Carbon dynamics in aboveground biomass of co-dominant plant species in a temperate grassland ecosystem: same or different?

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Summary

- Understanding the role of individual organisms in whole-ecosystem carbon (C) fluxes is probably the biggest current challenge in C cycle research. Thus, it is unknown whether different plant community members share the same or different residence times in metabolic ($t_{\text{metab}}$) and nonmetabolic (i.e. structural) ($t_{\text{nonmetab}}$) C pools of aboveground biomass and the fraction of fixed C allocated to aboveground nonmetabolic biomass ($A_{\text{nonmetab}}$).
- We assessed $t_{\text{metab}}$ $t_{\text{nonmetab}}$ and $A_{\text{nonmetab}}$ of co-dominant species from different functional groups (two bunchgrasses, a stoloniferous legume and a rosette dicot) in a temperate grassland community. Continuous, 14–16-d-long $^{13}$C-labeling experiments were performed in September 2006, May 2007 and September 2007.
- A two-pool compartmental system, with a well-mixed metabolic and a nonmixed non-metabolic pool, was the simplest biologically meaningful model that fitted the $^{13}$C tracer kinetics in the whole-shoot biomass of all species. In all experimental periods, the species had similar $t_{\text{metab}}$ (5–8 d), whereas $t_{\text{nonmetab}}$ ranged from 20 to 58 d (except for one outlier) and $A_{\text{nonmetab}}$ from 7 to 45%.
- Variations in $t_{\text{nonmetab}}$ and $A_{\text{nonmetab}}$ were not systematically associated with species or experimental periods, but exhibited relationships with leaf life span, particularly in the grasses. Similar pool kinetics of species suggested similar kinetics at the community level.

Introduction

Understanding the intricacies of carbon (C) dynamics in ecosystems – from (effect) traits of individual autotrophic and heterotrophic organisms/species and their interactions in synthesis and degradation pathways, to whole-ecosystem C fluxes – is probably the biggest current challenge in C cycle research (Lavorel & Garnier, 2002; Trumbore, 2006; Schmidt et al., 2011; Reichstein et al., 2014). In this context, the present study investigates C dynamics in the aboveground parts of different co-dominant plant species, which determine the first processes in the C cycle: they catalyze the rates of C fixation in the system (e.g. Anten & Hirose, 2003; Lattanzi et al., 2012a), and allocate C to the growth of aboveground and belowground plant parts, with eventual consequences for soil C turnover (De Deyn et al., 2008; Schmidt et al., 2011; Reichstein et al., 2014). The residence time ($t$, also termed the turnover time) of C in different components of the system is a main characteristic of C cycling. Integrated over pathways, it affects diverse functions, such as C storage in aboveground biomass or soil (Carvalhais et al., 2014), or delays/time lags between the fixation of C and its release in respiratory processes (Taneva et al., 2006; Adair et al., 2008; Brüggemann et al., 2011; Epron et al., 2012). Aboveground biomass is composed of metabolic and nonmetabolic (i.e. structural) C pools which serve contrasting functions at the ecosystem scale: metabolic pools support plant growth and autotrophic respiration, whereas nonmetabolic pools consist of end-products of metabolism, serve dedicated roles (e.g. as cell wall materials) and eventually pass on to the litter fraction, henceforth serving as substrate for the heterotrophic food chain.

