TECHNISCHE UNIVERSITÄT MÜNCHEN
Lehrstuhl für Grünlandlehre

Carbon residence time in above-ground and below-ground biomass of a grazed grassland community

Inga Schleip

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

genehmigten Dissertation.

Vorsitzender : Univ.-Prof. Dr. J. Kollmann

Prüfer der Dissertation:

1. Univ.-Prof. Dr. J. Schnyder
2. Univ.-Prof. Dr. W. W. Weisser

Die Dissertation wurde am 17.10.2012 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 12.01.2013 angenommen.

Abstract

Aims

The mean residence time of carbon (MRT_C) in the biomass of grassland ecosystems is an important factor in the terrestrial and global carbon (C) cycle, since grasslands cover about 40% of the world's land area. However, studies quantifying MRT_C in the field are scarce. The general aim of the present thesis research was to quantify MRT_C in above-ground species specific and community scale biomass and below-ground particulate organic matter of a grazed grassland ecosystem in different seasons. The specific aims were to (i) test, if MRT_C in structural above-ground plant tissue is determined by the tissue residence time, represented by the leaf life-span. In this context, (ii) a detailed analysis of leaf life-span was necessary. Furthermore (iii) MRT_C in below-ground particulate organic matter was compared to MRT_C in above-ground biomass and seasonal effects on C partitioning to below-ground assessed.

Materials and Methods

All investigations took place in a temperate humid pasture, that was continuously grazed by cattle for more than six years and dominated by the grasses *Lolium perenne* and *Poa pratensis* and the dicots *Taraxacum officinale* and *Trifolium repens*. MRT_C of each species and bulk above-ground biomass and below-ground particulate organic matter (>0.2 mm, including root phytomass) were determined with continuous $^{13}CO_2/^{12}CO_2$-labelling in open-top chambers in three different seasons of two years. A two pool model, consisting of a labile pool and a structural pool was fitted to above-ground tracer time courses revealing MRT_C for labile and structural compounds. The MRT_C of below-ground and bulk above-ground (living *plus* dead) biomass was determined by fitting single exponential decay functions to the tracer time courses. Leaf life-span of the four dominant species was measured.

Results and Discussion

MRT_C of the structural living above-ground C pool was related to the leaf life-span, though it was shorter (approx. 30 days compared to approx. 40 days). The latter discrepancy was possibly related to net growth and/or structural C deposition after full leaf expansion. Since MRT_C of the labile above-ground C pool was on average 5 days, MRT_C of bulk (labile *plus* structural) living above-ground biomass was approx. 20 days. MRT_C in below-ground

particulate organic matter was approx. 1 year in both seasons and thus 6 times longer than MRT_C in living *plus* dead above-ground biomass (2 months on average). It is suggested that this difference relates to a large amount of dead organic material in relation to live root biomass in the below-ground C compartment.

There were no systematic seasonal or interspecific differences in the C residence time above- or below-ground or in leaf life-span. Furthermore, leaf life-spans were short when compared with data from plants growing in less disturbed habitats. It is proposed that these results are a consequence of the adaptation of the community to its years-long history of intensive grazing. The frequent defoliation could lead to a similar shortening of payback-times for all species and observation periods, forcing individuals to construct leaves at low costs with short and similar leaf life-spans and consequently short and similar C residence times.

In autumn a higher percentage of total assimilated C was deposited below-ground (50%) than in spring (35%). This represented the only systematic seasonal effect in this study and reflected the different developmental stages of the sward in spring (mobilization of C for growth and reproductive development) and autumn (deposition of C to stores).

Conclusions

The results of this study indicate that the disturbance history of a sward has important implications for the MRT_C in above-ground biomass. This is due to the influence of defoliation frequency on the leaf life-span via the trade-off between long leaf life-span and short payback times of construction costs, and the linkage of leaf life-span and MRT_C.

Zusammenfassung

Zielsetzung

Die mittlere Verweildauer von Kohlenstoff (MRT_C) in der Biomasse von Grasland-Ökosystemen ist eine wichtige Komponente im terrestrischen und globalen Kohlenstoffkreislauf, da etwa 40% der globalen Landfläche von Grasland bedeckt ist. Dennoch gibt es kaum Studien, die MRT_C im Freiland quantifiziert haben. Das übergeordnete Ziel dieser Arbeit war es, die MRT_C in oberirdischer pflanzenart-spezifischer und gesamter oberirdischer Biomasse und in unterirdischer körniger organischer Substanz („particulate organic matter“) eines beweideten Grasland-Ökosystems in verschiedenen Jahreszeiten zu bestimmen. Die spezifischen Ziele waren es, (i) zu testen, ob die MRT_C in der oberirdischen strukturellen Biomasse durch die Verweildauer des strukturellen Pflanzengewebes selbst, repräsentiert durch die Blattlebensdauer, bestimmt war. In diesem Zusammenhang war (ii) eine detaillierte Analyse der Blattlebensdauer vonnöten. Des Weiteren wurde (iii) die MRT_C in der unterirdischen körnigen Biomasse mit der MRT_C in der oberirdischen Biomasse verglichen und der Einfluss der Jahreszeit auf die Verteilung von Kohlenstoff (C) zu der unterirdischen Biomasse hin untersucht.

Material und Methoden

Alle Untersuchungen fanden auf einer feucht-gemäßigten Weide statt, die seit 6 Jahren von Rindern beweidet war und von den Gräsern *Lolium perenne* und *Poa pratensis* und den Dikotylen *Taraxacum officinale* und *Trifolium repens* dominiert wurde. Die MRT_C jeder Art und der gesamten oberirdischer Biomasse und von unterirdischer körniger Biomasse (>0.2 mm mit Wurzelbiomasse) wurde mit Hilfe von kontinuierlicher $^{13}CO_2/^{12}CO_2$-Markierung in offenen Kammern in drei verschiedenen Perioden innerhalb von zwei Jahren bestimmt. Ein 2-Pool-Modell, bestehend aus einem löslichen Pool und einem strukturellen Pool wurde an die Markierungsverläufe der oberirdischen Biomasse angepasst und so die MRT_C des labilen und strukturellen Pools bestimmt. Die MRT_C der unterirdischen sowie der gesamten oberirdischen Biomasse (lebend und tot) wurde mit dem Fit einer einfach-exponentiellen Zerfallsfunktion an die Markierungsverläufe bestimmt. Zudem wurde die Blattlebensdauer der vier dominanten Arten gemessen.

Ergebnisse und Diskussion

Die MRT_C in dem lebenden oberirdischen strukturellen C-Pool stand in engem Zusammenhang mit der Blattlebensdauer, allerdings war sie kürzer (ca. 30 Tage gegenüber 40 Tagen). Diese Diskrepanz stand wahrscheinlich in Zusammenhang mit Netto-Wachstum und/oder Deposition von strukturellem C nach Beendigung des Blattwachstums. Da die MRT_C des löslichen Pools im Mittel 5 Tage betrug, war die MRT_C der gesamten oberirdischen Biomasse (löslich und strukturell) ungefähr 20 Tage. Die MRT_C der unterirdischen körnigen Biomasse betrug in etwa ein Jahr in beiden analysierten Jahreszeiten und war damit 6 mal so lang wie die MRT_C in der lebenden und toten oberirdischen Biomasse (2 Monate im Mittel). Es wird vorgeschlagen, dass dies durch den großen Anteil an totem organischem Material in der unterirdischen Biomasse im Verhältnis zur lebenden Wurzelbiomasse zustande kommt.

Es gab keine systematischen Unterschiede zwischen den Jahreszeiten und Arten in der MRT_C von unterirdischer und oberirdischer Biomasse und in der Blattlebensdauer. Zudem waren die Blattlebensdauern kurz im Vergleich zu Beobachtungen anderer Autoren in weniger gestörten Habitaten. Dies könnte eine Konsequenz aus der Anpassung des Bestandes an die jahrelange intensive Beweidung sein. Ständige Entblätterung könnte für alle Arten und in allen Untersuchungszeiträumen gleichermaßen zu einer Verkürzung der Rückerstattungszeit für die Blatt-Konstruktionskosten führen und damit die Pflanzen zu günstig produzierten Blättern mit einer kurzen und ähnlichen Blattlebensdauer und folglich einer kurzen und ähnlichen MRT_C zwingen.

Im Herbst wurde ein größerer Anteil an assimiliertem C an die unterirdische Biomasse verteilt (50%) als im Frühjahr (35%). Dies stellte den einzigen systematischen Effekt der Jahreszeit in der gesamten Studie dar und reflektierte den Entwicklungsstand des Bestandes im Frühjahr (Mobilisation von C für Wachstum und Reproduktion) und im Herbst (Einlagerung von C in Speicher).

Schlussfolgerungen

Die Ergebnisse dieser Arbeit legen nahe, dass das langjährige Störungsregime eines Grasland-Bestandes Auswirkungen auf die MRT_C in der oberirdischen Biomasse hat. Dies kommt durch den Einfluss der Entblätterungshäufigkeit auf die Blattlebensdauer zustande, der durch den Zielkonflikt zwischen langer Blattlebensdauer und kurzer Rückerstattungszeit für die Blattkonstruktionskosten gegeben ist, und die Verbindung zwischen Blattlebensdauer und MRT_C.

Contents

List of Figures

List of Tables

1. Introduction

This study is concerned with the carbon (C) residence time at species and community scale in above-ground biomass and in below-ground particulate organic matter of a temperate grazed grassland ecosystem. Terrestrial C cycle research and as part of it, mean residence times of C in terrestrial compartments, have become of major interest, since the relationship between increasing CO_2 concentration in the atmosphere and global climate warming has been recognized (Schimel, 1995). Grasslands contribute significantly to the terrestrial C cycle, since they cover about 40% of the world's land area - excluding Antarctica and Greenland – and contain about one third of the whole terrestrial C (White *et al.*, 2000). Therefore, knowledge of the C residence time in grassland C compartments is crucial for the understanding of the C cycle in the terrestrial and the global biosphere.

The mean residence time of C (MRT_C) in the above-ground biomass determines the time lag between fixation of C in the leaves and its deposition to below-ground via roots and leaf litter fall or its loss as CO_2 via respiration. Globally, about 2/3 of terrestrial C is found below-ground (Schlesinger, 1997) reflected by relatively high root/shoot ratios in temperate grassland ecosystems (approx. 4/1; Jackson *et al*., 1996; Mokany *et al*., 2004). Due to the transformation of plant litter into more persistent compounds, below-ground carbon accumulation accounts for most of an ecosystem's capacity to store organic carbon within a few years (Jones & Donelly, 2004). Below-ground C turnover is thought to be generally much slower (MRT_C 1 – 5 years; Klumpp *et al*., 2007; Personeni & Loiseau, 2004; Van Kessel *et al*., 2006) than above-ground C turnover.

Above-ground C turnover has been studied under controlled conditions in whole shoot biomass (Atkinson & Farrar, 1983; Prosser & Farrar, 1981), in shoot carbohydrates (Farrar & Farrar, 1986; Farrar, 1989; Lattanzi *et al*., 2012) and in shoot C pools feeding respiration (Lehmeier *et al*., 2008; Carbone & Trumbore, 2007; Bahn *et al*., 2009) revealing MRT_C in shoot C compartments in the dimension of days. However, to the author's best knowledge, there is no study quantifying the C residence time in above-ground biomass in the field and no study reporting on MRT_C in both above- and below-ground biomass in the field.

Carbon and tissue residence time

Essentially all organic carbon in terrestrial ecosystems derives from CO_2 that is photosynthetically assimilated by plants. Almost all of this organic carbon is eventually returned back to the atmosphere via respiration. After fixation in plants there are two principal fates of C. Either C serves as substrate for respiration or it is incorporated in structural biomass during growth. The second way is of particular interest, since most C is present in structural compounds (approx. 70% in *Lolium perenne*, Robson & Deacon, 1978) and in minimum half of all assimilated C is used for synthesis of structural biomass (*L. perenne*, Lehmeier *et al*., 2008; Lehmeier *et al*., 2012). C bound in structural compounds is not recycled within the plant (Robson & Deacon, 1978), but is lost from plants with the shedding of dead shoot and root tissue. This material then becomes available as substrate for heterotrophic respiration.

The residence time of structural C within the plant, i.e. the time lag between the incorporation of C in structural compounds during growth and the loss from the plant via litter fall, should be very closely related to the residence time of the structural tissue itself. In grasslands, where above-ground biomass mainly consists of leaves (including lamina and sheath tissues), the residence time of structural tissue is determined by the longevity of the leaves, i.e. the leaf life-span. Therefore, one should expect a close relationship between the leaf life-span in a community and the mean residence time of C in the structural tissue and probably the whole above-ground biomass.

Leaf life-span

Leaf life-span is a trait of great ecological meaning (Kikuzawa & Lechowicz, 2011) with, as I hypothesize (see above), major implications for ecosystem C cycling. Across species gradients, short leaf life-span is associated with both low leaf mass per unit area and high photosynthetic capacity per unit leaf mass (Reich *et al*., 1992; Wright *et al*., 2004). Conversely, long leaf life-span is related to a longer payback time, defined as the relation of construction costs to daily photosynthesis (Williams *et al*., 1989; Navas *et al*., 2003; Coste *et al*., 2011). Leaf life-span has therefore been considered indicative of the species' trade-off between productivity (rate of resource acquisition) and persistence (resource retention) (Westoby *et al*., 2002; Hikosaka, 2005).

Ryser & Urbas (2000) found a negative correlation between leaf life-span and ecological indices of disturbance frequency, across several grass species, noting that the nutrient conservation often associated with greater longevity was superimposed by the influence of disturbance frequency. This suggests that long-lived leaves would be disadvantageous when leaf survival is limited by regular disturbance, possibly because (parts of) leaves are lost from the plant before complete pay-back of construction costs. Accordingly Lemaire *et al.* (2009) argue that long-lived leaves have a higher probability of defoliation than short-lived leaves under a given grazing intensity. Further, it is proposed that severe disturbance generally leads to lower functional diversity (e.g. intermediate disturbance theory: Grime, 1973; Biswas & Malik, 2010; Duru *et al.*, 2012). For these reasons, I hypothesize that the leaf life-span of the dominant species in a habitat with frequent defoliation, such as intensively grazed grassland, lies in a narrow range and is rather short. This would also affect the mean residence time of C in the above-ground biomass, provided that leaf life-span was a major determinant of MRT_C in the above-ground biomass, as hypothesized here. Then similar patterns as for leaf life-span could be expected and thus also a more or less common and rather short MRT_C in the above-ground biomass.

Overview and aims

All investigations of the present study took place in a temperate humid grassland ecosystem at the Grünschwaige Grassland Research Station in three investigation periods with relatively similar temperatures over two years (two autumns and one spring). The sward had been grazed continuously by cattle for more than 6 years and was dominated by the perennial grasses *Lolium perenne* and *Poa pratensis*, the rosette forming herb *Taraxacum officinale* and the stoloniferous legume *Trifolium repens*.

The general aim of this work was to quantify C residence times in above- and below-ground C compartments and to gain a better understanding of their determinants, including the leaf life-span and seasonal, interannual and interspecific effects. C residence time of living shoots at species scale and bulk above-ground biomass of the community was analyzed and brought into relation with the measured leaf life-span. In this context, I performed a detailed analysis of leaf life-span, which also addressed methodological aspects. C residence times of below-ground particulate organic matter was compared to above-ground C residence time and seasonal effects on C partitioning to below-ground were analyzed.

In particular, the questions in this thesis were addressed in three main parts:

In chapter 3, a detailed analysis of leaf life-span was performed and the following specific questions were addressed: (1) is the leaf life-span of co-dominant species in an intensively grazed pasture similar? (2) Are there seasonal and/or interannual changes in the leaf life-span? (3) Is the species comparison, e.g. between grasses and dicots, altered by different leaf death definitions? And lastly, (4) how does the leaf life-span found in this work relate to published leaf-life span values of dominant species in similarly disturbed grasslands?
These questions were answered by studying the leaf life-span of the four co-dominant species in the analyzed pasture in different seasons and compiling data from the literature, both of the same species growing relatively undisturbed, and of other species of similarly grazed grasslands. The analysis of the influence of different leaf death definitions on interspecific comparison of leaf life-span was necessary, because comparisons between published studies were complicated by different operational definitions of leaf death.

In chapter 4, the man residence time of C in above-ground biomass was analyzed and the following questions were answered: (1) Is the carbon residence time in structural tissue of grassland plants similar to the leaf life-span? (2) Are there seasonal, interannual and/or interspecific differences in MRT_C in above-ground biomass? And related to that (3) how do above-ground species-specific MRT_C compare to MRT_C on a community scale?
To this end, three 15 to 16 day-long continuous ^{13}C-labelling experiments (also termed dynamic labeling, Ratcliffe & Shachar-Hill, 2006) were conducted in two seasons over two years. For that purpose a recently developed open-top chamber system was used (Gamnitzer *et al*., 2009), which allowed for precisely controlled application of ^{13}C-labelled CO_2 under ambient CO_2 concentration in the field. A two pool carbon turnover model of shoot biomass, which included a labile, well mixed C pool and a structural C pool, was fitted to the C tracer time courses of living shoots of the four studied species and the living bulk above-ground biomass. In the model the structural pool was fed by the labile pool and followed a first-in-first-out mechanism. Model-derived MRT_C were compared to the leaf life-span data. This procedure gave rise to the last question: (4) Is the proposed two pool C model adequate for the description of the C tracer time courses of living above-ground biomass?

