago sativa* increased after clipping. Also retranslocation of reserve C was affected by clipping and shading, but the effects varied between both treatments. The redistribution of reserve C after clipping is determined not only by the lower substrate supply but also by the C demand for the regrowth of new shoots. Concretely, clipping induces a higher demand of reserve C for new growing shoots to compensate the lower availability of assimilates due to lower photosynthesis. This happened at expense of other C pools in the plant. In contrast, after shading only the lower substrate supply determines the redistribution of reserve C. After shading, the main observed effect was a higher utilization of stored C for maintenance respiration. These differences indicate that after clipping the removal of biomass is more important for the retranslocation of stored C than limited substrate supply. The results also show that soil microbial biomass decrease after clipping and shading, because of limited C release by roots. The CO$_2$ efflux from soil declined after shading for *Lolium perenne*. The CO$_2$ efflux decreased more after shading compared to clipping, because of a higher growth respiration in response to removal of biomass. For *Lolium perenne* a decrease in soil CO$_2$ efflux was observed only after shading but not after clipping. This indicates that the non-legume *Lolium perenne* and the legume *Medicago sativa* have different mechanisms to cope with clipping. While *Lolium perenne* utilizes stored C mainly for shoot regrowth, *Medicago sativa* has also a high demand of stored C for N$_2$ fixation. This reduces the effect of limited substrate supply and removal of plant biomass on stored C of *Medicago sativa* after defoliation.
7. References


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Eidesstattliche Erklärung

Hiermit erkläre ich, die vorliegende Diplomarbeit selbst verfasst und keine anderen Quellen und Hilfsmittel als die angegebenen verwendet, sowie alle wörtlich und sinngemäß übernommen Stellen in der Arbeit gekennzeichnet zu haben.

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Ort, Datum                          Andreas Schmitt