Conceptually, the aboveground metabolic C pool can be considered as the composite of all C compounds that are available for shoot metabolism, including recycled (stored and mobilized) materials. This comprises the nonstructural carbohydrates (mainly sucrose, starch and fructan), soluble proteins, amino acids and organic acids (ap Rees, 1980; Poorter & Bergkotte, 1992; Gebbing et al., 1998; Plaxton & Podestá, 2006; Lattanzi et al., 2012b; Lehmeier et al., 2013). Studies of $\tau$ in metabolic C pools supplying respiration or growth have described these pools as well-mixed, homogeneous reservoirs (e.g. Lattanzi et al., 2005; Carbone & Trumbore, 2007; Gamnitzer et al., 2009). Quantitative tracer studies with grass plants in controlled environments have indicated similar $\tau$ in several major components of metabolic C. Thus, $\tau$ of C in the carbohydrate (fructan) store (Lattanzi et al., 2012b) and in the pools supplying growth (Lattanzi et al., 2005) and respiration (Lehmeier et al., 2010a,b) of Lolium perenne ranged in the order of 2–5 d. Carbone & Trumbore (2007) found a $\tau$ value of 4–5 d for C substrates feeding aboveground respiration of grasses in a Californian mountain meadow. The C pool supplying autotrophic soil respiration of an alpine grassland had a
The nonmetabolic pool of aboveground plant parts can be thought of as the composite of all end-products of plant C metabolism in shoot tissue that cannot be recycled and metabolized further by the plant. It is generally accepted that this pool is made up mainly of cell wall constituents (lignin, cellulose, hemicellulose, pectin and protein bound in cell walls), but that it also includes minor amounts of other organic compounds, such as lipids, waxes and phenolics (Chapin et al., 1990; Poorter et al., 1997; Matile, 2000). As C becomes locked when it enters the nonmetabolic biomass, the nonmetabolic pool behaves as a nonmixed or layered pool, to which C is successively added during tissue growth (e.g., Helliker & Ehleringer, 2002), and eventually lost when senesced tissue is shed in the form of litter (Schneider et al., 2006). In the aboveground biomass of intensively managed grassland systems, the bulk of the nonmetabolic biomass is associated with leaves (Jones & Lazenby, 1988), and the synthesis of nonmetabolic biomass is connected with the expansion and differentiation of structural tissue during leaf growth (MacAdam & Nelson, 1987; Maurice et al., 1997). Continuous leaf production and senescence determine leaf turnover and the associated flux of nonmetabolic C to the litter pool (Thornley, 1998; Schneider et al., 2006).

In mixed plant communities, C cycling studies should seek an understanding of how different coexisting plant species or functional groups affect ecosystem C dynamics. However, to our knowledge, potential differences between coexisting species in the C kinetics of aboveground biomass have not yet been investigated, at least in grasslands. Between-species differences in functional traits may add between-species variation in plant C kinetics. Such variation may be connected with tissue life span and related traits (Funk & Cornwell, 2013), differences in the kinetic properties and relative sizes of metabolic and nonmetabolic pools, and allocation between above- and belowground plant parts. In what concerns leaf life span, however, Schleip et al. (2013) found some variation, but no systematic difference between coexisting, co-dominant species of different functional groups in an intensively managed grassland ecosystem. Given that observation, we would not expect systematic species-related differences in τ of nonmetabolic C. However, such a supposition has not been verified.

To shed light on these questions, this study asked whether co-dominant plant species from different functional groups – coexisting in the same grassland community – differ (or not) in the residence time of C in aboveground metabolic (τ_{metabol}) and nonmetabolic (τ_{nonmetabol}) pools, and in the allocation of assimilated C to the shoot nonmetabolic pool (A_{nonmetabol}). We also discuss the relationships between the kinetic characteristics of nonmetabolic C and leaf life span. Four co-dominant perennials were considered: two bunchgrasses (L. perenne and P. pratensis), a rosette-forming dicot (T. officinale) and a stoloniferous legume (T. repens). The approach involved continuous \(^{13}\text{CO}_2/^{12}\text{CO}_2\) labeling of a mixed grassland community at ambient concentration in three experimental periods, period sampling of individual shoots of the four species, analysis of tracer content, the estimation of metabolic C as hot water-soluble C plus C in starch and mobilizable protein, and compartmental modeling of the tracer kinetics in the aboveground biomass of the species. The analysis compared different models and assessed their various underlying assumptions.

Materials and Methods

Location

The study was performed at Grünschwaige Grassland Research Station (48°23′N, 11°50′E, 435 m above sea level) near Freising, Germany. The climate of the site is temperate humid, with a mean annual air temperature of 9°C and mean annual precipitation of 775 mm. Details of climate, soil characteristics and pasture management were reported by Schnyder et al. (2006).

The labeling experiments were performed in the middle of paddock number 8, a permanent pasture paddock that is grazed by Limousin cattle from late April to late October every year. The sward state was kept stable by maintaining the canopy height at c. 7 cm by periodic adjustments of grazing pressure through targeted changes in animal stocking density. The sward is dominated by L. perenne, P. pratensis, T. officinale L. and T. repens (Schleip et al., 2013), accounting for c. 80% of the standing biomass. These species are frequent (co-) dominant members of intensively managed permanent pastures in temperate humid climates. The area had not been fertilized since sowing in 1999, and only received excreta returned to the pasture by the grazing cattle, and atmospheric deposition. In each experimental period (see later), cattle were excluded from the labeling site for c. 2 wk before the start of labeling.