In chapter 5, the carbon residence time in the below-ground particulate organic matter (>2 mm of diameter, POM) and in the bulk above-ground biomass and the C partitioning to below-ground were investigated. This chapter addressed the following specific questions: (1) Are there seasonal differences in MRT_C in POM or carbon partitioning to below-ground? And (2) how does the carbon turnover of below-ground particulate organic matter compare to that of above-ground biomass?
To this end, carbon tracer time courses of POM and bulk above-ground biomass, which were produced in the same labelling experiments as mentioned above, were fitted with single exponential decay functions revealing MRT_C of POM and bulk above-ground biomass.

The thesis is organized as follows: Materials and Methods of all observations and experiments are compiled together (chapter 2), whereas the results of each of the three main parts are followed directly by a specific discussion section. The interrelationships of all parts are considered in the final summarizing discussion (chapter 6).

2. Materials and Methods

Location

All observations and experiments took place on a grazed pasture paddock (paddock number 8) at the Grünschwaige Grassland Research Station (435 m.a.s.l.) near Freising, Germany. The climate of the area is temperate humid, with a mean annual air temperature of 9 °C and mean annual precipitation of 775 mm. For more details (including climate, soil characteristics and management practices) see Schnyder *et al*. (2006). All experiments were performed in the middle of the paddock, which was continuously grazed by cattle since sowing in 1999 maintaining a nominal compressed sward height of 7 cm throughout the growing season. The sward was dominated by the four studied species, the two perennial grasses *Lolium perenne* and *Poa pratensis* and the dicots *Taraxacum officinale*, a rosette forming herb, and *Trifolium repens*, a stoloniferous legume. Those four species are frequent members of intensively managed pasture communities in temperate humid climates. Grasses accounted for approx. 70% of the standing dry matter biomass. The area has not been fertilized since sowing, and only received nutrients in the form of faeces from the grazing cattle and atmospheric deposition. Investigations took place in three different periods, autumn 2006 and spring and autumn 2007. Two weeks before the beginning of each measurement period, grazing cattle was excluded from the measurement site.

Leaf life-span measurements

Measurement periods

Interspecific differences in leaf life-span, phyllotherm –the delay between the appearances of successive leaves on a tiller expressed in thermal time– and number of live leaves, as well as the effects of different operational definitions of leaf death and the validation of measurement methods, were all assessed over a 3-month-period during autumn 2007 (27 Aug. – 28 Nov. 2007). For this, 10 individuals per species were chosen in each of two transects, where individual refers to a main tiller in the grass species, a single plant in *T. officinale*, and a stolon in *T. repens*. Due to losses the initial number of 20 individuals decreased, so that in the

end data from 16, 7, 18 and 20 individuals of *L. perenne*, *P. pratensis*, *T. officinale* and *T. repens* were analysed.

Seasonal effects were assessed on six to twelve individuals per species over three observation periods with relatively similar temperatures, each lasting 16 days, in autumn 2006 (28 Aug. – 13 Sep. 2006) and spring 2007 (13 May – 29 May 2007) and autumn 2007 (10 Sep. – 26 Sep. 2007). These individuals grew inside open top chambers placed in the same pasture (Gamnitzer *et al*. 2009). Mean air temperature was near identical in- and outside the chambers (Gamnitzer *et al*. 2009). In autumn 2007, the number of live leaves, phyllotherm and leaf life-span was compared for plants growing inside and outside of the chambers. No significant chamber effect was observed.

Data collection

Measurements were performed on average-sized individuals marked with little plastic rings. Observations were made on alternate days, except in late autumn of 2007, when observation intervals were increased up to nine days due to low temperatures. At each observation date, the following parameters were recorded:

Number of leaves

The number of live leaves (n_L) was recorded for every individual on every observation date. Five operational definitions of leaf death were used: a leaf was considered dead when either 5, 25, 50, 75 or 100% of its total area (length, in grasses) was chlorotic. The corresponding number of live leaves is referred to as $n_{L\text{-}5}$, $n_{L\text{-}25}$, $n_{L\text{-}50}$, $n_{L\text{-}75}$ and $n_{L\text{-}100}$, respectively. In autumn 2006, $n_{L\text{-}25}$ was not measured but interpolated as $n_{L\text{-}5} + 0.4 * (n_{L\text{-}100} - n_{L\text{-}5})$, a relationship inferred from the observations in 2007. Senescence classification was done by eye.

Phyllotherm

At each observation new leaves were marked by a dot of paint. In grasses and *T. officinale* a leaf was defined as 'new' when its tip was first visible, and in *T. repens*, when the still-folded leaflets were fully visible (*i.e.* uncovered by the stipules). The average of newly appeared leaves per individual (n_A) was estimated as the total number of newly appeared leaves divided by the number of individuals observed.

The phyllotherm (t_{Phyll}) was then calculated as

$$t_{Phyll} = gdd_{OP} / n_A \quad (1)$$

where gdd_{OP} is the sum of growing degree days of the observation period. The uncertainty of phyllotherm was calculated by Gaussian error propagation of the uncertainty of n_A. Importantly, Eq. (1) is also suitable for datasets including individuals with no leaf appearance during the observation period.

Growing degree days (gdd) were calculated as

$gdd = \Sigma (T - \vartheta_0)$, with values of $(T - \vartheta_0) < 0$ set to 0 (2)

where T is the daily mean soil temperature (in ^{o}C) at 10 cm depth and ϑ_0 is the base temperature, below which it is assumed that leaves do not grow. Hourly mean soil temperature at 10 cm depth was measured by a soil temperature sensor (Th2f, UMS GmbH, München, Germany). Using soil temperature accounted for the fact that apices and leaf growth zones of the studied species were located just below or in close vicinity to the soil surface (Peacock, 1975; Davies & Thomas, 1983). Additionally air temperature at 50 cm above soil surface was recorded (Mini-Psychrometer MP 101, Gottfried Herzig, Braunschweig, Germany).

Assuming a linear relationship between leaf appearance rate and temperature in the range of the measurements, the base temperature was calculated for each species, during autumn 2007, as the x-intercept of the linear regression of leaf appearance rate against temperature (average $R^2 = 0.87$). Estimates were close to 4 °C for grasses and *T. officinale* and 6 °C for *T. repens*. These results are similar to published values for grasses (Lemaire *et al.*, 2000; Berone *et al.*, 2007) and *T. repens* (Chapman, 1983). To enable interspecific comparisons for the same observation periods, the same base temperature of 4 °C was used for all species.

Leaf life-span

Leaf life-span (t_L) was calculated as

$t_L = t_{Phyll} * n_L$ (3)

according to the above-mentioned leaf death criteria, and correspondingly denoted as $t_{L\text{-}5}$, $t_{L\text{-}25}$, $t_{L\text{-}50}$, $t_{L\text{-}75}$ and $t_{L\text{-}100}$ (*i.e.* substituting $n_{L\text{-}5}$ into equation (3) gives $t_{L\text{-}5}$, and so on). The uncertainty of t_L was calculated by Gaussian error propagation of the uncertainties of t_{Phyll} and n_L. The n_L was first averaged over the observation period for every individual, and then over all individuals per species. In *T. repens*, only the number of live leaves (n_L) of the time before late October were used. Expressing leaf life-span in gdd rather than days reduced the coefficient of variation by approx. 50% in the present study.

Equation (3) is based on the fact that many grassland species show a succeeding type of leaf production (Kikuzawa, 1984) and progressive senescence (Leopold, 1961), where plants, mainly consisting of leaves, have growing and senescing leaves at the same time. This mechanism keeps the morphology of plants in the vegetative state relatively unaltered and leads to a close interrelationship between the leaf life-span, the phyllotherm and the number of live leaves on the tiller. These dynamics have been thoroughly investigated in grass species (Lemaire & Chapman, 1996; Matthew *et al.*, 2001), and equation (3) has been validated by Lemaire & Agnusdei (2000) for C3 and C4 grasses of a grazed community. For dicotyledonous plants with a succeeding type of leaf production and progressive senescence the same dynamics should be expected.

Validation of equation (1) and equation (3)

Estimated phyllotherms were nearly identical when calculated with either equation (1) or as the thermal time between the appearance of two successive leaves in an individual, during the three-month observation period in autumn 2007. Measures of uncertainty –standard deviation, standard error or confidence interval– were also similar. Likewise, leaf life-span estimated with equation (3) compared well to leaf life-span estimated as the thermal time between the appearance and death of individual leaves, except for *T. repens* (Figure 1). Uncertainties were generally higher when calculated with equation (3). The reason for the divergent estimates in *T. repens* was a departure from steady state conditions, as n_L of *T. repens* started to decrease in late October 2007, probably due to low temperatures. This accelerated leaf senescence and in consequence, shortened leaf life-span, when estimated for individual leaves. In contrast, n_L entering equation (3) were primarily influenced by temperatures before late October (for method validation, values of n_L of the whole observation period were used).

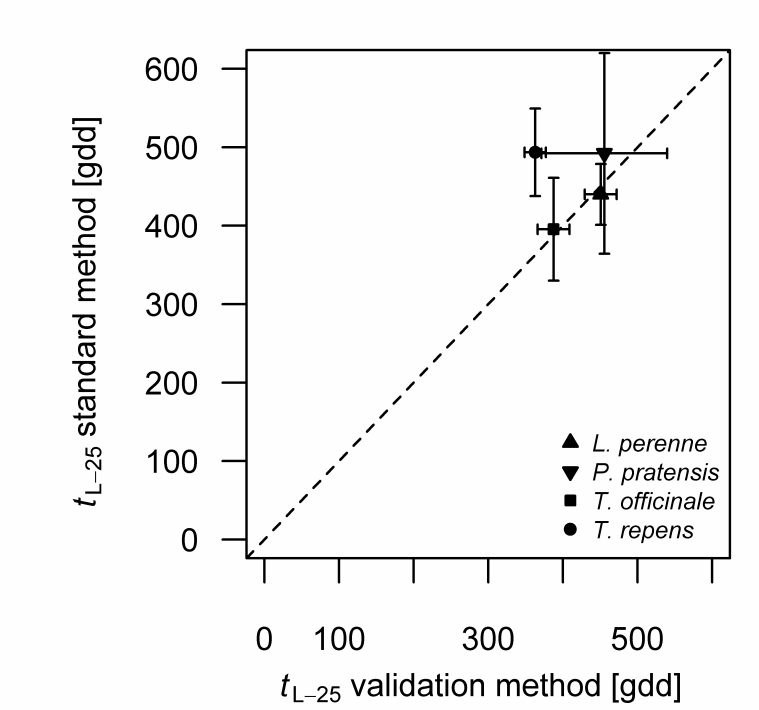

Figure 1: Comparison of the leaf life-span (t_{L-25}) calculated with the standard method (t_{L-25} = t_{Phyll} * n_{L-25}) and validation method (t_{L-25} = thermal time (gdd) between leaf appearance and 25% of senescence for individual leaves averaged per species) in the three-month observation period in autumn 2007. The pattern was similar for all leaf life-span definitions. The base temperature used was 4 °C for all species. Numbers are means of 16, 7, 18 and 20 individuals of *Lolium perenne*, *Poa pratensis*, *Taraxacum officinale* and *Trifolium repens*, respectively, and error bars denote 95%-confidence intervals.

Labelling experiments

Labelling procedure

Three continuous labelling experiments with open-top chambers, each lasting 15 or 16 days, were carried out in autumn 2006 (28 Aug. – 13 Sep. 2006) and spring and autumn 2007 (13 May – 29 May 2007, 10 Sep. – 26 Sep.2007).

A detailed description of the chamber system for labelling was presented by Gamnitzer *et al*, 2009. In short, four Plexiglas chambers, open at their tops to the atmosphere ("open-top chambers") were flushed with air containing CO_2 of the desired concentration and carbon isotopic composition. CO_2 concentration inside the chamber was 366, 367 and 375 μmol mol^{-1} CO_2 at noon in autumn 2006, spring 2007 and autumn 2007, respectively, which was similar to ambient conditions. Carbon isotope composition (presented as $\delta^{13}C = R_{sample}/R_{standard} - 1$, where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios in the sample and in the international VPDB standard, respectively) of CO_2 inside the chamber was -43.8‰, -46.9‰ and -47.8‰ (assimilation weighted mean), respectively and was kept constant (± 0.4‰ SD) throughout the whole labelling durations. The labelling CO_2 was depleted in ^{13}C compared to ambient CO_2, which had a $\delta^{13}C$ of -8.3‰ and -8.5‰ in spring and autumn 2007, respectively (autumn 2006 not measured).

In autumn 2006 two chambers were used for labelling of two sites (in close proximity) throughout a 15 days-long period. In spring and autumn 2007 another labelling scheme was used: In both seasons ten sites were labelled individually for 1, 2, 4, 8 and 16 days with two replications each, respectively (Gamnitzer *et al.* 2009). All labelling was performed within the 16-day long periods with four open-top chambers. Two chambers were used to label two sites for the entire 16 day-long periods, the other two rotated between sites for the shorter labelling durations. In that way, replicates of labelling durations resulted not only from different sites, but also from the labelling at different dates. Each morning at sunrise each chamber was watered with the equivalent of the previous day's evaporation plus an extra 20% to account for run-off (5 – 10 mm in total). Mean air temperature was nearly identical in- and outside the chambers in autumn 2006 and spring 2007 and approx. 1°C warmer inside the chambers in autumn 2007. Relative humidity inside the chambers was approx. 20% less than outside due to the feeding of dry air (inside: approx. 51% on average over 24 h, ambient: approx. 71% on

average over 24 h). But, overall, the modifications of climatic conditions inside the open-top chambers were quite modest.

Harvests and Sample preparation

Shoots and bulk above-ground biomass

In all experiments four (2006) to eight (2007) replicate plants per species and three to four community level samples (only in 2007) per sampling time were harvested out of the chambers. Harvests took place during eight (2006) or seven (2007) sampling times for species-specific and five sampling times for community scale samples within the respective whole labelling period. The plants were sampled daily in the beginning of the labelling period (where largest changes in f_{old} were expected) and less frequent towards the end of the labelling period. In addition eight control plants per species and six control samples at community scale were harvested outside of the chambers. All samples were harvested in the dark to prevent an uptake of ambient CO_2 by the plants, since the chambers had to be removed to gain access to the canopy. Harvests of shoots took place approx. 3.5 – 5.5 h after sunset. Average sized individuals (a mature tiller in grasses as defined in De Visser *et al.* (1993), a single plant in *T. officinale*, and a stolon in *T. repens*) were cut close to soil surface and immediately cooled in a transportable cooling box. After transfer to the laboratory green, senescing and senescent leaves (grasses and *T. officinale*) or stolon sections bearing green, senescing and senescent leaves (*T. repens*) were separated. Senescing leaves (partly green, partly senescent) were discarded. For community scale samples squares of 14 cm width and length per chamber were cut close to soil surface before sunrise and immediately cooled in a transportable cooling box. Species and community scale samples were oven dried at 105 °C for 1 hour and at 60 °C for 56 h. In the dried state community samples were separated into living and dead material according to colour. All dried species and community samples were weighed and ground to a homogeneous fine powder in a ball mill and dried again for 24 h at 60 °C.

Below-ground biomass

In spring and autumn 2007 eight soil cores of 5 cm diameter and 20 cm depth were taken inside the chambers before sunrise during five sampling times within the whole labelling period. In addition, eight control samples from outside the chambers were taken. Due to the shallow soils (gravel at ≥ 20 cm depth) it was assumed that more than 90% of root and soil

organic matter was present in the top 20 cm of the soil. After harvests samples were immediately cooled in a transportable cooling box and transported to the laboratory. There remaining stubbles were removed from the root blocks. Then root blocks were crushed and immediately washed and sieved using sieves with mesh sizes of 1 mm, 0.63 mm and 0.2 mm. The material retained by each sieve was washed until the water ran clear. Where necessary, density flotation in water was used to separate organic material from mineral substrate. Similar to Personeni & Loiseau (2004) I defined the material > 1 mm as coarse particulate organic matter including root phytomass (POM_c), the fraction between 0.63 and 1 mm as medium particulate organic matter (POM_m) and the fraction between 0.2 and 0.63 mm as fine particulate organic matter (POM_f). Similar to above-ground samples, all POM samples were oven dried at 105 °C for 1 hour and at 60 °C for 56 h. All dried samples were weighed and ground to a homogeneous fine powder in a ball mill and dried again for 24 h at 60 °C.

Elemental and isotope analysis

Aliquots of 0.70 ± 0.05 mg of each sample were weighed into tin cups (IVA Analysentechnik e.K., Meerbusch, Germany) and combusted in an elemental analyzer (NA 1110; Carlo Erba Instruments, Milan, Italy), interfaced (Conflo III, Finnigan MAT, Bremen, Germany) to a continuous-flow isotope-ratio mass spectrometer (CF- IRMS; Delta Plus, Finnigan MAT).