\(^{13}\text{CO}_2/^{12}\text{CO}_2\)-labeling experiments

Continuous \(^{13}\text{CO}_2/^{12}\text{CO}_2\) labeling experiments were performed in September 2006 (29 August–13 September), May 2007 (13–29 May) and September 2007 (10–26 September). The technical components, the procedures and the performance of the methodology used in these experiments have been described in Gaminzter et al. (2009). Briefly, open-top chambers (OTCs) made of clear acrylic glass were used as labeling cuvettes. Each OTC covered an area of 0.83 m\(^2\), and had a volume of 660 l. The OTCs were placed on collars, which had been forced c. 12 cm into the soil c. 2 wk before labeling began. The concentration and C isotope composition of CO\(_2\) inside the OTCs was controlled and monitored quasi-continuously using an infrared gas analyzer (LI7000; Li-Cor, Lincoln, NE, USA) and an online stable isotope ratio mass spectrometer (Delta Plus Advantage; Thermo Electron, Bremen, Germany) interfaced with a gas chromatic column (Gasbench II; Thermo Electron) (Schnyder et al., 2004; Gaminzter et al., 2009). The C isotope composition is presented as \(δ^{13}C = R_{\text{sample}}/R_{\text{standard}} - 1\), where \(R_{\text{sample}}\) and \(R_{\text{standard}}\) are the \(^{13}\text{C}:/^{12}\text{C}\) ratios in the sample and in the international VPDB standard, respectively. Calibration of the mass spectrometric measurements paid attention to the equal treatment of samples and calibration gas (see Schnyder et al., 2004).
The CO₂ from two gas cylinders (δ¹³C = −3.64‰ and δ¹³C = −48.51‰), treated identically to chamber headspace air samples, allowed for the correction of instrument drift and cross-calibration with a second mass spectrometer (which was used for plant tissue analysis, see later). The precision (standard deviation, SD) of sample repeats was 0.10–0.17‰.

The CO₂ concentration inside the OTCs was maintained between 365 and 376 μmol mol⁻¹ (measured at noon; Table 1), close to ambient conditions. Within each of the three experimental periods, the variation (SD) of CO₂ concentration between the OTCs on the different plots (n = 2–4) was 2–3 μmol mol⁻¹, and day-by-day variation (SD, n = 12–16) was 3–6 μmol mol⁻¹. The δ¹³C of CO₂ inside the OTCs (δOTC) was near constant at −43.8‰–46.9‰ and −47.9‰ (assimilation-weighted mean) in September 2006, May 2007 and September 2007, respectively. The variations in δOTC between the different plots (c. 0.1‰ SD, n = 2–4) and from day to day (0.1–0.3‰ SD, n = 12–16) were very small. δOTC was ¹³C-depleted by 35–39‰ relative to ambient daytime CO₂ (δamb, −8.3‰ and −8.5‰ in May and September 2007). This provided a labeling signal that was > 100 times stronger than the (external) measurement precision, which integrated all errors in the system, including the precision of the isotope ratio mass spectrometer in the field (Schnyder et al., 2004; Gamnitzer et al., 2009).

In September 2006, two replicate plots were labeled for a total duration of 14 and 15 d, respectively. In both May and September 2007, 10 plots were labeled individually for 1, 2, 4, 8 or 16 d, with two replicates each. While two OTCs were used for the 16-d-long labeling (analogously to September 2006), another two OTCs were rotated between different plots for the shorter labeling durations, in such a way that the two replicates of a given labeling interval (e.g. 8 d) were not run simultaneously but within the 16-d period (see Supporting Information Fig. S1 for details of labeling schedule). Nonlabeled ‘control’ plots were marked outside of the OTCs. The effects of lateral cross-contamination of labeling CO₂ with nonlabeled air (or vice versa) between the inside and outside of the OTCs were negligible (Gamnitzer et al., 2011).

Environmental conditions

Each chamber was equipped with a set of sensors, including air temperature/relative humidity (1400-104; Li-Cor), soil temperature (Tsoil; 1400-103, Li-Cor; c. 5 cm depth) and photosynthetic photon flux density (PPFD; LI-190SZ quantum sensor interfaced with photomultiplier MV-100, Li-Cor; mounted near the OTCs). These environmental parameters were continuously monitored during the experimental periods. During daytime tracer application, the mean air temperature inside the OTCs was slightly higher than outside (+0.4°C in September 2006, +0.3°C in May 2007 and +1.4°C in September 2007), but relative humidity was lower (c. 42% inside vs c. 69% outside), as all OTCs were fed with dry air.

The effects of OTCs on environmental conditions were analyzed by Gamnitzer et al. (2009). Possible soil moisture stress in the OTCs was prevented by daily watering at dawn with the equivalent of the previous day’s evapotranspiration plus an extra 20% to account for possible percolation loss.

Aboveground green biomass (determined from three or four squares of 0.14 × 0.14 m² on each plot) was near-constant within the experimental periods in May and September 2007 (P = 0.22 and 0.51 for slope of linear regression, data not shown; data for 2006 were not available).

Harvests and sample preparation

In all experiments, individual shoots of the co-dominant species L. perenne, P. pratensis, T. officinale and T. repens were collected from the labeled plots and from the nonlabeled control plots. In all labeled plots, sampling was performed at the end of the nominal labeling durations (14–15 d in September 2006; 1, 2, 4, 8 and 16 d in May and September 2007). In addition, we collected samples at intervals in the plots with the longest labeling durations, that is, after 1, 2, 3, 5, 8 and 11 labeling days in September 2006, after 3 and 12 labeling days in May 2007, and after 3 and 11 labeling days in September 2007 (see also Fig. S1). During these additional samplings, particular care was taken to minimize canopy disturbance and, in total, only 0.8–2.6% of standing green biomass was removed from these plots.

On each sampling day, two to four average-sized shoots of each species were collected per plot 3.5–5.5 h after sunset. Sampled shoots consisted of mature grass tillers, single rosettes (T. officinale) or stolons (T. repens), and comprised all age classes of leaves (growing, mature, senescing and fully senesced leaves). Shoots were cut at their base (grasses and T. officinale), close to the soil surface, or proximal to the last fully senesced leaf (stolons of T. repensis), and stored in plastic bags on ice inside an insulation box. After transfer to the laboratory, green (living) and senesced (dead) leaf tissue (grasses and T. officinale) or stolon sections

| Table 1 Concentration and isotopic composition of CO₂ inside the open-top chambers (OTCs) |
|---------------------------------|-----------------|-----------------|-----------------|
| CO₂ (μmol mol⁻¹) inside OTCs at noon (12:00–13:00 h local time) | Overall mean: 365.1 | 366.9 | 375.7 |
|                                | Overall SD      | 3.2             | 6.2             | 4.6             |
|                                | Day-to-day SD   | 3.2             | 6.2             | 3.6             |
|                                | Plot-to-plot SD | 1.8             | 2.5             | 3.1             |
| δOTC (‰, assimilation-weighted mean) | Overall mean: −43.78 | −46.94 | −47.88 |
|                                | Overall SD      | 0.10            | 0.29            | 0.22            |
|                                | Day-to-day SD   | 0.09            | 0.27            | 0.20            |
|                                | Plot-to-plot SD | 0.07            | 0.15            | 0.10            |

The overall means and SD (across all plots and days), plot-to-plot SD (SD between the plots, n = 2–4, averaged across all days) and day-to-day SD (SD between the days, n = 12–16, averaged across the simultaneously labeled plots) in each experimental period are given.

δ¹³C inside the OTCs showed c. 1‰ diurnal variation during the light period (Gamnitzer et al., 2009). For each plot, daily mean δOTC was calculated from the time course of δOTC, weighted by net assimilation at the respective time (calculated from CO₂ concentration difference between OTC inlet and outlet, analogous to night-time respiration measurements by Gamnitzer et al., 2009).
bearing green and senesced leaf tissue (T. repens) were divided. All samples were placed in a forced draft oven for 1 h at 105°C (to arrest metabolism), and then dried at 60°C for 56 h, weighed and ground to a homogeneous fine powder in a ball mill.

Unlabeled control samples collected outside the OTCs were used as end-member in the two-member isotopic mixing model used in labeling data evaluation. In most cases (six of eight comparisons of species by experimental period combinations in 2007), these control samples had the same C mass as plants growing inside the OTCs for the maximum labeling duration of 16 d.