Each sample was measured against a laboratory working CO_2 gas standard, which had previously been calibrated against a secondary isotope standard (IAEA-CH6 for ^{13}C, accuracy of calibration ± 0.06‰ SD). After every tenth sample a solid internal laboratory standard (fine ground wheat flour) was run to estimate the precision of the isotope analysis. The solid internal laboratory standard had a similar C/N ratio as the respective sample material and had previously been calibrated against the international IAEA-CH6 standard. The precision of sample repeats was 0.15‰ SD.

$\delta^{13}C$ of community scale bulk above-ground biomass (living *plus* dead) was calculated as the C mass-weighted mean of living and dead biomass. $\delta^{13}C$ of bulk below-ground POM (POM_c *plus* POM_m *plus* POM_f) was calculated as the C mass-weighted mean of the single POM fractions.

Data analysis

The fractions of labelled and unlabelled C in plant tissue or particulate organic matter (f_{new} and f_{old}) were calculated similarly to Schnyder and De Visser (1999) from the $\delta^{13}C$ of the labelled plant samples (δ_{plant}) according to mass balance considerations as

$$f_{new} = (\delta_{plant} - \delta_{old}) / (\delta_{new} - \delta_{old}) \quad (4)$$

$$f_{old} = 1 - f_{new} \quad (5)$$

where δ_{old} and δ_{new} are the ^{13}C signatures of non-labelled plant tissue of control plants and of plant tissue entirely grown in the labelled atmosphere, respectively. Since the labelling duration was too short to achieve isotopic equilibrium of the plants under the new labelled atmosphere, δ_{new} was estimated from C isotope discrimination (Δ) and the carbon isotope signature of the CO_2 inside the chambers (δ_{lab}) as in Schnyder *et al*. (2003):

$$\delta_{new} = (\delta_{lab} - \Delta) / (1 + \Delta) \quad (6)$$

The discrimination, Δ, was calculated with unlabelled plant tissue and the ^{13}C signature of the unlabelled ambient air CO_2 during the day (δ_{amb}) as

$$\Delta = (\delta_{amb} - \delta_{old}) / (1 + \delta_{old}) \quad (7)$$

with the assumption that the C isotope discrimination was not influenced by the conditions inside the open top chambers and therefore the same for labelled and unlabelled plant tissue.

Models for carbon tracer time courses

Two pool model for shoots and living bulk above-ground biomass

The living shoots of the species were considered to consist of two pools, a first one called labile pool, built of all material, which can be remobilised within the plant and a second one called structural pool. In the structural pool all material is contained, that cannot be remobilised, and is lost from green plant tissue during senescence (Figure 2a). In this work, the sum of the labile and structural pool refers to only living plant biomass, i.e. green tissue in case of above-ground biomass. The structural pool is assumed to consist of cellulose, hemicellulose, lignin, pectin and structural proteins and the labile pool is assumed to consist of all other materials such as non-structural carbohydrates (glucose, fructose, sucrose, fructan, starch), organic acids, amino acids and soluble proteins.

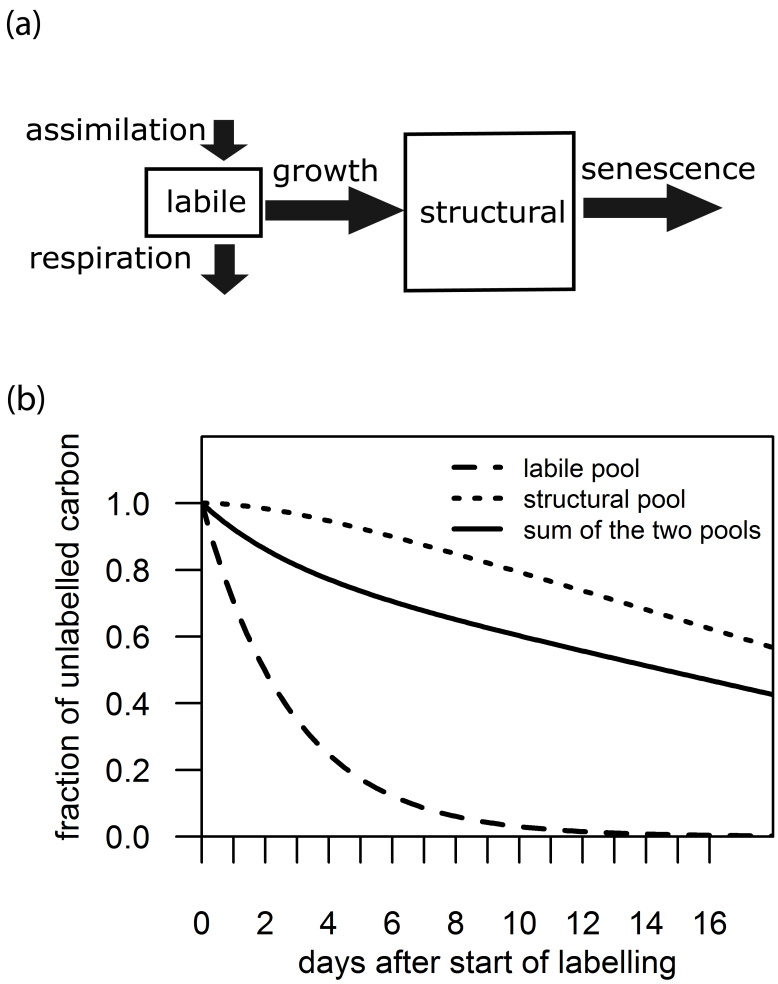

Figure 2: Two-pool model of carbon in the shoot and (b) theoretical carbon tracer kinetics of the two pools and the shoot (i.e. the sum of the two pools) with the following parameterization: mean residence time of C in the labile pool: 2 days; ratio structural carbon relative to total carbon = 75%; leaf life-span = 35 days.

Carbon enters the labile pool *via* photosynthesis and is lost by respiration or incorporated in the structural pool during growth and differentiation processes. The labile pool is assumed to be homogenous and well-mixed. The dilution by labelled carbon would therefore follow a one-term exponential decay function in carbon tracer experiments (Figure 2b). In contrast, carbon entering the structural pool stays in it until it is lost during senescence. The time span between the incorporation of C into structural material and its loss represents the mean residence time of carbon in the structural tissue. This time span is hypothesised to be similar to the leaf life-span. The structural pool therefore represents a first-in-first-out mechanism and the dilution of unlabelled carbon in the isolated structural pool would therefore follow a linear decay function in carbon tracer experiments. However, since carbon enters the structural pool

via the labile pool, the C flow into the structural pool is not totally labelled from the first day of labelling on, but contains the isotopic footprint of the labile pool. Thus, the dilution of unlabelled carbon in the structural pool contains both an exponential and a linear part. The dilution of unlabelled carbon of the whole shoot then is represented by the sum of both pools. The shape of the C tracer time courses of the whole shoot is determined by three parameters: the mean residence time of C in the labile pool ($MRT_{\text{C-Labile}}$) (or halftime or turnover rate), the relative ratio of both pools and the mean residence time of C in the structural pool, $MRT_{\text{C-Structural}}$. The dilution of unlabelled carbon in the shoots is linked to the increase of labelled carbon as (compare equation 5)

$$f_{old}(t) = 1 - f_{new}(t) \quad (8)$$

and the increase of the fraction of labelled carbon in the shoots and the community scale above-ground biomass is given by

$$f_{new}(t) = (1 - a) * (1 - \text{EXP}(-(1/MRT_{\text{C-Labile}}) * t)) + a * 1/MRT_{\text{C-Structural}} * (t + MRT_{\text{C-Labile}} * \text{EXP}(-(1/MRT_{\text{C-Labile}}) * t) - MRT_{\text{C-Labile}}) \quad (9)$$

where a is the fraction of the structural pool in total C. The first summand represents the labile pool, weighted by (1- a) and the second summand represents the structural pool, weighted by a. The turnover rate of the labile and structural pool equals $1/MRT_{\text{C-Labile}}$ and $1/MRT_{\text{C-Structural}}$, respectively. Equation (2) was fitted to the carbon tracer time courses and $MRT_{\text{C-Structural}}$ and $MRT_{\text{C-Labile}}$ were optimized in terms of a minimized RMSE with the Software Table Curve (Systat Software GmbH, Erkrath, Germany).

The value of a was approximated by extracting hot water soluble carbon and starch carbon from the samples and defining the leftover as structural carbon similar as in Grimoldi *et al.* (2005). In detail, 10 mg dry mass of eight samples per species and community biomass in spring and autumn 2007 were extracted with 2 ml of distilled water for 10 min at 93 °C and for 45 min at room temperature (Schnyder & de Visser, 1999). After centrifugation (10000g for 15 min), 300 µl of the supernatant were pipetted into Tin Cups and oven dried at 60 °C. The amount of carbon was then determined in an elemental analyzer, using sulphanilamide (Merck, Darmstadt, Germany) as a standard. Finally, the residual pellets were hydrolysed in a mixture of 5 ml of dimethylsulfoxide (DMSO) and 1.25 ml HCl (8M) for 30 min at 60 °C. Starch was determined colorimetrically after neutralization with 1.25 ml NaOH (8M) and equilibration with citric buffer (0.112 M; pH = 4) by an enzymatic test-combination (Cat. Nr. 207748, Boehringer, Mannheim, Germany). Structural C was estimated as

$C_{Structural} = C_{Total} - (C_{Soluble} + C_{Starch})$ (10)

and a as

$a = C_{Structural} / C_{Total}$ (11)

$MRT_{C\text{-}Structural}$ was compared to the leaf life-span (measured as described above), defined as the time from leaf appearance till the beginning of senescence ($t_{L\text{-}5}$), in accordance with the harvest of living green plant material for C isotope analysis, and expressed in days.

One pool model for particulate organic matter and bulk above-ground biomass

The carbon tracer time courses of bulk above- and below-ground biomass were fitted with one term exponential decay functions revealing MRT_C as

$f_{old}(t) = \text{EXP}(-1/MRT_C * t)$ (12)

The absolute amount of new carbon at each labelling day t (C_{new}, g m^{-2}) was calculated as

$C_{new}(t) = f_{new}(t) * C(t)$ (13)

where $C(t)$ is the carbon mass of each sample at day t. Net carbon partitioning to below-ground at time t ($NCP(t)$) was calculated as

$NCP(t) = C_{new\text{-}below\text{-}ground}(t) / (C_{new\text{-}below\text{-}ground}(t) + C_{new\text{-}above\text{-}ground}(t))$ (14)

Statistical Analysis

All statistical tests were performed with the statistical software R (R Development Core Team, 2011) or Excel (Microsoft Corporation, Redmond, USA).

Leaf life-span, phyllotherm and number of live leaves

To test for normal distribution and equal variances, the Andersen Darling Test and Fisher's F-Test were used, respectively. While all n_L were normally distributed, phyllotherm and leaf life-span could not be tested due to the calculation procedures (no "real" replicates). When calculated with the validation methods (see above) on individuals (phyllotherm) and cohorts of individual leaves (leaf life-span), both, phyllotherm and leaf life-span were normally distributed. Inequality of variances was evident, when equation (3) and the validation method for estimating leaf life-span were compared. For the comparison of two means Students t-test (equal variances) or Welch-Test (unequal variances) and for the comparison of several means analysis of variance (ANOVA) and pair wise t-tests with Bonferroni adjustments were performed, all at 95% significance level.

Mean residence times of carbon

Species and seasonal effects on the modelled MRT_C were tested with one-way ANOVA on summary data (Heiberger, 2009). Due to species and season interactions each season and species was then tested separately with Tukey´s range test and Duncan´s test to compare several means (da Silva, 2010). Both tests gave the same results. Differences between $MRT_{C\text{-}Structural}$ and measured leaf life-span were analysed by subtracting the $MRT_{C\text{-}Structural}$ from the corresponding measured leaf life-span. A t-test (null hypothesis: mean of difference = 0) was performed with the mean of the derived (normally distributed) differences.

3. Common leaf life-span of co-dominant species in a continuously grazed temperate pasture

Results

Weather

Daily soil temperature at 10 cm depth averaged 17.6 °C, 17.9 °C and 15.0 °C in the 16-day observation periods in autumn 2006, spring 2007 and autumn 2007, respectively. Mean daily air temperatures (at 50 cm above soil surface) were 15.3 °C, 15.1 °C and 11.5 °C, respectively. Air and soil temperatures during the three-month observation period in autumn 2007 are shown in Figure 3a. The temperatures in autumn 2007 and spring 2007 were not different from long-time averages. Conversely, temperatures during autumn 2006 were about 2-3°C warmer than usual. Mean daily temperatures during observations never exceeded 21 °C, but temperatures were close to or below the base temperature of *T. repens* (6 °C) from mid October 2007 onwards. By mid November 2007 temperatures had dropped below the base temperature of all species (Figure 3a).

Influence of the definition of leaf death on estimated leaf life-span

As expected, different operational definitions of leaf death had a significant effect on the estimated number of live leaves. On average, $n_{L\text{-}100}$ was 35% higher than $n_{L\text{-}5}$. This difference was directly translated to the estimated leaf life-span (Equation (3), Figure 4). Importantly, the magnitude of the difference between definitions was not constant. For instance, $t_{L\text{-}100}$ was 10 to 85% longer than $t_{L\text{-}5}$, depending on season and species. The differences were quite variable, but tended to be greater in grasses than in dicots and greater in autumn than in spring (data not shown).

Henceforth, I defined the number of live leaves as $n_{L\text{-}25}$ and leaf life-span as $t_{L\text{-}25}$, the leaf life-span measured from leaf appearance till 25% of leaf senescence (as defined by Diemer *et al.*, 1992, and as discussed below).

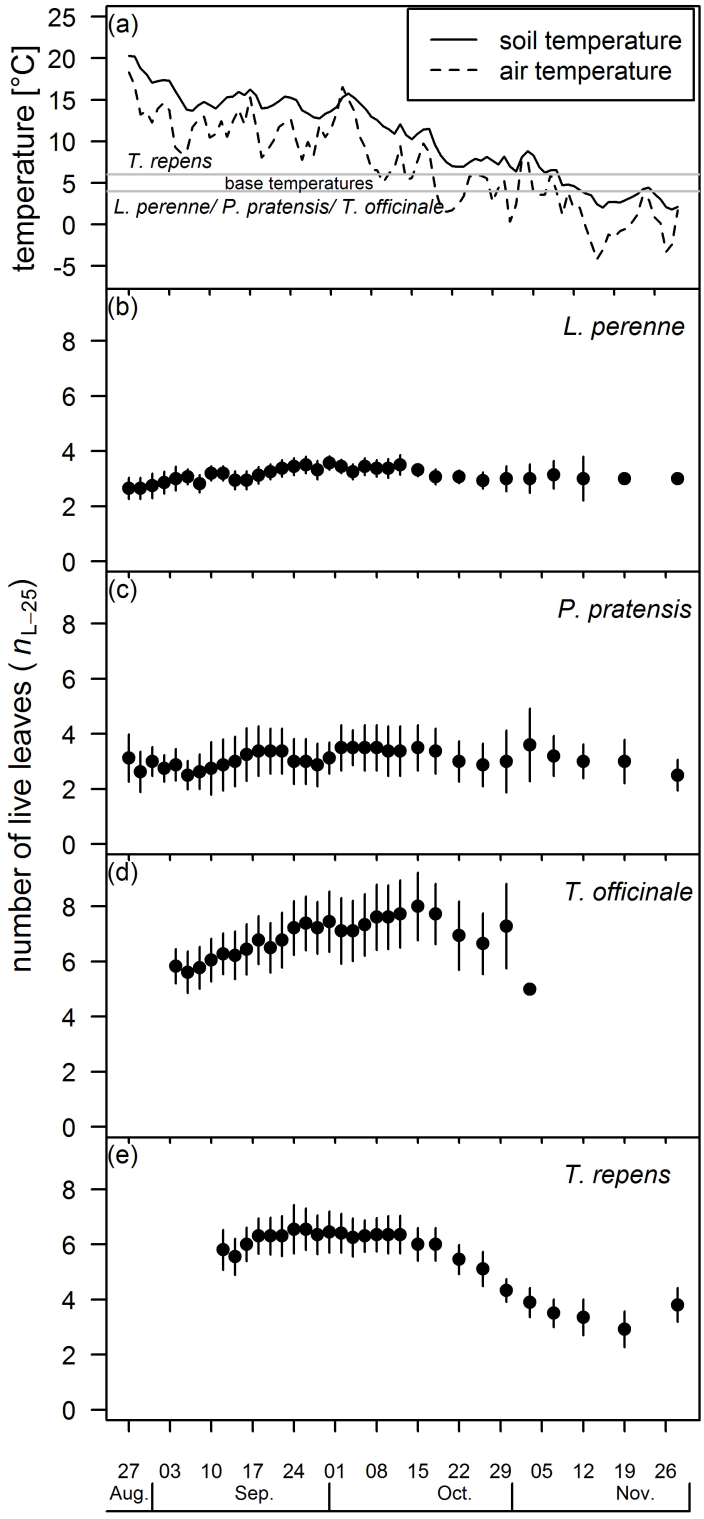

Figure 3: (a) Daily mean temperatures of soil and air (10 cm below, solid line, and 50 cm above soil surface, dashed line, respectively) and (b)–(d) the number of live leaves per individual with less than 25% chlorotic leaf area (n_{L-25}) in the three-month observation period in autumn 2007. The pattern of n_L over time was the same for all n_L definitions. Sample sizes were 16, 7, 18 and 20 individuals of *Lolium perenne*, *Poa pratensis*, *Taraxacum officinale* and *Trifolium repens*, respectively. Error bars denote the 95%-confidence interval of the mean.

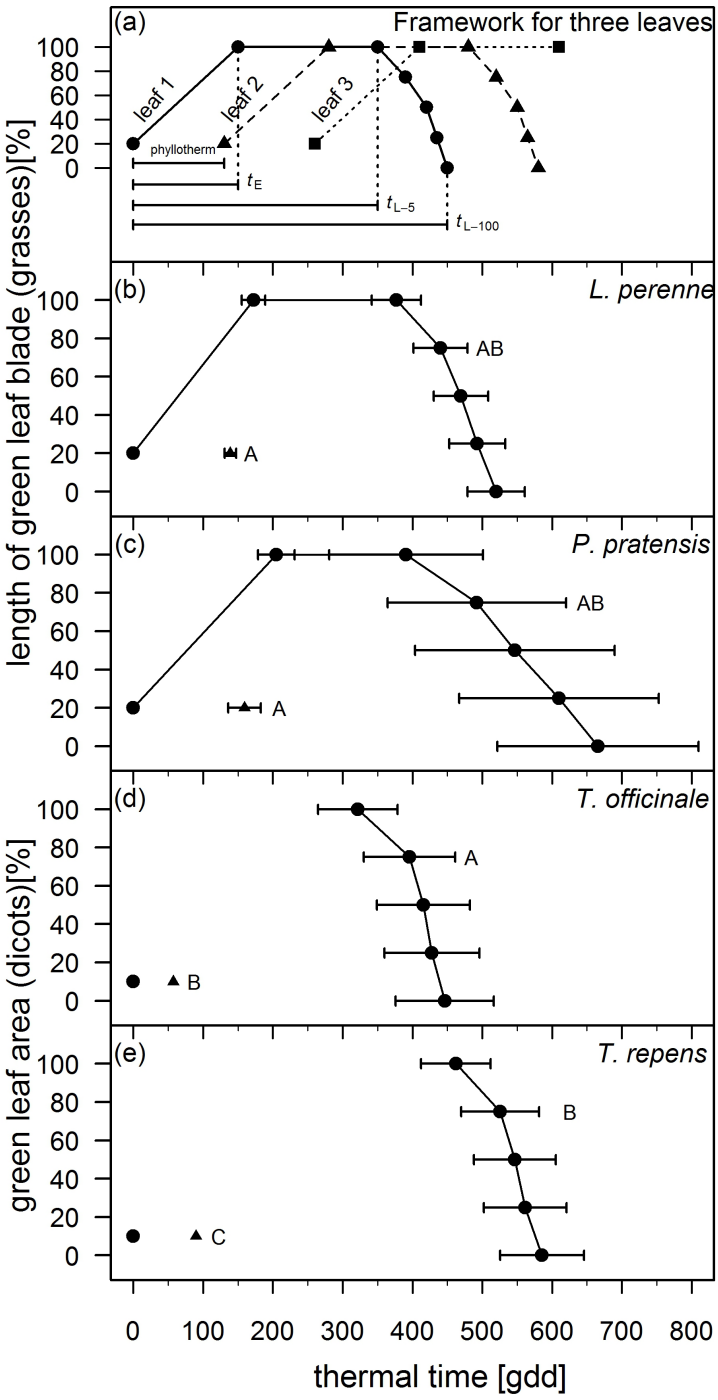

Figure 4: Principal scheme of the figure structure (a) and the phyllotherm, leaf life-span (all analysed definitions) and leaf elongation duration (t_E) of the four study species (b-e). Leaf elongation duration was only measured for grasses with the assumption that leaf elongation ended when the ligule was fully developed. Accordingly, there are no datapoints for leaf maturity in the dicots. Error bars denote 95%-confidence intervals. Sample sizes were 16, 7, 18 and 20 individuals of *Lolium perenne*, *Poa pratensis*, *Taraxacum officinale* and *Trifolium repens*, respectively. Variation of phyllotherm (solid triangles) in the dicot species was very small, therefore error bars do not show in the plot. Lower case letters indicate significant interspecific differences (alpha = 5%) of the phyllotherm and the leaf life-span. Since this study focuses on the leaf life-span measured till 25% of leaf senescence (t_{L25}), statistical information is only provided for this definition. The base temperature used was 4 °C for all species. Grass leaves appeared, when they had approx. 20% of their final length. In the dicots, leaves became visible, when leaf size ranged from 5% to 20% of its final size.

Leaf life-span

There were no differences of leaf life-span between species in any season, except for a 25% shorter leaf life-span of *T. officinale* than *T. repens* in the three-month observation period in autumn 2007 (Figure 4). Also, no significant seasonal influence on leaf life-span was evident (Figure 5). Consequently, seasons did not elicit a clear effect on species rankings for leaf life-span.

Number of live leaves

The number of live leaves per individual showed a clear pattern in interspecific comparison: The grasses had similar n_L of approx. 3, while n_L in the dicots was twice that of the grasses at approx. 7 and 6 in the autumns in *T. officinale* and *T. repens*, respectively (Figure 3b–e and Figure 5c). There was no seasonal influence on the n_L of grasses, but dicots had a smaller n_L in spring (approx. 5 and 4 in *T. officinale* and *T. repens*, respectively) than in autumn (Figure 5c). This effect led to a smaller difference between n_L of grasses and dicots in spring. The n_L of grasses was stable during the whole 3-month observation period (Figure 3b–e), while that of *T. repens* decreased by approx. 50% in late fall when temperatures were close to or below 6 °C. *T. officinale* had approx. 20% more leaves in the middle of the measurement period than at the beginning.

Phyllotherm

The phyllotherm of grasses ranged between 150 and 200 gdd (disregarding two values which had very high uncertainties), with little seasonal variation. The phyllotherm of dicots was about half as long in both autumns (approx. 90 gdd), but 130 to 155 gdd in spring 2007 (Figure 4, Figure 5b). In consequence, the difference between grasses and dicots was more pronounced in the two autumn periods. During the three-month observation period in autumn 2007, *T. officinale* had a shorter phyllotherm than *T. repens*, but this was not evident during other observation periods. The phyllotherm was stable over time in all species, when calculated with species-specific base temperatures of 4 °C for *L. perenne*, *P. pratensis*, *T. officinale* and 6 °C for *T. repens*, respectively (data not shown). When calculated with 4 °C base temperature, the phyllotherm of *T. repens* increased somewhat towards the end of the observation period.

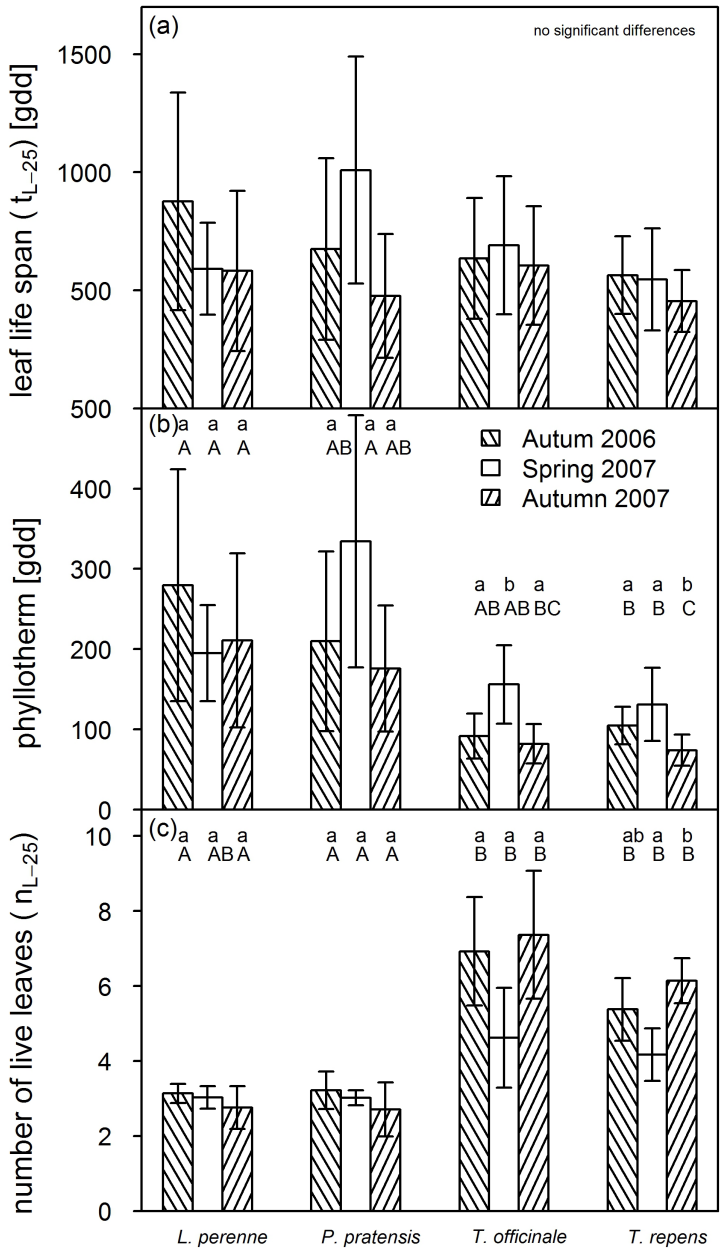

Figure 5: (a) Leaf life-span (t_{L-25}), (b) phyllotherm and (c) number of live leaves per individual (n_{L-25}) in the short observation periods. Sample sizes were 8, 7, 6 (*Lolium perenne*), 7, 12, 8 (*Poa pratensis*), 7, 8, 7 (*Taraxacum officinale*) and 8, 10, 8 (*Trifolium repens*) individuals in autumn 2006, spring 2007 and autumn 2007, respectively. Error bars denote 95%-confidence intervals and capital and small letters indicate significant (α = 5%) interspecific differences in one season and seasonal differences of one species, respectively. The base temperature used was 4 °C for all species.

Discussion

The need for a standard operational definition of leaf life-span

Valid comparisons of leaf life-spans across studies and between species must rely on a meaningful and standardized (common) operational definition of leaf 'birth' and 'death'. This is not trivial, as evident from the diverse definitions adopted by different authors working with the same species (Table 1 and references therein). As shown here, the requirement for a standard definition of leaf life-span is particularly important in grasses, in which the time-spans between first appearance and full expansion, and onset and completion of senescence are relatively long. Some investigators defined leaf life-span as the time between full expansion and beginning of senescence, while others defined it as the time between the emergence of the leaf tip from the encircling sheaths of older leaves and complete senescence (Table 1). Such a wide range of definitions can produce a spuriously wide range of leaf life-spans. For instance, in the case of *P. pratensis* (Figure 4c) the latter definition meant a leaf life-span three times longer that of the former.

Leaf life-span can be defined as the time elapsed between leaf construction and leaf senescence (yellowing). In grasses, the appearance of a given piece of leaf tissue is delayed relative to its first construction. This is because new tissue is produced at the leaf base, encircled by sheaths of older leaves. A grass leaf becomes visible when it already has about 20-25% of its final length (Skinner & Nelson, 1994; Durand *et al.*, 1999, this study). For this reason, the time between the appearance of the leaf tip above the encircling leaf sheaths and the time when 25% of the leaf blade area has senesced (that is $t_{L\text{-}25}$) approximates well the longevity of the piece of leaf tissue produced at the time of leaf appearance. This definition provides clear and simple rules (cf. Diemer *et al*., 1992). Since periods of expansion and senescence have a similar duration, this definition yields similar leaf life-spans measured as the time between full leaf expansion and complete senescence (*e.g.* Ryser & Urbas, 2000). A problem with the latter definition is that the duration of leaf senescence is very variable (Diemer *et al*., 1992). In the present study, the coefficient of variation (CV) of leaf senescence duration was three times larger than that of leaf elongation duration.

When the dicots are compared with the grasses, particularly *P. pratensis*, the relative rate of their senescence was faster and more uniformly distributed across the leaf. As a result, leaf life-spans defined for 25% to 100% of senescence differed very little (and did not change my conclusions regarding species differences in leaf life-span). Also, leaf 'birth' was recorded at

the time when they had reached approx. 5 – 20% of their final area. For these reasons, I argue for the same operational definition of leaf life-span for grasses and dicots.

Although different authors have used different definitions of leaf life-span (Table 1), their data could still be compared with mine due to the observations of the progression of senescence in the present studies. This enabled calculation of leaf life-spans for the whole range of leaf life-span definitions used by others (see below).

Similar and short leaf life-span

The results of this study support the hypothesis that the leaf life-span (here expressed in growing degree days) of the dominant species of intensively grazed grasslands is short and closely similar. Significantly, the four study species did not differ in their leaf life-span within or between observation periods, except for *T. officinale* in autumn 2007, whose leaf life-span was 25% shorter than that of *T. repens*. The leaf life-span of the four species ranged between approx. 400 and 520 gdd (base temperature: 4 ^{o}C) with a general mean of 463 (± 56 CI) gdd in the three-month observation period in autumn 2007, when measurements were most precise.

The results of the presented thesis differ from those of others who examined the same species in the absence of defoliation or under low to moderate defoliation intensities (Table 1). The leaf life-span values in my study were on average 30% lower (range: 3 to 73%) than those observed by others for the same species under less disturbed conditions (Table 1). The grazing regime in my study gave place to defoliation intervals of 35 to 50 days (Wade 1991), depending on the season, comparable to cutting regimes of four to five cuts per year. Leaf life-spans in the presented thesis were also low compared to those of species adapted to less disturbed habitats (Ryser & Urbas, 2000). Furthermore, the observed range of leaf life-spans in the present work was narrow compared to that of grassland species growing in less disturbed conditions, where leaf life-span varied by a factor > 2 (Ryser & Urbas, 2000; Maire *et al.*, 2009; Pontes *et al.*, 2010; Al Haj Khaled *et al.*, 2005; Diemer *et al.*, 1992).

Table 1: Leaf life-span (t_L) of *Lolium perenne*, *Poa pratensis*, *Taraxacum officinale* and *Trifolium repens* in published work and the absolute differences relative to this study. Different authors have used different t_L definitions. Ryser *et al.* (2000): maturity till end of senescence ($t_{L\text{-}mat\text{-}100}$); Maire *et al*. (2009) and Pontes *et al*. (2010): maturity till beginning of senescence ($t_{L\text{-}mat}$); Al Haj Khaled *et al.* (2005) and Sturite *et al.* (2007): appearance till end of senescence ($t_{L\text{-}100}$); Diemer *et al*. (1992): appearance till 25% of senescence ($t_{L\text{-}25}$). For calculation of the differences, t_L of the present study was expressed in the same definition and unit and calculated with the same base temperature as in the respective cited work.

Species	t_L in days	t_L in gdd[a]	$t_{L\text{-}Ref.} - t_{L\text{-}this\ study}$ in days	$t_{L\text{-}Ref.} - t_{L\text{-}this\ study}$ in gdd[a]	Defoliation regime	Reference	t_L definition
L. perenne	33		+1		no cutting	Ryser *et al.* (2000)	($t_{L\text{-}mat\text{-}100}$)
	33	584	+14	+305	three cuts per year	Maire *et al*. (2009)	($t_{L\text{-}mat}$)
		765		+59	not specified	Al Haj Khaled *et al*. (2005)	($t_{L\text{-}100}$)
		408		+129	three cuts per year	Pontes *et al*. (2010)	($t_{L\text{-}mat}$)
P. pratensis	47		+9		no cutting	Ryser *et al*. (2000)	($t_{L\text{-}mat\text{-}100}$)
	59	1044	+42	+791	three cuts per year	Maire *et al*. (2009)	($t_{L\text{-}mat}$)
		769		+516	three cuts per year	Pontes *et al*. (2010)	($t_{L\text{-}mat}$)
T. officinale	66		+26		one cut per year	Diemer *et al*. (1992)	($t_{L\text{-}25}$)
		800		+198	not specified	Al Haj Khaled *et al*. (2005)	($t_{L\text{-}100}$)
T. repens	61		+13		one cut per year	Diemer *et al*. (1992)	($t_{L\text{-}25}$)
	59		+7		three cuts per year	Sturite *et al*. (2007)	($t_{L\text{-}100}$)

[a] growing degree days, base temperature = 0°C.

I suggest that the homogenising influence of the intense disturbance regime, *i.e.* grazing pressure, enforced a convergence to similar and rather short leaf life-span, which would enable individuals to maintain a positive carbon balance in the face of shortened payback times. This could explain why Ryser *et al*. (2000) and Maire *et al*. (2009) found that *P. pratensis* had a 40 – 80% longer leaf life-span than *L. perenne* at low defoliation intensity, while the same species had short and indistinguishable leaf life-spans in the intensively grazed habitat of the presented thesis. Similarly narrow ranges of leaf life-span were observed in a grazed community of the flooding *pampa* in Argentina (Lemaire & Agnusdei, 2000). In that study, leaf life-span of C3 species ranged from 335 – 425 gdd in winter, to 390 – 530 gdd in autumn, to 395 – 595 gdd in spring (base temperature: 0°C). Interestingly, *S. neesiana* – a subordinate species of low abundance – departed from this trend and always had longer leaf life-spans of 600 – 745 gdd (Agnusdei, 1999). This supports the view that a long leaf life-span may weaken competitive ability in highly disturbed habitats. Data from intensively grazed grasslands in the UK lends further support to this explanation: the leaf life-span of *L. perenne* and *T. repens* ranged from 24 to 37 days and 30 to 36 days, respectively, the shorter leaf life-span always observed in the highest grazing intensities (estimated from Figure 2 of Parsons *et al.*, 1991).