Estimation of metabolic carbon content

The sum of hot water-soluble C, starch C, and C in remobilizable protein, precipitated during the hot water extraction, was determined as an estimate of metabolic C. For the determination of soluble C, aliquots of 10 mg dry mass were placed in capped Eppendorf vials, extracted with 2 ml of distilled water for 10 min at 93°C and then shaken for 45 min at room temperature (Grimoldi et al., 2005). After centrifugation (at 9500 g for 15 min), 300 μl of the supernatants were pipetted into tin cups and oven dried at 60°C. The C content was determined with an enzymatic analyzer (NA 1110; Carlo Erba Instruments, Milan, Italy), using sulfuranilamide (Merck, Darmstadt, Germany) as a reference. Starch C in the residual pellets was determined by an enzymatic test (cat. no. 207748; Boehringer, Mannheim, Germany). Remobilizable protein C, precipitated during hot water extraction, was estimated on the basis of the nitrogen (N) content of the residual pellets, which had a similar C:N ratio to the samples (IVA Analysentechnik e.K., Meerbusch, Germany) and combusted in an elemental analyzer (NA 1110; Carlo Erba Instruments) interfaced (Conflo III; Finnigan MAT, Bremen, Germany) to a continuous-flow isotope-ratio mass spectrometer (CF-IRMS; Delta Plus, Finnigan MAT). Samples were measured against a laboratory working reference CO2 gas, previously calibrated against a secondary isotope standard (IAEA-CH6; precision of calibration, ± 0.06%/oo SD). A solid internal laboratory standard (fine ground wheat flour, which had a similar C:N ratio to the samples) was used for the correction of instrument drift. The precision (SD) of sample repeats was 0.15%/oo.

Fractions of labeled C

The fractions of labeled (‘new’) and unlabeled (‘old’) C in plant tissue (f_labeled and f_unlabeled, with f_labeled + f_unlabeled = 1) were calculated from the δ13C of the samples (δ_sample) as in Schnyder (1992):

\[ f_{\text{labeled}} = (\delta_{\text{sample}} - \delta_{\text{old}}) / (\delta_{\text{new}} - \delta_{\text{old}}) \]  
\[ \text{Eqn 1} \]

\[ \delta_{\text{old}} \] and \( \delta_{\text{new}} \) represent the species- and experimental period-specific δ13C of nonlabeled (control) and fully labeled plant tissue, respectively. As the labeling duration was too short to achieve isotopic saturation of total shoot C with labeled C, \( \delta_{\text{new}} \) was estimated as:

\[ \delta_{\text{new}} = (\delta_{\text{OTC}} - \Delta) / (1 + \Delta) \]  
\[ \text{Eqn 2} \]

The C isotope discrimination \( \Delta \) was obtained for each species and experimental period as:

\[ \Delta = (\delta_{\text{amb}} - \delta_{\text{old}}) / (1 + \delta_{\text{old}}) \]  
\[ \text{Eqn 3} \]

This estimation used the assumption that \( \Delta \) was not altered by the conditions inside the OTCs, and therefore was identical for labeled and unlabeled plant tissue of a given species in a given experimental period. The uncertainty/error of this assumption on estimates of \( f_{\text{labeled}} \) must have been very small, as all plants in the OTCs were well watered, the CO2 concentration in the OTCs was close to ambient and \( \delta_{\text{OTC}} \) was virtually constant. The vapor pressure gradient between the OTCs and outside (0.4–0.5 kPa) corresponded to a 1–2%/oo decrease in \( \Delta \) in investigations of Auerswald et al. (2010) and Köhler et al. (2010). At the same site, Schnyder et al. (2006) observed a maximum range of \( \Delta \) in pasture biomass of 1.6%/oo among dry and wet periods of multiple years (including the centennial drought year 2003). However, these relationships probably exaggerate the effect of vapor pressure on \( \Delta \), as vapor pressure co-varied with soil water deficit in these investigations, a factor that was excluded in the present work. Nevertheless, if the vapor pressure effect was fully expressed, the eventual variations in \( \Delta \) translated into a variation of \( f_{\text{labeled}} \) of < 3% (Gamnitzer et al., 2009).

Modeling pool turnover and allocation

Compartmental models have proven to be useful for quantitative interpretations of tracer dynamics from organisms to ecosystems (Moorthy & Jarman, 1975; Borland & Farrar, 1988; Toal et al., 2000; Nägele et al., 2010; Lee et al., 2011; Schnyder et al., 2012). They represent biological systems via a discrete number of functionally distinct C pools, where each is exchanging with other pools in the system and with the (abiotic) environment, according to a specific linkage pattern (Jacquez, 1972).