How could grazing intensity affect leaf life-span? One possibility is that under intense grazing individuals with long leaf life-span are eliminated and/or out-competed by individuals with shorter leaf life-span leading to a narrower genetic pool. However, genotypic variation within species in leaf life-span appears to be limited, at least in *L. perenne* (Al Haj Khaled *et al*., 2005; Berone, 2005). Alternatively, reductions of leaf life-span could be a product of phenotypic plasticity. Results from Parsons *et al*. (1991) would agree with this view, since in that study morphogenesis of the plants was measured immediately after different grazing intensities were imposed, leaving no time for genotypic selection. Further evidence for shorter leaf life-span under more severe defoliation has been found in C4 grasses (Boggiano *et al.*, 2001; Neto *et al*., 2002; Sousa *et al*., 2010; Sousa *et al*., 2011). Thus, the scarce available studies support the view that the similarity of leaf life-span would be mainly a matter of phenotypic plasticity in response to grazing pressure.

Interaction of phyllotherm and number of live leaves in grasses vs. dicots

Similar leaf life-span values were attained with different organization strategies in grasses and dicots of this study*: L. perenne* and *P. pratensis* had fewer leaves and longer phyllotherms than *T. officinale* and *T. repens*. This contrast was most evident in the comparison of *T. officinale vs.* the grasses. Evidence for a longer phyllotherm in grasses than in dicots (*Dactylis glomerata* vs. *T. officinale*) can be found in Calviere & Duru (1995), but to my knowledge the interaction between phyllotherm and n_L has not been explicitly noted before.

The differences between grasses and dicots of the presented study in n_L and phyllotherm might be related to the location of leaf meristems. In grasses, the leaf meristem is located at the base of the tiller, while in dicots it is spread over the leaf area (Esau, 1977). Thus, after defoliation grass leaves keep growing since the meristem is retained, while in dicots meristematic parts of the leaf are lost and growth is immediately constrained (Parsons *et al*., 1991). In this situation, to compensate for the missing meristematic tissue, it might be advantageous to build new leaves more frequently. Alternatively, the difference may reflect different growth habits. Both *T. officinale* (a rosette forming dicot) and *T. repens* (a stoloniferous species) thrive in relatively short, open swards. In this situation, it might be advantageous to organize leaf tissue in a larger number of smaller organs, with which plants may rapidly occupy empty spaces. In comparison, the more erect grasses might benefit more from organizing leaf tissue into fewer, longer organs that can compete more effectively in taller canopies.

4. Is the carbon residence time in the above-ground biomass of a temperate pasture determined by the leaf life-span?

Results

Weather

Inside the open-top chambers daily soil temperature at 5 cm depth averaged 16.2 °C, 16.4 °C, and 14.2 °C during the observation periods in autumn 2006, spring 2007 and autumn 2007, respectively (Figure 6). Mean daily air temperatures (at 50 cm) were 15.6 °C, 14.9 °C and 12.7 °C, respectively. Temperatures in autumn 2006 were 2-3 °C warmer and temperatures in autumn 2007 and spring 2007 were not different from long-time averages.

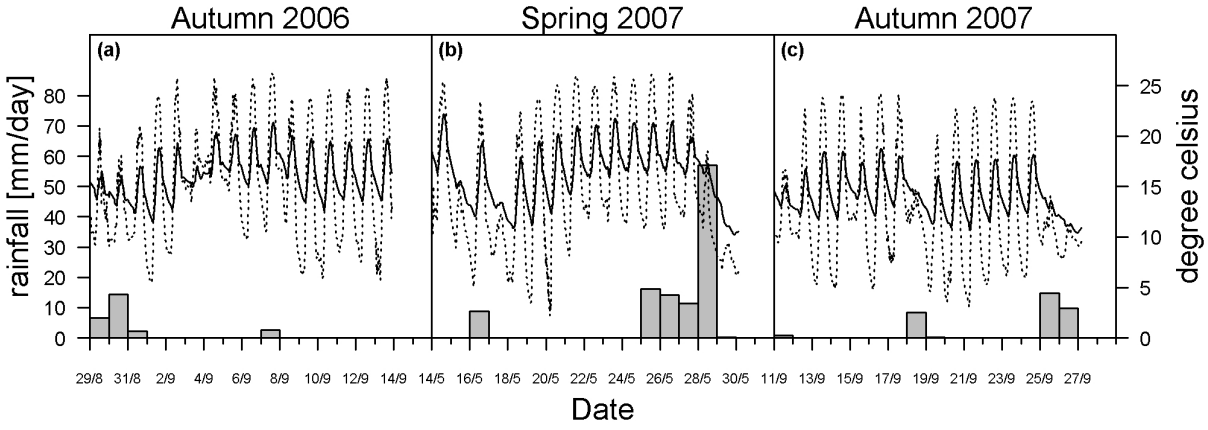

Figure 6: Soil temperatures at 5 cm below soil surface (continuous line) and air temperatures at 50 cm above soil surface (dashed line) inside the open-top chambers during the ^{13}C-labelling experiments in (a) autumn 2006, (b) spring 2007 and (c) autumn 2007. Bars indicate the sum of daily rainfall at the weather station Eichenried, 7 km away from the study site in the same landscape unit.

Hot water extractable carbon

Hot water extractable *plus* starch C was on average 35% in individual species and 28% at community scale with little variation between species and seasons (Table 2). Thus the fraction of the structural pool, *a*, was approx. 65% in individual species and 72% at community scale. The higher value of *a* in community scale samples was related to the differing harvest times. Individual species were harvested early in the night, when the content of soluble carbon was higher than shortly before sunrise, when community scale samples were harvested (Farrar & Farrar, 1985).

Equation (9) was fitted to the C tracer time courses with the overall average $a = 0.65$ of the dominant species and 0.7 of community scale datasets. Fitting equation (9) with dataset-specific values of *a* changed $MRT_{\text{C-Structural}}$ and $MRT_{\text{C-Labile}}$ by not more than 5% and 15%, respectively, and did not change any conclusions. For sensitivity analysis, *a* was varied within the measured range from 0.6 to 0.7 for species specific and from 0.65 to 0.75 for community scale datasets.

Table 2: Hot water extractable plus starch carbon relative to total C (in %) for shoots of the four studied species and bulk community above-ground biomass in 2007. Numbers are means of 8 replicate samples (± SE).

	spring 2007	autumn 2007
L. perenne	33 (± 1)	40 (± 2)
P. pratensis	31 (± 1)	35 (± 1)
T. officinale	34 (± 1)	31 (± 1)
T. repens	34 (± 1)	38 (± 1)
community	29 (± 1)	27 (± 1)

Carbon tracer time courses

Fully senesced leaves, still attached to the tillers, showed no tracer incorporation during labelling. The labelling kinetics of living shoots were similar for all four studied species and the community scale samples (Figure 7): after the last day of labelling on average 46% ± 5% SD of carbon remained unlabelled. Only in *P. pratensis* in spring 2007 and *T. officinale* in autumn 2006 exceptionally much C remained unlabelled after the last day of labelling (64% and 57%, respectively). In both cases, this percentage was significantly higher (*P. pratensis*: α = 5%; *T. officinale*: α = 10%) than for the same species in the other seasons and the other species in the same season.

The fit of the two-pool model (Equation (9)) to the C tracer time courses yielded significant results for the mean residence times of C in the labile and the structural pool. Calculations of the Bayesian Information Criterion (BIC) showed that the two-pool model was mostly equivalent (six datasets out of twelve) or better (four datasets out of twelve) or worse (two datasets out of twelve) than a one-pool model with first order kinetics, i.e. one term exponential decay function (on average over all datasets BIC of -45 and -48 for one- and two-pool model, respectively).

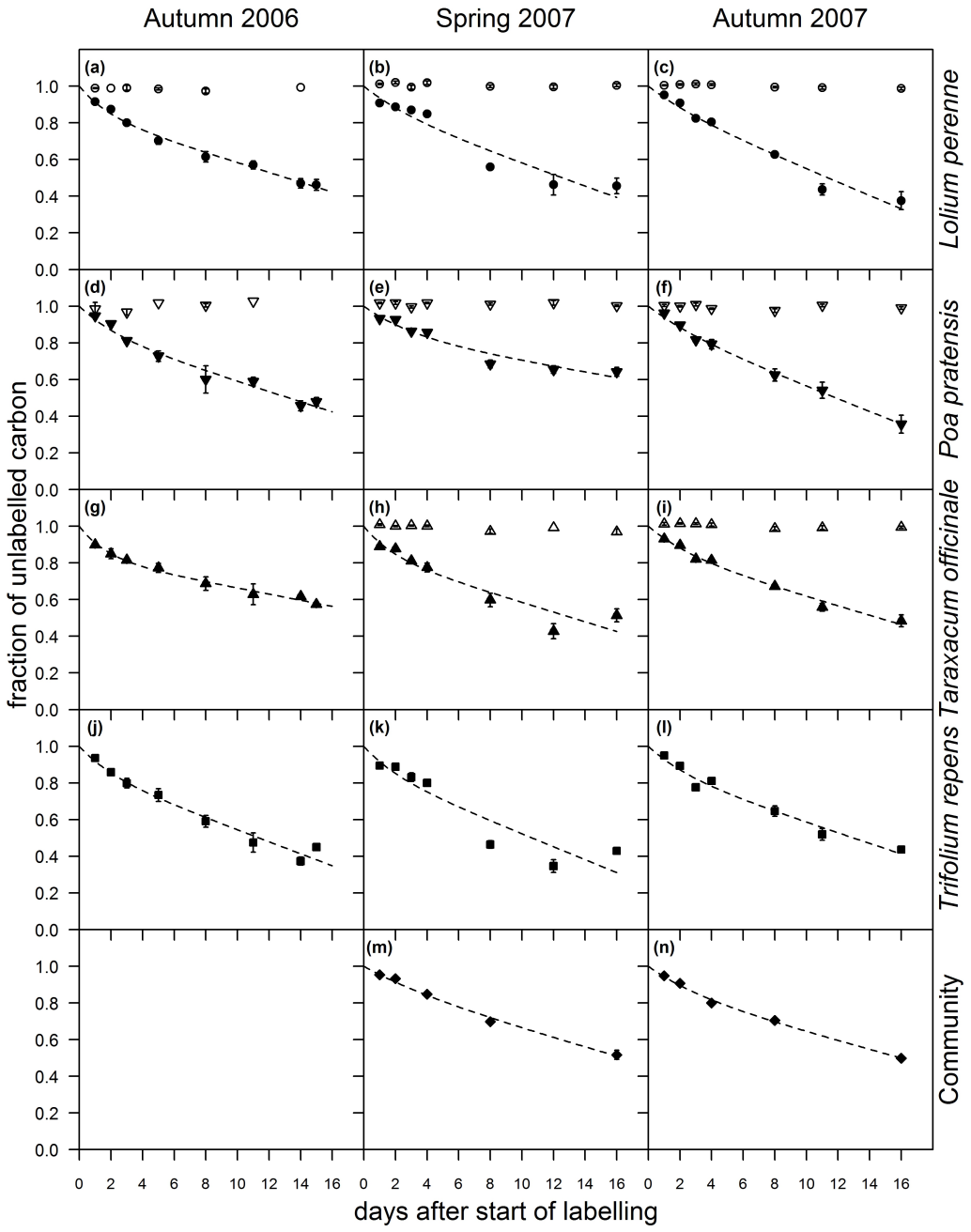

Figure 7: (a) – (l) The fraction of unlabelled carbon in the living shoots (closed symbols) and senesced leaves (open symbols) of the single species. Each value of living shoots is the mean of two (last two days of labelling) to four (all other days) replicate plants in autumn 2006 and seven to eight replicate plants in spring 2007 and four (days 3 and 12 after start of labelling) to eight (all other days) in autumn 2007 (± SE). Each value of senesced leaves is the mean of two to three plants in autumn 2006 and four to eight plants in spring and autumn 2007 (± SE). In autumn 2006, no senesced leaves of *T. officinale* were collected and in case of *T. repens*, samples of senesced leaves were contaminated with pieces of green stolon and are therefore not shown. (m) – (n) The fraction of unlabelled carbon in the total living above-ground biomass of the community. Data points are means of six (last day of labelling in spring 2007) to eight replicate plants (± SE). No community scale samples were collected in 2006. (a) – (n) The dashed line indicates model fits with 65% and 70% of structural carbon relative to total carbon, *a*, for single species and community scale samples, respectively. Due to harvests at different times of day, community samples had less water soluble carbon and thus fits of community scale samples had to be compared to fits of species samples with *a* of species samples + 0.05.

Mean residence times of carbon

The MRT_C in the labile pool, derived from the model fits with a = 0.65, ranged from 4 to 6 days (Table 3) and no interspecific or seasonal effects and no difference between community and species scale datasets were detected. The MRT_C in the structural pool, $MRT_{C\text{-}Structural}$, ranged from 21 to 38 days for all species and community scale datasets (n=14) with two exceptions, *T. officinale* in autumn 2006 and *P. pratensis* in spring 2007, where modeled $MRT_{C\text{-}Structural}$ was greater than 100 days, albeit with large uncertainties (Table 4). Besides this there were no clear systematic seasonal or interspecific differences in $MRT_{C\text{-}Structural}$.

Sensitivity of model results to the fraction of structural C in total C

Modelled mean residence times of C, MRT_C, were sensitive to the ratio of structural C relative to total C, a, in the model fits (Table 3 and Table 4). The lower a was set, e.g. 0.6 instead of 0.65, the longer were MRT_C of the labile and the structural pool. Uncertainties of modelled MRT_C reacted in opposite ways for both pools in response to a: the lower a was set, the less certain were modelled MRT_C of the structural pool and the more certain modelled MRT_C of the labile pool.

Table 3: Modelled mean residence times of C in the labile pool of *Lolium perenne*, *Poa pratensis*, *Taraxacum officinale*, *Trifolium repens* and the total above-ground biomass (community). No statistically significant differences ($\alpha = 0.05$) between species, seasons nor years were detected. Numbers in brackets denote ± SE. Due to harvests at different times of day, community samples had less water soluble carbon and thus MRT_C of community scale samples has to be compared to MRT_C of species samples with the ratio of the structural pool of species specific samples + 0.05.

	autumn 2006	spring 2007	autumn 2007
ratio of structural pool = 0.6 (community 0.65)			
L. perenne	4.9 (± 0.3)	6.0 (± 1.4)	6.3 (± 1.0)
P. pratensis	5.5 (± 0.6)	7.2 (± 0.9)	6.7 (± 0.5)
T. officinale	5.2 (± 0.5)	4.6 (± 1.0)	6.3 (± 0.5)
T. repens	5.1 (± 0.7)	4.5 (± 1.4)	5.8 (± 0.7)
community	-	7.7 (± 0.7)	6.4 (± 0.5)
ratio of structural pool = 0.65 (community 0.7)			
L. perenne	4.1 (± 0.3)	5.2 (± 1.3)	5.5 (± 0.9)
P. pratensis	4.7 (± 0.6)	6.1 (± 0.9)	5.8 (± 0.4)
T. officinale	4.3 (± 0.4)	3.9 (± 0.9)	5.4 (± 0.4)
T. repens	4.3 (± 0.6)	3.9 (± 1.4)	5.0 (± 0.7)
community	-	6.5 (± 0.6)	5.3 (± 0.4)
ratio of structural pool = 0.7 (community 0.75)			
L. perenne	3.3 (± 0.2)	4.4 (± 1.2)	4.7 (± 0.9)
P. pratensis	3.9 (± 0.5)	5.1 (± 0.8)	4.8 (± 0.4)
T. officinale	3.4 (± 0.3)	3.2 (± 0.9)	4.5 (± 0.4)
T. repens	3.6 (± 0.6)	3.3 (± 1.3)	4.1 (± 0.6)
community		5.4 (± 1.0)	4.3 (± 0.4)

Table 4: Mean residence time of carbon in the structural pool of *Lolium perenne*, *Poa pratensis*, *Taraxacum officinale*, *Trifolium repens* and the total above-ground biomass (community). Small letters indicate significant (global $\alpha = 0.05$) seasonal differences of one species / community and capital letters indicate significant (global $\alpha = 0.05$) interspecific (including community) differences in one season. Due to harvests at different times of day, community samples had less water soluble carbon and thus MRT_C of community scale samples has to be compared to MRT_C of species samples with the ratio of the structural pool of species specific samples + 0.05.

	autumn 2006	spring 2007	autumn 2007
ratio of structural pool = 0.6 (community 0.65)			
L. perenne	41 (± 3.7) $^{a\ A}$	28 (± 9.5) $^{a\ A}$	21 (± 4.1) $^{a\ A}$
P. pratensis	36 (± 6.5) $^{a\ A}$	200 (± 225) $^{a\ A}$	22 (± 2.0) $^{a\ A}$
T. officinale	199 (± 121) $^{a\ A}$	43 (± 15.1) $^{a\ A}$	39 (± 5.7) $^{a\ A}$
T. repens	27 (± 4.2)$^{a\ A}$	25 (± 8.9) $^{a\ A}$	32 (± 6.3) $^{a\ A}$
community	-	33 (± 4.5) $^{a\ A}$	37 (± 4.5) $^{a\ A}$
ratio of structural pool = 0.65 (community 0.7)			
L. perenne	36 (± 2.4)$^{a\ A}$	28 (± 7.7) $^{a\ A}$	21 (± 3.6) $^{a\ B}$
P. pratensis	34 (± 4.7)$^{a\ A}$	131 (± 78.4)$^{a\ A}$	22 (± 1.7)$^{a\ AB}$
T. officinale	104 (± 24.9)$^{a\ B}$	38 (± 10.3)$^{b\ A}$	36 (± 4.2)$^{b\ A}$
T. repens	26 (± 3.3) $^{a\ A}$	24 (± 7.2) $^{a\ A}$	31 (± 4.9) $^{a\ AB}$
community		33 (± 3.8) $^{a\ A}$	36 (± 3.3) $^{a\ A}$
ratio of structural pool = 0.7 (community 0.75)			
L. perenne	34 (± 1.7)$^{a\ A}$	27 (± 6.3)$^{a\ A}$	22 (± 3.1) $^{a\ B}$
P. pratensis	32 (± 3.5)$^{a\ A}$	84 (± 28.6)$^{b\ B}$	23 (± 1.5)$^{a\ AB}$
T. officinale	71 (± 8.8)$^{a\ B}$	34 (± 7.6)$^{b\ A}$	34 (± 3.2)$^{b\ A}$
T. repens	25 (± 2.7)$^{a\ A}$	24 (± 6.1)$^{a\ A}$	29 (± 3.8)$^{a\ AB}$
community		33 (± 3.2)$^{a\ A}$	35 (± 2.5)$^{a\ A}$

Leaf life-span and $MRT_{C\text{-}Structural}$

Within the margins of error, $MRT_{\text{C-Structural}}$ was not significantly different from measured leaf life-span, when all datasets were considered (Figure 8). But, when the two exceptional datasets (see above) were disregarded, $MRT_{\text{C-Structural}}$ was on average 10 days (= 25%) shorter than leaf life-span (P = 0.033).