We tested different compartmental models. The simplest models consisted of one well-mixed pool or one nonmixed (also termed layered or 'first-in, first-out') pool. A biologically meaningful model included at least a metabolic and a nonmetabolic pool (as discussed in the Introduction). We tested two-pool models with either both pools well mixed,
or one well mixed and one nonmixed. A three-pool model was developed from the latter two-pool model by splitting the metabolic pool into a ‘transport’ and a ‘storage’ pool, similar to Lehmeyer et al. (2010a). The multiple-pool models were tested unconstrained, or constrained by independent measurements of metabolic C content (see later).

As shown in the Results and Discussion sections, the simplest biologically meaningful shoot biomass model that fitted the data of all species × experimental period combinations (except for one case) was the two-pool compartmental model with a well-mixed metabolic pool and a nonmixed nonmetabolic pool (the ‘proposed model’, Fig. 1). In this model, photosynthetically fixed C (\(F_{\text{assim}}\)) enters the system via the metabolic pool. From there, C is either incorporated into the shoot nonmetabolic pool (\(F_{\text{nonmetab}}\)) or is lost by shoot and root respiration and allocation to roots and rhizosphere (\(F_{\text{root+resp}}\)). The model is a steady-state model in which pool sizes and fluxes are constant (\(F_{\text{nonmetab}} = F_{\text{dead}}\) and \(F_{\text{assim}} = F_{\text{nonmetab}} + F_{\text{root+resp}}\)). The verisimilitude of this model conception is verified in the Results and Discussion sections.

In this model, the tracer kinetics in the shoot biomass of each species are determined by three parameters: the (mean) residence time of C in the metabolic (\(\tau_{\text{metab}}\)) and nonmetabolic (\(\tau_{\text{nonmetab}}\)) pools, and the relative size of both pools, represented by the contributions (weights) of the metabolic (\(w_{\text{metab}}\)) and nonmetabolic (\(1 - w_{\text{metab}}\)) pools to total C in shoot biomass. The increase in the fraction of labeled C in the living shoot biomass is given by (see Methods S1 for a derivation):

\[
\frac{f_{\text{labeled}}(t)}{t} = w_{\text{metab}}(1 - \exp(-t/\tau_{\text{metab}})) + (1 - w_{\text{metab}})\frac{1}{\tau_{\text{nonmetab}}}(t - \tau_{\text{metab}})(1 - \exp(-t/\tau_{\text{metab}})) \tag{Eqn 4}
\]

The first summand represents metabolic C, weighted by its contribution \(w_{\text{metab}}\) to total shoot C; the second summand describes nonmetabolic C, accordingly weighted by \(1 - w_{\text{metab}}\). The fraction \(w_{\text{metab}}\) was estimated as given earlier for the labeling experiments in 2007. For September 2006, such data were not available, and the average of the former values was used. In the model runs, both \(\tau_{\text{nonmetab}}\) and \(\tau_{\text{metab}}\) were optimized in terms of a minimized root-mean-square error for the tracer kinetics of each species in each experimental period. To enable interspecies comparisons, a complementary model was fitted for each species to the (pooled) tracer kinetics data of the other three species in the respective experimental period.

All fluxes are given relative to total shoot C (nonmetabolic + metabolic C), denoting the rate of C transfer per unit of (green) shoot C, and are obtained as:

\[
F_{\text{nonmetab}} = (1 - w_{\text{metab}})/\tau_{\text{nonmetab}} \quad \text{and} \quad Eqn 5
\]

\[
F_{\text{assim}} = w_{\text{metab}}/\tau_{\text{metab}} \quad \text{Eqn 6}
\]

\(F_{\text{assim}}\), the relative photosynthesis (or tracer uptake, cf. Lattanzi et al., 2012a) rate, is the rate of CO\(_2\) fixation per unit of (green) shoot C. The proportion of fixed C allocated to the shoot nonmetabolic pool (\(A_{\text{nonmetab}}\)) is given by:

\[
A_{\text{nonmetab}} = F_{\text{nonmetab}}/F_{\text{assim}} \quad \text{Eqn 7}
\]

As the model is a steady-state model, we investigated how far model predictions were dependent on environmental variables. Thus, to account for day-to-day variation in the environmental conditions, the tracer kinetics were also analysed (analogous to Eqn 4) as a function of accumulated \(T_{\text{soil}}\) (threshold temperature 0°C) and accumulated PPFD. For the comparison of results obtained with the different independent variables, the \(\tau\) values were scaled by the average daily \(T_{\text{soil}}\) and PPFD in the respective experimental period.

**Statistics**

All model fits and statistical data evaluations were carried out in R (R Development Core Team, 2014). The nonlinear least-squares estimates of the model parameters of the (nonlinear) pool models were determined from the tracer kinetics data with the NLS function from the NLS2 package (Grothendieck, 2013). The Akaike information criterion (AIC) was determined for each model in each species × experimental period combination. ΔAIC is the AIC of a tested model minus that of the proposed model. ΔAIC > 0 indicates that the tested model was less likely to represent the observed data than the proposed model. The standard error (SE) of \(\tau\) was determined by the nls fitting routine. Gaussian error propagation was used to estimate the SE of \(F_{\text{assim}}\) and \(A_{\text{nonmetab}}\). Differences in pool characteristics between species or experimental periods were investigated by two-sample \(t\)-tests and paired \(t\)-tests where appropriate.
Results

Environmental conditions

Daily mean air and soil temperatures (15–16°C) were similar and practically the same in September 2006 and May 2007 (Table 2). In September 2007, temperatures were c. 2–3°C cooler. The daily mean relative humidity of air was similar in all experimental periods (48–52%). However, the daily mean PPFD differed between the periods; it was highest at 40 mol m⁻² d⁻¹ in May 2007, intermediate in September 2006 (34 mol m⁻² d⁻¹) and lowest in September 2007 (25 mol m⁻² d⁻¹). In all periods, daily PPFD was relatively constant for most days, except for a few cloudy days with strongly reduced radiation (for time series of TSoil and PPFD during the experimental periods, see Fig. S1).

Carbon tracer time courses

The green shoot biomass of the four species, L. perenne, P. pratensis, T. officinale and T. repens, experienced strong continuous label incorporation. Tracer incorporation was strongest at the beginning of labeling: 4–11% of all green shoot biomass was labeled on the first labeling day (Fig. 2, open and closed symbols). By the end of the 14–16-d-long labeling periods, 36–64% of all C in the shoot biomass of all species was labeled. Tracer administration and incorporation were precise; the variability in label contents was 4.2% (average SD, n = 2–4) for replicate plants within a plot, and 3.2% (average SD, n = 2) between replicate plots. In each experimental period, the general pattern of the labeling kinetics was similar for all species. Notably, senesced leaves, still attached to live shoots, did not contain any label; even at the end of the labeling experiments, no significant amount of tracer was detected in these leaves (Fig. 2, gray crosses).

Metabolic carbon content

The metabolic C content, ωmetab, of the different species ranged between 360 and 480 mg g⁻¹ of total shoot C in May and September 2007, with an average of 440 mg g⁻¹ (Fig. 3). In September 2007, all species agreed within 95% confidence intervals (CIs). In May 2007, ωmetab was 17% lower in the grasses than in the dicots. In the grasses, ωmetab was 18% lower in May than in September, but, in the dicot species, the difference between the experimental periods was not significant.

| Table 2 Environmental conditions during the labeling experiments |
|-------------------------------|---------------|-----------------|-----------------|
|                             | September 2006 (n = 15) | May 2007 (n = 16) | September 2007 (n = 16) |
| Air temperature (°C)        | 15.7 ± 1.8      | 15.4 ± 3.6      | 12.9 ± 1.9      |
| Relative humidity (%)       | 52.5 ± 5.2      | 52.6 ± 4.0      | 48.3 ± 3.2      |
| Soil temperature (Tsoil) (°C)| 16.2 ± 1.2      | 16.4 ± 2.3      | 14.1 ± 1.1      |
| PPFD (mol m⁻² d⁻¹)          | 34 ± 6          | 40 ± 17         | 25 ± 10         |

1Mean inside the open-top chambers (OTCs) during the labeling experiments ± day-by-day SD across all OTCs.
2Mean during the labeling experiments ± day-by-day SD.

Model fits

The proposed model (Fig. 1) provided a fair fit to the C tracer kinetics (Fig. 2, solid lines) in all species and all experimental periods, except for T. officinale in September 2006. In the latter, ωmetab was not analyzed in this experimental period, and with the use of the mean of the two other labeling periods, the fitting routine did not provide a convergent solution. However, a significant fit was obtained when ωmetab < 400 mg g⁻¹ C was used (see Fig. S2). For the proposed model, AIC indicated a clearly better performance than the model with the one layered pool (ΔAIC = 4.5–35), and similar (seven of 12 comparisons with |ΔAIC| < 4) or better (five of 12 comparisons with ΔAIC = 4.6–20) performance than the model with the one well-mixed pool (Table 3). Both versions of the two-pool model (both pools well mixed, or one well-mixed pool and one layered pool) showed similar AIC (ΔAIC < 3). Failure of the fitting routine became more frequent with model complexity (increasing number of fitting parameters). Thus, the model with three parameters could only be fitted in four of 12 datasets (in which ΔAIC = 0–2, indicating that the unconstrained model was not superior to the proposed model). The model with four parameters did not fit to any of the datasets (Table 3).