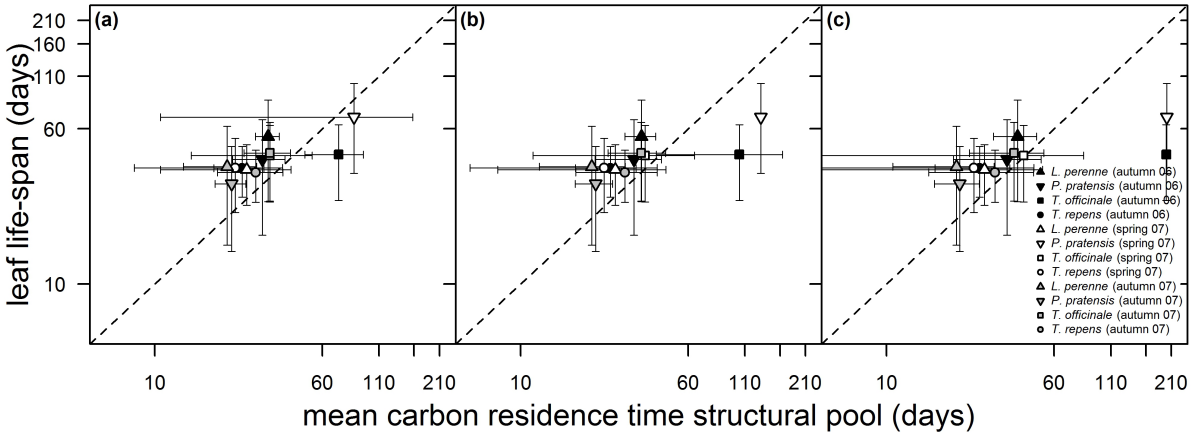

Figure 8: Comparison of modelled MRT_C in the structural pool and measured leaf life-span. (a), (b) and (c) show modelled MRT_C with 70%, 65% and 60% structural carbon relative to total carbon, respectively. The different species are indicated by the different symbols and different seasons by different shades. Axes are set logarithmically for better overview.

Discussion

Performance of the two pool model

The good fits (average $R^2 = 0.97$) of the two pool model to the datasets and the comparison of the Bayesian Information Criterion for less complex (1-pool) models confirmed that the proposed two pool model adequately reflected the basic mechanisms underlying C turnover in the shoots of the analysed grassland species and community. Experimental support for the proposed first-in-first-out mechanism of carbon in the structural pool was given by the fact that fully senesced leaves, which were still attached to the tillers and had probably senesced during the labelling period, incorporated no tracer after the full labelling time. Obviously, leaves dying during the labelling experiment had incorporated their structural carbon during growth before the experiment.

Sensitivity of model results to the fraction of structural C in total C

One important parameter in the fitting of the model was the *a*-value, the ratio of the structural pool relative to total C. I varied this parameter within the range of values determined for the residue obtained after hot water extraction. There was a clear effect of the *a*-value on the modelled mean residence time of C in the non-structural and the structural pool: the higher *a* (i.e. the more structural material was assumed to be present in the shoot), the shorter $MRT_{C\text{-}Structural}$ and $MRT_{C\text{-}Labile}$. This sensitivity mirrors differences in the assignment of chemical compounds with intermediate MRT_C to the two pools. Hexoses and free amino acids are known to have relatively short MRT_C of less than 4 days (Lehmeier *et al*., 2008; Lattanzi *et al*., 2012) and must be contained in the rapidly turning over labile pool. Conversely, cell wall compounds like cellulose, hemicelluloses and structural proteins form part of the structural pool. Fructans of *L. perenne* leaf blades have also shown MRT_C of less than 4 days (Lattanzi *et al*., 2012; Borland and Farrar, 1988); however, some fructan pools are assumed to serve as longer-term to seasonal stores which much longer MRT_C (Pollock *et al*., 1989; Schnyder, 1993). Soluble proteins of leaves have a MRT_C of 5 to 12 days (Simpson *et al*., 1981; Dungey & Davies, 1982; Lehmeier, Wild & Schnyder. unpublished). When *a* is relatively small (0.6), slowly turning over proteins and fructans might form part of the labile pool increasing $MRT_{C\text{-}Labile}$. When *a* is relatively large (0.7), the slowly turning over proteins and fructans are attributed to the structural pool decreasing its $MRT_{C\text{-}Structural}$.

Carbon residence time in the structural biomass is related to the leaf life-span

There was a clear relationship between the carbon residence time in the structural pool, $MRT_{C\text{-}Structural}$, and the measured leaf life-span, though $MRT_{C\text{-}Structural}$ seemed to be systematically shorter than leaf life-span (Figure 8). With two exceptions (see above), the overall average of $MRT_{C\text{-}Structural}$ and leaf life-span was 30 and 40 days, respectively. Supposing a significant difference between the two parameters (see Results): What could have been responsible for this 10 day discrepancy?

There are two main factors regarding assumptions of the two-pool model, which could have caused such a divergence between estimates of leaf life-span and $MRT_{C\text{-}structural}$: (i) deviations from the steady state-assumptions due to net standing biomass production of the sward, and (ii) imprecision in the first-in-first-out mechanism underlying the structural C pool. Probably, both factors contributed to the difference between $MRT_{C\text{-}Structural}$ and leaf life-span.

Although I found no evidence for an imbalance between growth rate and senescence rate during the labelling experiment (based on biomass harvests), measurements of leaf length revealed that young leaves were approx. 10% longer than old leaves in the spring experiment.

Net standing biomass production, i.e. a higher growth than senescence rate, would lead to a greater incorporation of C into plant biomass compared to the loss of C via respiration and senescence. The consequence would be a faster dilution of old C with labelled C than in a system under steady-state. Under continuous labelling, the turnover estimate of the labile pool is less susceptible to this imbalance, since this pool is well-mixed. Consequently, the isotopic composition of the labile pool is directly imprinted on the C, which is lost from the pool during growth and respiration. In contrast, the C turnover estimates of the structural pool can be biased by net standing biomass production. C, which is lost from the structural pool during senescence, has the isotopic imprint of C, which was incorporated a leaf life-span before. Thus, the loss of old C from the structural pool can be accompanied by an unproportionate higher incorporation of new C into the structural pool and consequently distort the tracer kinetics of the structural pool in the direction to a faster turnover. In fact, simple exemplifications with Excel showed that a shape as in *L. perenne* in spring 2007, where tracer time course seemed to form a plateau in the first days of labelling, can be caused by the effect of net biomass production on the tracer kinetics of the structural pool. But notably, this explanation for the difference between leaf life-span and $MRT_{C\text{-}Structural}$ does not apply to the autumns.

There was good general support for the first-in-first-out principle of C in the structural pool in the data of this study, since fully senesced leaves, which were still attached to the tillers and had probably senesced during the labelling period, had not incorporated tracer during labelling (see above). Nevertheless, some C incorporation in structural biomass of leaves might have continued after full tissue expansion (Maurice *et al*., 1997 for *Festuca arundinacea*), meaning that carbon incorporation in structural biomass of growing and developing tissue does not follow an on-and-off mechanism, but occurs over a longer period of time, including the periods of cell division, expansion and differentiation. In strict terms, this mechanism is inconsistent with the first-in-first-out principle. There is also a possibility that some C may be remobilised from cell walls during the last stages of senescence (Mohapatraa *et al*., 2010; Halgren & Banowetz, 2012). However, such a mechanism would not be observable in my datasets, since shoot samples contained only green tissue and senescing leaves (partly green, partly senescent) were not included in shoot samples.

Mean residence times of carbon in above-ground biomass

Mean residence times of the labile pool, $MRT_{\text{C-Labile}}$ were very similar between all species and seasons. $MRT_{\text{C-Labile}}$ varied between approx. 4 and 6 days. This is well in the order of mean residence times of carbohydrate pools in source leaves of *L. perenne* under controlled conditions (2-5 days; Lattanzi *et al*., 2012) and similar to mean residence times of C in pools feeding respiration of grassland plants (Lehmeier *et al*., 2008; Carbone & Trumbore, 2007; Bahn *et al*., 2009). To the author´s best knowledge, this is the first report on the $MRT_{\text{C-Structural}}$. Hence, the values of $MRT_{\text{C-Structural}}$ obtained in this study can not be compared to the literature. The MRT_{C} in the whole shoots or the bulk living above-ground biomass ($MRT_{\text{C-Structural+Labile}}$) is a function of the MRT_{C} in the structural and labile pool and the fraction of assimilated C atoms incorporated in the structural C pool. In this study after on average 5 days in the labile pool, C was either respired or incorporated in the structural pool, where it stayed on average for another 30 days. The ratio of C atoms reaching the structural pool relative to total assimilated C is given by the carbon use efficiency, the fraction of fixed C that is effectively incorporated in biomass. Assuming that 50% of assimilated C was incorporated in the structural pool (Lehmeier *et al*., 2008 for *L. perenne*), the average MRT_{C} of whole above-ground biomass (structural *plus* labile C) would be 20 days (0.5 * 5 d + 0.5 *(30 d + 5 d) = 20 d).

Despite the variability in MRT_C and leaf life-span, there were no systematic influences of species and season or year. This gave rise to a more or less common MRT_C in all analyzed seasons. A characteristic of the presented study was the relatively similar temperature in all experimental periods. Since higher temperatures accelerate respiration (James, 1953; Forward, 1960) and growth (Robson, 1972), it can therefore be expected that at higher temperatures C turnover might have been faster and MRT_C in above-ground biomass shorter than observed here. The lack of interspecific differences also meant that MRT_C of community scale was well reflected by MRT_C of the dominant species.

5. Carbon residence time in below-ground particulate organic matter and carbon partitioning to below-ground

Results

Chemical properties of the different POM fractions

There was a clear trend in chemical properties and in the fraction of unlabelled C of POM fractions. The fine POM fraction (> 0.2 mm and < 0.63 mm) had the smallest carbon, but the greatest nitrogen content, the smallest C/N ratio and contained the most unlabelled carbon after the last day of labelling (Table 5).

Table 5: Properties of coarse, medium and fine particulate organic matter (POM_c >1 mm including root phytomass; 0.63 < POM_m < 1 mm; 0.2 mm < POM_f < 0.63 mm) and of roots attached to plants. f_{old} denotes the fraction of unlabelled carbon.

	spring 2007			autumn 2007			autumn 2006	
	POM fraction			POM fraction				
	POM_f	POM_m	POM_c	POM_f	POM_m	POM_c	soil	roots*
Nitrogen content (% of dry mass)	1.6	1.7	1.0	2.0	1.7	1.0	0.43	1.1
Carbon content (% of dry mass)	27	37	41	36	41	43	4.9	42
Carbon / nitrogen ratio	17	22	41	18	24	42	11.3	44
f_{old} after last day of labelling (%)	98	96	94	98	97	96	100	82
Contribution to whole POM C mass (%)	29	9	63	30	10	60		

* single washed roots, attached to individual plants of *L. perenne*, *P. pratensis*, *T. officinale* and *T. repens*; weighted mean of all species

Seasonal differences in pool sizes

In spring 2007 the ecosystem contained more above- and below-ground biomass than in autumn 2007 (Table 6). Further, in autumn there were more dead leaves in the above-ground biomass and the below-ground C to above-ground C ratio was one third greater than in spring.

Table 6: Biomass and C mass in the ecosystem in spring and autumn 2007. Numbers in brackets are: (± SE; number of replicates).

	spring 2007	autumn 2007
whole POM biomass [g m^{-2}]	1647 (±26; 49)	1308 (±19; 48)
whole POM C mass [g m^{-2}]	572 (±19; 48)	518 (±16; 42)
whole shoot biomass [g m^{-2}]	341 (±12; 38)	219 (±7; 45)
whole shoot C mass [g m^{-2}]	139 (±5; 38)	82 (±3; 45)
living / total ratio of shoot biomass	0.58	0.38
living shoot biomass [g m^{-2}]	198	83
living shoot C mass [g m^{-2}]	81	31
below (POM) / above (bulk) C mass ratio	4.1	6.3

MRT_C in POM and bulk above-ground biomass

Both bulk above-ground biomass and below-ground POM showed a distinct tracer time course, though the rate of tracer incorporation was very different between fractions. In POM 95.5% and 96.7% of carbon remained unlabelled, while in the bulk above-ground biomass (including litter) 71% and 82% of carbon remained unlabelled after 16 days of labelling (Figure 9a).

The fit of a single exponential decay function on the tracer time courses showed that the mean residence time of C, MRT_C, in POM was six to eight times longer than MRT_C of bulk above-ground biomass (Table 7). There were no seasonal differences between MRT_C in POM, but MRT_C in bulk above-ground biomass was longer in autumn than in spring.

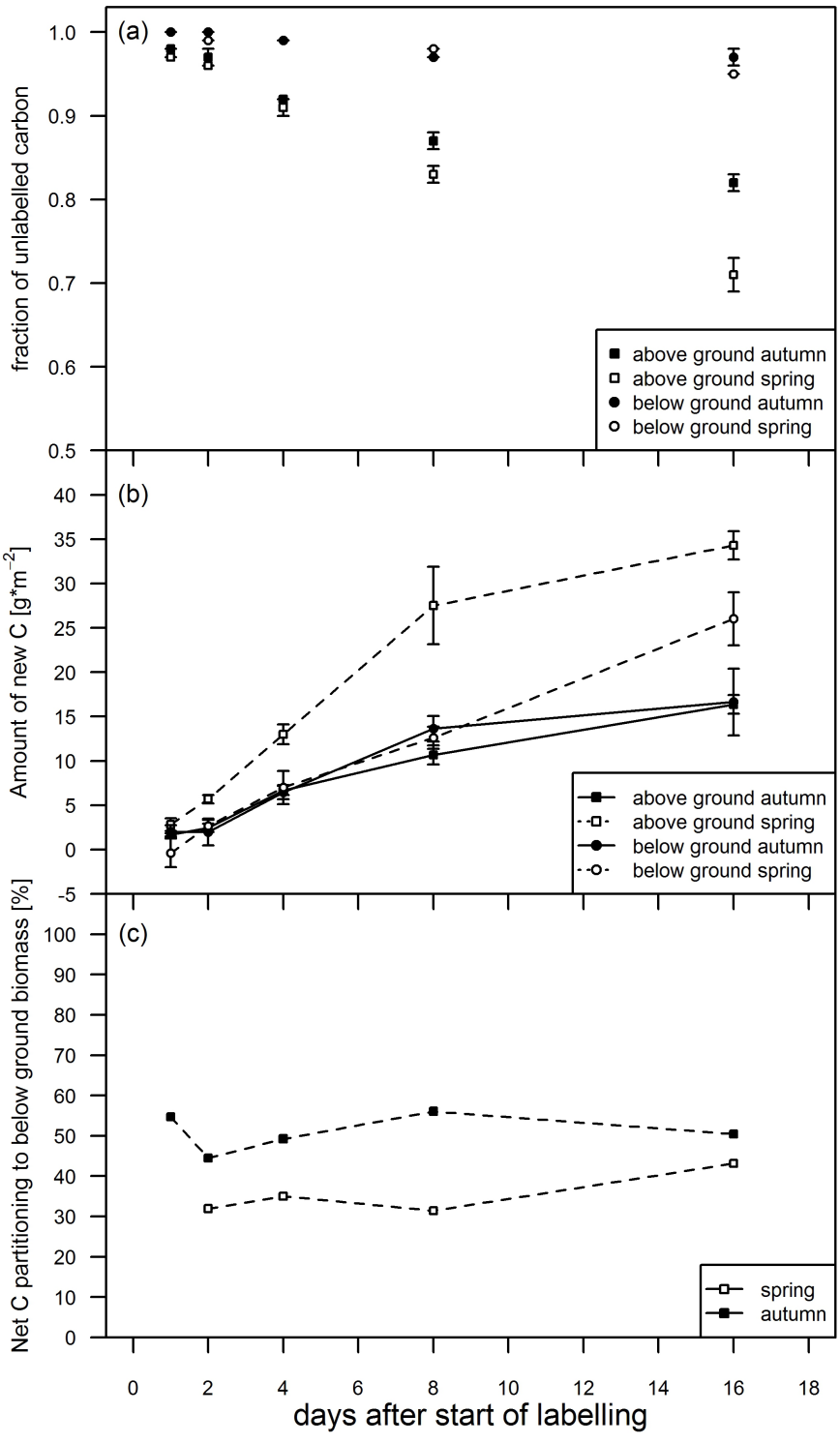

Figure 9: (a) The fraction of unlabelled carbon in bulk above-ground biomass (living *plus* dead) and below-ground POM, (b) the accumulation of labelled C in the above- and below-ground biomass and (c) net C partitioning to below-ground in spring and autumn 2007. Data points in (a) and (b) are means of eight samples and error bars denote SE.

Table 7: Mean residence times of C [days] (± SE) in below- and above-ground (dead *plus* living) biomass in spring and autumn 2007 derived with single exponential decay functions. Different small letters indicate significant differences (α = 5%) between seasons and different capital letters indicate significant differences between compartments.

	spring	autumn
below-ground biomass	358 (± 20)^{aA}	416 (± 49)^{aA}
above-ground biomass	46 (± 1)^{aB}	71 (± 4)^{bB}

New carbon accumulation and partitioning to below-ground

The absolute amount of labelled "new" carbon in the above-ground biomass in spring was twice as high as in autumn (Figure 9b). In contrast, the accumulation of new carbon below-ground was similar in both seasons. The ratio of new carbon being incorporated in below-ground (relative to above- *plus* below-ground new C accumulation) was relatively stable in both spring and autumn, when the first data point was excluded (Figure 9c). In spring less carbon was transferred below-ground than in autumn: the mean ratio of days 2, 4, 8 and 16 was 35% (±5 SE) and 50% (±5 SE) in spring and autumn, respectively.

Discussion

Decay continuum of particulate organic matter along a size gradient

The grading of the POM fraction properties in this study, i.e. decreasing C/N ratio, decreasing carbon percentage and increasing f_{old} with decreasing particle size, confirm the current theory of a root litter decay continuum along a particle size gradient, as discussed in Personeni & Loiseau (2004). In the presented study, C and N content, C/N ratio and f_{old} of the coarse fraction were closest to original root phytomass, while chemical properties and f_{old} of the finest fraction were closest to values of the soil (Table 5). This is consistent with results of Personeni & Loiseau (2004) and Loiseau & Soussana (1999). Mechanisms behind the C/N ratio decrease with decreasing particle size are thought to be carbon mineralisation along with litter decay resulting in an increased N concentration as well as the colonisation of litter with microbes of low C/N ratios (Personeni & Loiseau, 2004).

Below-ground POM is a big, but metabolically inactive carbon pool

In this study the carbon pool of below-ground biomass, here POM *plus* root phytomass, was four (spring) to six times (autumn) larger than the carbon pool of bulk above-ground biomass (Table 6). This is a common feature of grassland ecosystems (Jackson *et al*., 1996; Mokany *et al*., 2006). But POM properties (see above) and mean residence times of C in POM biomass suggest that most parts of POM were decaying dead material and only a small fraction was metabolically active living root biomass. In the presented study the mean residence time of C in the POM pool was approx. 1 year, which is similar to that reported for similar POM fractions (Klumpp *et al*., 2007; Personeni & Loiseau, 2004; Van Kessel *et al*., 2006) and was six to eight times longer than in the bulk above-ground biomass. MRT_C in POM represents a weighted mean of the MRT_C in different C pools and their respective mass. How much might living metabolically active roots have contributed to total POM C?

Lehmeier *et al*. (2008) found that root respiration was fed by the same pools with the same MRT_C as shoot respiration in studies on *L. perenne* under controlled conditions. If this finding is applied to the ecosystem studied here, it would imply that MRT_C in the labile pool was the same in roots as in above-ground biomass (chapter 4). Further, if the relationship between MRT_C of structural tissue and the tissue residence time (leaf or root life span) was a general one, as proposed, then MRT_C in structural tissue of roots should be related to root life-span.

According to the shoot and root phytomer data of Matthew et al. (2001), grass root longevity is approx. twice that of leaf life-span. Consequently, C residence time in below-ground living root biomass was approx. twice that of MRT_C in living above-ground biomass. This would suggest that only a small percentage of POM was living metabolically active roots and that the difference in MRT_C of above- and below-ground biomass was not caused by a different functioning of metabolically active pools, but related to a huge amount of (slowly) decaying material in the below-ground POM compartment.

Seasonal effects on MRT_C and C partitioning

The MRT_C in total POM was similar in the two seasons, but MRT_C in bulk above-ground biomass was longer in autumn than in spring (Figure 9 and Table 7). This difference in MRT_C of bulk above-ground biomass can be completely assigned to the 30% higher percentage of living material in the bulk above-ground biomass in spring (Table 6). In autumn, bulk above-ground biomass contained more unlabelled dead leaves than in spring leading to a greater dilution of label in the total of living and dead leaves in autumn. When all tracer in bulk above-ground biomass was attributed to the living leaves, the fraction of unlabelled carbon in living leaves after the last day of labelling was very similar, 48% and 46% in spring and autumn, respectively (compare chapter 4). Therefore, the seasonal difference in the tracer time course of bulk above-ground biomass was not a consequence of a different behaviour of metabolically active plant pools, but of different proportions of living and dead above-ground biomass. This and the fact, that there was more biomass on the ground in spring than in autumn (Table 6), lead to a greater amount of new C in the above-ground biomass in spring.
Below-ground, there were hardly any differences in the amount of new carbon in POM between seasons. But, since there was more new C above-ground in spring than in autumn, the net C partitioning to below- ground was different between seasons: approx. 35% of new carbon was deposited below-ground in spring, in contrast to approx. 50% in autumn. Since the net carbon partitioning was relatively stable after the first day of labelling, allocation of new carbon seemed to be a good proxy for the allocation pattern of total carbon. The presented results on carbon partitioning are in accordance with results of Belanger *et al*. (1992) on *Festuca arundinacea* in the field. In their work carbon partitioning to roots was higher in late summer and autumn than in spring. These seasonal allocation patterns reflect the developmental phases of the swards at the times of observations. In general, there is a growth peak of temperate grasslands in spring and carbohydrate reserves are mobilized by the plants

to feed leaf growth and reproductive development (Parsons, 1988). In autumn plants allocate more carbon to roots and below-ground stores which support survival in winter and regrowth in spring.

6. General Discussion

In the following, the questions addressed in the presented thesis are answered in a summarizing way, in particular the report on mean residence times of C, MRT_C, in above-ground and below-ground biomass, the relationship of MRT_C in above-ground biomass and tissue residence time, i.e. leaf life-span and the effects of season and species. At last the influence of grazing on MRT_C shall be discussed.

Is the mean residence time of carbon in above-ground biomass determined by the leaf life-span?

The MRT_C in bulk living above-ground biomass was approx. 20 days, and thus shorter than the leaf life-span of the dominant species (on average 40 days), since (1) some C (assumed approx. 50%; Lehmeier *et al*., 2008) was directly respired from the labile pool after on average 5 days and (2) even the MRT_C in the structural pool was 25% shorter than the leaf life-span. The latter discrepancy may be a consequence of net growth in spring and/or deposition of C after full leaf expansion. In this study, this lead to a 50% shorter MRT_C in bulk living above-ground biomass compared to the leaf life-span. This has to be taken into account, when the MRT_C in bulk living above-ground biomass is approximated via the leaf life-span. The $MRT_{C\text{-}Labile}$ in this study (on average 5 days) compared well to the range previously reported for carbohydrate pools in source leaves of *L. perenne* in controlled/artificial environments (Lattanzi *et al*., 2012) and pools feeding respiration of grassland plants (Lehmeier *et al*., 2008; Carbone & Trumbore, 2007; Bahn *et al*., 2009). This suggests that $MRT_{C\text{-}Labile}$ is quite constant in grassland ecosystems. To the author's best knowledge, this is the first report of MRT_C for structural and whole above-ground biomass. Therefore these results cannot be compared with literature. Although $MRT_{C\text{-}Structural}$ was shorter than the leaf life-span, both parameters were obviously related. This agrees with the view that $MRT_{C\text{-}Structural}$ is mechanistically connected with leaf (or root) life-span. Due to the large variation in leaf life-span between species and ecosystems (weeks to years, Wright *et al*. 2004), $MRT_{C\text{-}Structural}$ most probably also varies in a similar range between ecosystems.

Below-ground biomass is a big, but metabolically inert C pool

The MRT_C in below-ground POM was much longer than in above-ground biomass (approx. 1 year compared to approx. 2 months; for valid comparison, bulk (living *plus* dead) above-ground biomass was taken). The dry or C mass below-ground was four to six times that of above-ground, which is in accordance with published root/shoot ratios for temperate grasslands (Jackson *et al*., 1996; Mokany *et al*., 2006). However, the absolute amount of new C was relatively similar above- and below-ground (Figure 9). Probably this resulted from a huge amount of decaying material in the below-ground POM compartment (Loiseau & Soussana, 1999; Personeni & Loiseau, 2004). This view is also supported by (1) approximations of MRT_C in living below-ground root biomass, which was estimated to be approx. twice that of the MRT_C in above-ground biomass and thus much shorter than measured MRT_C in POM and (2) the grading of POM fractions regarding their C/N ratios reflecting the progression of decay with decreasing particle size. All this suggests that only a small percentage of below-ground POM was living metabolically active roots and that the difference in MRT_C of above- and below-ground biomass was not caused by a different functioning of metabolically active pools, but by a huge amount of decaying material in the below-ground POM compartment.

Common leaf life-span and C residence time

A remarkable finding, in all chapters, was the absence of clear seasonal, interannual or interspecific effects on leaf life-span (chapter 3) and MRT_C in structural and labile above-ground (chapter 4) and in below-ground (chapter 5) C pools, which gave rise to common community scale values for leaf life-span (40 days) and C residence time in living above- (labile: 5 days; structural: 30 days; bulk: approx. 20 days) and below-ground biomass (1 year). This pattern suggests the existence of some general homogenizing influence on all species that was similar in all seasons. It is proposed that the sward's history of years-long intense grazing was such a homogenizing force, since it kept the sward in a similar state (nominal compressed sward height of 7 cm) throughout all the years. Frequent defoliation leads to shortened payback-times and therefore, plants supposedly decrease their costs for leaf construction, in order to maintain a positive C balance. It is known that short payback-times, defined as the relation of construction costs to daily photosynthesis, are related to short leaf life-span (Williams *et al*., 1989; Navas *et al*., 2003; Coste *et al*., 2011). Thus, the frequent

intense defoliation, which affected all species in all observation periods in a similar way, could explain why leaf life-span was relatively short and more homogeneous between species than in other reports (chapter 2) and why both, leaf life-span and MRT_C, showed no systematic seasonal or interspecific differences (all chapters).

The mechanisms underlying the relative homogeneity in leaf life-span and MRT_C are not well known, but could involve selection of adapted genotypes and phenotypic responses. For leaf life-span however, room for intraspecific variability seems to be limited at least in *L. perenne* (Al Haj Khaled *et al*., 2005; Berone, 2005), while there is evidence for phenotypic plasticity of leaf life-span in response to defoliation in the literature (Parsons *et al*., 1991; Boggiano *et al*., 2001; Neto *et al*., 2002; Sousa *et al*., 2010; Sousa *et al*., 2011). Thus, the available studies support the view, that the absence of clear interspecific, seasonal and interannual differences in leaf life-span and MRT_C was a matter of phenotypic plasticity in response to grazing pressure. Strong support to this view is also given by the fact that despite missing systematic interspecific, seasonal and interannual effects, leaf life-span and $MRT_{C\text{-}Structural}$ were variable within a certain range.

The only clear seasonal difference in this study appeared in the interaction between above- and below-ground C compartments. In autumn, more C was allocated below-ground than in spring (50% compared to 30% of total C, respectively), similar to observations of Belanger *et al.* (1992). This was also mirrored by the higher ratio of dead leaves in autumn than in spring. These were the only parameters, where the influence of the developmental stage of the sward (spring growth with C-mobilization vs. autumn C storage; Parsons, 1988) was apparent.

Transferability of the results in at the time grazed pastures

The paddock, in which the presented studies took place, had been grazed by cattle for more than six years, but during observations the animals were excluded from the site. Thus, leaf life-span and MRT_C measurements report on undefoliated plants. How would those parameters be altered in a grazed pasture in comparison to the values derived in my study? Lemaire *et al*. (2009) reviewed defoliation parameters in pasture grasses: a leaf is defoliated on average once in its lifetime under intense grazing with a severity of approx. 50% of its lamina blade removed (Mazzanti & Lemaire, 1994) or 35% of its extended tiller height (Wade *et al.*, 1989). In consequence, approx. 35% of structural carbon in the grasses is lost from the above-ground biomass after a time period similar to half the leaf life-span. Therefore in the

presented study, grazing would lead to a reduction of leaf life-span and MRT_C in above-ground biomass by approx. 5 days. ($MRT_{\text{under grazing}}$ = 0.65 * MRT + 0.35 * MRT/2; for leaf life-span accordingly)

Conclusions and outlook

The view that the leaf life-span determines the MRT_C could partially be supported, since $MRT_{C\text{-Structural}}$ was related to the leaf life-span, though shorter. It would be very interesting to analyze $MRT_{C\text{-Structural}}$ in species and ecosystems with a greater variation in leaf life-span, in order to validate the relationship between $MRT_{C\text{-Structural}}$ and the leaf life-span. Here, also the proposed mechanisms causing the named discrepancy between $MRT_{C\text{-Structural}}$ and the leaf life-span – net standing biomass production and C deposition after full leaf expansion – could be addressed. Approximating MRT_C in bulk living above-ground biomass via the leaf life-span proofed to be a non- trivial challenge, since some C is directly respired from the labile C pool and thus the fraction of C incorporated in the structural pool has to be known to deduce MRT_C in bulk living above-ground biomass from MRT_C of its components (labile and structural C). This has to be taken into account, when the leaf life-span is taken as a proxy for MRT_C in above-ground biomass.

There were strikingly little differences in the MRT_C between species, seasons and years and the dominant species well reflected the MRT_C of the community. The only seasonal effect was a greater net C allocation to below-ground in autumn compared to spring reflecting the sward's developmental stage. It is suggested that the history of intense grazing had a homogenizing influence on the leaf life-span as well as the MRT_C between seasons, years and species in the analysed sward. The mechanism underlying the phenomenon is not well understood, but could well involve effects of grazing pressure (and related disturbance) on selection of adapted genotypes and phenotypic responses. Clearly, further studies focusing on such putative mechanisms are needed.

Bibliography

Agnusdei M (1999) *Analyse de la dynamique de la morphogenèse foliaire et de la défoliation de plusieurs espèces de graminées soumises à un paturage continu dans unde communauté végétale de la Pampa Humide (Argentine)*. PhD dissertation, Insitut National Polytechnique de Lorraine, Nancy, France.

Al Haj Khaled R, Duru M, Theau J P, Plantureux S, Cruz P (2005) Variation in leaf traits through seasons and N-availability levels and its consequences for ranking grassland species. *Journal of Vegetation Science* 16: 391–398.

Atkinson CJ, Farrar JF (1983) Allocation of photosynthetically-fixed carbon in *Festuca ovina* L. and *Nardus stricta* L. *New Phytologist* 95: 519–531.

Bahn M, Schmitt M, Siegwolf R, Richter A, Brüggemann N (2009) Does photosynthesis affect grassland soil-respired CO_2 and its carbon isotope composition on a diurnal timescale? *New Phytologist* 182: 451–460.

Belanger G, Gastal F, Warembourg FR (1992) The effects of nitrogen fertilization and the growing season on carbon partitioning in a sward of Tall Fescue (*Festuca arundinacea* Schreb). *Annals of Botany* 70: 239–244.

Berone, G. D. (2005) *Morphogenetical characterization of two Lolium perenne and Bromus stamineus cultivars*. Master thesis, Universidad Nacional de Mar del Plata, Balcarce, Argentina (in Spanish).

Berone GD, Lattanzi FA, Colabelli MR, Agnusdei MG (2007) A comparative analysis of the temperature response of leaf elongation in *Bromus stamineus* and *Lolium perenne* plants in the field: Intrinsic and size-mediated effects. *Annals of Botany* 100: 813–820.

Biswas SH, Malik AU (2010) Disturbance effects on species diversity and functional diversity in riparian and upland plant communities. *Ecology* 91: 28–35.

Boggiano P, Maraschin GE, Nabinger C, Riboldi J, Cadenazzi M (2001) Herbage allowance and nitrogen fertilization effects on morphological characteristics of *Paspalum notatum* Flugge. In: Gomide JA, Mattos WRS & Da Silva SC (Eds.), Proceedings *of the XIX international Grassland Congress: grassland ecosystems: an outlook into the 21st century* (pp 60–61). Sao Pedro, Sao Paulo, Brazil.

Borland AM, Farrar JF (1988) Compartmentation and fluxes of carbon in leaf blades and leaf sheaths of *Poa annua* L. and *Poa x jemtlandica* (Almq.) Richt. *Plant, Cell and Environment* 11: 535–543.

Calviere I, Duru M (1995) Leaf appearance and senescence patterns of some pasture species. *Grass and Forage Science* 50, 447–451.

Carbone MS, Trumbore SE (2007) Contribution of new photosynthetic assimilates to respiration by perennial grasses and shrubs: residence times and allocation patterns. *New Phytologist* 176: 124–135.

Chapman DF (1983) Growth and demography of *Trifolium repens* stolons in grazed hill pastures. *Journal of Applied Ecology* 20: 597–608.

Coste S, Roggy JC, Schimann H, Epron D, Dreyer E (2011) A cost-benefit analysis of acclimation to low irradiance in tropical rainforest tree seedlings: leaf life span and payback time for leaf deployment. *Journal of Experimental Botany* 62: 3941–3955.

Da Silva, LJ (2010) Laercio: Duncan test, Tukey test and Scott-Knott test. R package version 1.0-1. http://CRAN.R-project.org/package=laercio

Davies A, Thomas H (1983) Rates of leaf and tiller production in young perennial ryegrass plants in relation to soil temperature and radiation. *Annals of Botany* 57: 591–597.

De Visser R, Vianden H, Schnyder H (1997) Kinetics and relative significance of remobilized and current C and N incorporation in leaf and root growth zones of *Lolium perenne* after defoliation: assessment by ^{13}C and ^{15}N steady-state labelling. *Plant Cell and Environment* 20: 37–46.

Diemer M, Körner C, Prock S (1992) Leaf life spans of wild perennial herbaceous plants: a survey and attempts at a functional interpretation. *Oecologia* 89: 10–16.

Dungey NO, Davies DD (1982) Protein turnover in the attached leaves of non-stressed and stressed barley seadlings. *Planta* 154: 435–440.

Durand JL, Schäufele R, Gastal F (1999) Grass leaf elongation rate as a function of developmental stage and temperature: morphological analysis and modelling. *Annals of Botany* 83: 577–588.

Duru M, Theau JP, Cruz P (2012) Functional diversity of species-rich managed grasslands in response to fertility, defoliation and temperature. *Basic and Applied Ecology* 13: 20–31.

Esau K (1977) Anatomy of seed plants. 2nd edn. John Wiley & Sons, New York.

Forward DF (1960) Effect of temperature on respiration. In: W. Ruhland (Ed.): *Enyclopedia of plant physiology.* Vo. 12. (pp 234–258). Springer Verlag, Berlin.

Farrar JF (1989) Fluxes and turnover of sucrose and fructans in healthy and deseased plants. *Journal of Plant Physiology* 134: 137–140.

Farrar SC, Farrar JF (1985) Carbon fluxes in leaf blades of barley. *New Phytologist* 100: 271–283.

Farrar SC, Farrar JF (1986) Compartmentation and fluxes of sucrose in leaf blades of barley. *New Phytologist* 103: 645–657.

Gamnitzer U, Schäufele R, Schnyder H (2009) Observing ^{13}C labelling kinetics in CO_2 respired by a temperate grassland ecosystem. *New Phytologist* 184: 376–386.

Grime JP (1973) Competitive exclusion in herbaceous vegetation. *Nature* 244: 310–311.

Grimoldi AA, Kavanova M, Lattanzi FA, Schnyder H (2005) Phosphorus nutrition-mediated effects of arbuscular mycorrhiza on leaf morphology and carbon allocation in perennial ryegrass. *New Phytologist* 168: 435–444.

Halgren A, Banowetz GM (2012). Life cycle expression analysis of three cell wall degradation-related genes in ethylene-treated grass. *Plant Growth Regulation* 66: 167–177.

Hikosaka K (2005) Leaf canopy as a dynamic system: Ecophysiology and optimality in leaf turnover. *Annals of Botany*, 95, 521–533.

Heiberger RM (2009). HH: Statistical Analysis and Data Display: Heiberger and Holland. R package version 2.1-32. http://CRAN.R-project.org/package=HH

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED (1996) A global analysis of root distributions for terrestrial biomes. *Oecologia* 108: 389–411.

James WO (1993) 'Plant respiration.' Clarendon Press: Oxford.

Jones MB, Donelly A (2004) Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO_2. New Phytologist 164: 423–439.

Kikuzawa K (1984) Leaf survival of woody plants in deciduous broad-leaved forests. 2. Small trees and shrubs. *Cancadian Journal of Botany* 61: 2133–2139.

Kikuzawa K, Lechowicz MJ (2011) Ecology of leaf longevity. Springer, New York.

Klumpp K, Soussana JF, Falcimagne R (2007) Long-term steady state ^{13}C labelling to investigate soil carbon turnover in grasslands. *Biogeosciences* 4: 385–394.

Lattanzi FA, Ostler U, Wild M, Morvan-Bertrand A, Decau ML, Lehmeier CA, Meuriot F, Prud'homme MP, Schäufele R, Schnyder H (2012) Fluxes in central carbohydrate

metabolism of source leaves in a fructan-storing C_3 grass: rapid turnover and futile cycling of sucrose in continuous light under contrasted nitrogen nutrition status. *Journal of Experimental Botany* 63: 2363–2375.

Lehmeier CA, Lattanzi FA, Schäufele R, Wild M, Schnyder H (2008) Root and shoot respiration of perennial ryegrass are supplied by the same substrate pools: assessment by dynamic ^{13}C labeling and compartmental analysis of tracer kinetics. *Plant Physiology* 148: 1148–1158.

Lehmeier CA, Lattanzi FA, Schnyder H (2012) Stores as substrate sources for respiration – effects of nitrogen stress and day length. In: Matyssek R, Schnyder H, Ernst D, Munch J-C, Osswald W, Pretzsch H (Eds.): *Growth and defence in plants: Resource allocation at multiple scales*. Ecological Studies, Springer (in press).

Lemaire G, Chapman DF (1996) Tissue flows in grazed plant communities. In: Hodgsons J, Illius AW (Eds.), *The ecology and management of grazing systems* (pp 3–36). Wallingford: CABI Publishing.

Lemaire G, Agnusdei M (2000) Leaf tissue turnover and efficiency of herbage utilization. In: Lemaire G, Hodgson J, De Moraes A, De F Carvalho PC, Nabinger C (Eds.): *Grassland ecophysiology and grazing ecology* (pp. 265–287). Wallingford, UK: CABI Publishing.

Lemaire G, Da Silva SC, Agnusdei M, Wade M, Hodgson J (2009) Interactions between leaf lifespan and defoliation frequency in temperate and tropical pastures: a review. *Grass and Forage Science* 64: 341–353.

Leopold AC (1961) Senescence in plant development. *Science* 134: 1727–1732.

Loiseau P, Soussana JF (1999) Elevated [Co_2], temperature increase and N supply effects on the accumulation of below-ground carbon in a temperate grassland ecosystem. *Plant and Soil* 212: 123–134.

Maire V, Gross N, Pontes L da S, Picon-Cochard C, Soussana JF (2009) Trade-off between root nitrogen acquisition and shoot nitrogen utilization across 13 co-occurring pasture grass species. *Functional Ecology* 23: 668–679.

Matthew C, Van Loo EN, Thom ER, Dawson LA, Care DA (2001) Understanding shoot and root development. In: *Proceedings of the XIX international Grassland Congress: grassland ecosystems: an outlook into the 21st century* (pp 19–27). Sao Pedro, Sao Paulo: Fundacao de Estudos Agrarios Luiz de Queiroz.

Maurice I, Gastal F, Durand JL (1997) Generation of form and associated mass deposition during leaf development in grasses: a kinematic approach for non-steady growth. *Annals of Botany* 80: 673–683.

Mazzanti A, Lemaire G (1994) Effect of nitrose fertilization upon herbage production of tall fescue swards continuously grazed by sheep. 2. Consumption and efficiency of herbage utilization. *Grass and Forage Science* 49: 352–359.

Mohapatra PK, Patro L, Raval MK, Ramaswamy NK, Biswal UC, Biswal B (2010) Senescence-induced loss in photosynthesis enhances cell wall β-glucosidase activity. *Physiologia Plantarum* 138: 346–355.

Mokany K, Raison RJ, Prokushkinz AS (2006) Critical analysis of root : shoot ratios in terrestrial biomes. *Global Change Biology* 12: 84–96.

Navas ML, Ducout B, Roumet C, Richarte J, Garnier J, Garnier E (2003) Leaf life span, dynamics and construction cost of species from Mediterranean old-fields differing in successional status. *New Phytologist* 159: 213–228.

Neto AFG, do Nascimento Junior D, Regazzi AJ, da Fonseca DM, Mosquim PR, Gobbi KF (2002) Morphogenetic and structural responses of *Panicum maximum* cv. Mombaca on different levels of nitrose fertilization and cutting regimes. *Revista Brasileira de Zootecnia* 31: 1890–1900.

Parsons AJ (1988) The effect of season and management on the growth of grass swards. In Jones MB, Lazenby A (Eds.): *The grass crop. The physiological basis of production.* Chapman and Hall Ltd, London, Great Britain.

Parsons AJ, Harvey A, Woledge J (1991) Plant–animal interactions in a continuously grazed mixture. I. Differences in the physiology of leaf expansion and the fate of leaves of grass and clover. *Jounal of Applied Ecology* 28: 619–634.

Peacock JM (1975) Temperature and leaf growth in *Lolium perenne* L. II. The site of temperature perception. *Journal of Applied Ecology* 12: 115–123.

Personeni E, Loiseau P (2004) How does the nature of living and dead roots affect the residence time of carbon in the root litter continuum? Plant and Soil 267: 129–141.

Pollock CJ, Cairns AJ, Collis BE, Walker RP (1989) Direct effects of low temperature upon components of fructan metabolism in leaves of *Lolium temulentum* L. *Journal of Plant Physiology* 134: 203–208.

Pontes L da S, Lonault F, Carrère P, Maire V, Andueza D, Soussana JF (2010) The role of plant traits and their plasticity in the response of pasture grasses to nutrients and cutting frequency. *Annals of Botany* 105: 957–965.

Prosser J, Farrar JF (1981) A compartmental model of carbon allocation in the vegetative barley plant. *Plant, Cell and Environment* 4: 303–307.

Ratcliffe RG, Shachar-Hill Y (2006) Measuring multiple fluxes through plant metabolic networks. *Plant Journal* 45: 490–511.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Reich PB, Walters MB, Ellsworth DS (1992) Leaf life-span in relation to leaf, plant and stand characteristics among diverse ecosystems. *Ecological Monographs* 62: 365–392.

Robson MJ (1972) The Effect of Temperature on the Growth of S170 Tall Fescue (*Festuca arundinacea*). 1. Constant temperature. *Journal of Applied Ecology* 9: 643–653.

Robson MJ, Deacon MJ (1978) Nitrogen deficiency in small closed communities of S24 Ryegrass. II. Changes in the weight and chemical composition of single leaves during their growth and death. *Annals of Botany* 42: 1199–1213.

Ryser P, Urbas P (2000) Ecological significance of leaf life span among Central European grass species. *Oikos* 91: 41–50.

Schimel DS (1995) Terrestrial ecosystems and the carbon cycle. *Global Change Biology* 1: 77–91.

Schlesinger WH (1997) Biogeochemistry: an analysis of global change, 2nd edn. Academic Press, San Diego, CA, 1997

Schnyder H (1993) The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling - a review. *New Phytologist* 123: 233–245.

Schnyder H, de Visser R (1999) Fluxes of reserve-derived and currently assimilated carbon and nitrogen in perennial ryegrass recovering from defoliation: the regrowing tiller and its component functionally distinct zones. *Plant Physiology* 119: 1423–1435.

Schnyder H, Schäufele R, Lötscher M, Gebbing T (2003) Disentangling CO_2 fluxes: direct measurements of mesocosm-scale natural abundance $^{13}CO_2/^{12}CO_2$ gas exchange, ^{13}C

discrimination, and labelling of CO_2 exchange flux components in controlled environments. *Plant, Cell and Environment* 26: 1863–1874.

Schnyder H, Schwertl M, Auerswald K, & Schäufele R (2006) Hair of grazing cattle provides an integrated measure of the effects of site conditions and interannual weather variability on $\delta^{13}C$ of temperate humid grassland. *Global Change Biology* 12: 1315–1329.

Simpson E, Cooke RJ, Davies DD (1981) Measurement of protein degradation in leaves of *Zea mays* using [^{3}H]acetic anhydride tritiated water. *Plant Physiology* 67: 1214–1219.

Skinner RH, Nelson CJ (1995) Elongation of the grass leaf and its relationship to the phyllochron. *Crop Science* 35: 4–10.

Sousa BML, do Nascimento Junior D, da Silva SC, Monteiro HCF, Rodrigues CS, da Fonseca DM, da Silveira MCT, Sbrissia AF (2010) Morphogenetic and structural characteristics of andropogon grass submitted to different cutting heights. *Revista Brasileira de Zootecnia* 39: 2141–2147.

Sousa BML, do Nascimento Junior D, Rodrigues CS, Monteiro HCF, da Silva SC, da Fonseca DM, Sbrissia AF (2011) Morphogenetic and structural characteristics of xaraes palisadegrass submitted to cutting heights. *Revista Brasileira de Zootecnia* 40: 53–59.

Sturite I, Henriksen TM, Breland TA (2007) Longevity of White Clover (*Trifolium repens*) leaves, stolons and roots, and consequences for nitrogen dynamics under northern temperate climatic conditions. *Annals of Botany* 100: 33–40.

van Kessel C, Boots B, de Graaff MA, Harris D, Blum H, Six J (2006) Total soil C and N sequestration in a grassland following 10 years of free air CO_2 enrichment. *Global Change Biology* 12: 2187–2199.

Wade MH (1991) Factors affecting the availability of vegetative *Lolium perenne* to grazing dairy cows with special reference to sward characteristics, stocking rate and grazing method. PhD dissertation, Universite de Rennes, France.

Wade MH, Peyraud JL, Lemaire G, Comeron EA (1989) The dynamics of daily area and depth of grazing and herbage intake of cows in a five day paddock system. Proceedings of the 16th International Grassland Congress, Nice, France, pp. 1111–1112. Paris, France: INRA.

Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ (2002) Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* 33: 125–159.

White RP, Murray S, Rohweder M (2000) Pilot Analysis of Global Ecosystems: Grassland Ecosystems. World Resources Institute, Washington, DC, USA

Williams KB, Field CB, Mooney HA (1989) Relationships among leaf construction cost, leaf longevity and light environment in rain forest plants of the genus Piper. *American Naturalist* 133: 198–211.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groon PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklass EJ, Villar R (2004) The worldwide leaf economics spectrum. *Nature* 428: 821–827.

Acknowledgements

The last years of my life were not only affected by the fascinating and demanding work for my doctorate, but also by the birth of my two children. To master both has not always been easy and I am grateful and would like to thank the many people, who helped me making it possible:

First of all, Prof. Hans Schnyder for the guidance, advice, confidence and understanding he gave me. I have learned a lot from him and I am sure: if there were more heads like him, the birthrate would be higher!

Ulrike Ostler, nee Gamnitzer, for the great collaboration, the numerous discussions and advice and the friendship

Fernando Lattanzi for the guidance and advice and the delicate meals in his house

Melitta Sternkopf for not only being such a competent and friendly secretary, but also the heart of the institute

Rudi Schäufele for the smooth running of many samples

Monika Michler, Angela Ernst-Schwärzli, Anja Schmidt and Erna Eschenbach for technical assistance in the laboratories, in the field and for many hours of washing roots

Richard Wenzel for skilful assistance with the labelling facility

Iris Köhler, Tom Gottfried and Claudia Landinger for their sampling assistance during the labelling experiments, even in nighttime hours! Tom Gottfried also for observing leaves, even in the first snow of 2007 (sorry for the frozen fingers…)

Wolfgang Feneis for providing quick help with computer problems

Alex Braun for accepting “fresh air” in every season of the year

And thanks to all the other colleagues, namely Karl Auerswald, Monika Kavanová, Agustín Grimoldi, Christoph Lehmeier, Melanie Wild, Sun Luanzi, Xiaoying Gong and Germán Berone for making the work at Grünlandlehre so fruitful and enjoyable

And last but not least thanks to all of my family, especially my husband Christoph for his encouragement, his love and for sharing his life with me

to my mom for always believing in me

and my kids for being there and never letting me forget that there is more to live than work