The proposed model fitted the C tracer data with an average residual SE of 0.06. The residual fraction of labeled C (observed $f_{labeled} –$ model fit) showed a uniform distribution in most (nine of 11) datasets (Fig. 4). However, in L. perenne and T. repens in May 2007, the residuals revealed a pattern. During the first 4 d of continuous labeling, the model fit progressively overestimated the observed tracer content and, between 8 and 12 d of labeling, the model fit underestimated the observed tracer content by c. 0.1. Compartmental models with more parameters could not be fitted in these particular cases.

Comparison of the fit to an individual species (Fig. 2, solid lines) with the fit to the bulked data of the other three species (dashed lines) showed indistinguishable curves within the 95% confidence bands (gray shaded areas) in six of 11 datasets. The deviations in the remaining five datasets did not show an obvious relationship with a particular species or experimental period (see also, later, the subsection ‘Influence of environmental conditions on model fits’).

Mean residence times of carbon in the metabolic and nonmetabolic pool

The mean residence time of C in the metabolic pool, $\tau_{metab}$, ranged from 5 to 8 d (Fig. 5a–c), with no significant difference between species in the different experimental periods (see also Table S1). However, in September 2007, the coolest of all the experimental periods, $\tau_{metab}$ was almost 2 d longer than in September 2006 and May 2007.

The residence time of C in the nonmetabolic pool, $\tau_{nonmetab}$ (Fig. 5d–f), was much longer than $\tau_{metab}$ and varied between 20 and 58 d in all species and experimental periods, except for P. pratensis in May 2007. In the latter, $\tau_{nonmetab}$ was c. 150 d, albeit that estimate was associated with a very large uncertainty.
Although \( \tau_{\text{nonmetab}} \) exhibited distinct variability (in particular in the grasses; Table S1), the data revealed no consistent effect of species or experimental period on \( \tau_{\text{nonmetab}} \). Grasses tended to have lower values of \( \tau_{\text{nonmetab}} \) in September 2007, but this effect was not found in the other periods.

**Relative photosynthesis rate**

The relative photosynthesis rate, \( F_{\text{assim}} \), ranged between 5.7% and 8.9% in the different species and experimental periods (Fig. 5g–i). Between-species variation was small and nonsignificant in September 2006 and 2007. Although the grasses seemed to exhibit a lower \( F_{\text{assim}} \) than the dicots in May 2007, the difference was not significant. In September 2007, the experimental period with the coolest conditions and lowest PPFD, \( F_{\text{assim}} \) was c. 18% lower than in September 2006 and May 2007.

**C allocation**

The model predicted that, on average of all species and experimental periods, 24% of all fixed C was allocated to shoot nonmetabolic biomass (Fig. 5j–l). However, \( A_{\text{nonmetab}} \) was variable (7–45% of all fixed C), in particular for the grasses.
Research

Influence of environmental conditions on the model fits

In all species and experimental periods, the fitting parameters $\tau_{\text{metab}}$ and $\tau_{\text{nonmetab}}$ were not influenced significantly by the independent variable (accumulated $T_{\text{soil}}$ or PPFD instead of labeling duration; see Table 4 and Fig. S3). In all cases, $\tau$ determined from the accumulated $T_{\text{soil}}$ and PPFD agreed with that predicted by the labeling duration within 95% CI. In all experimental periods, $T_{\text{soil}}$ and PPFD influenced $\tau_{\text{metab}}$ and $\tau_{\text{nonmetab}}$ in the same direction and magnitude in all species, with $\tau$ determined from the accumulated $T_{\text{soil}}$ and PPFD ranging within $-16\%$ to $+25\%$ (average of all species) of that predicted by the labeling duration. In general, $T_{\text{soil}}$ had a smaller influence than PPFD on the estimated $\tau$.

Potential drivers of bias in $f_{\text{labeled}}$ were investigated for the May 2007 data, where we had detected a seemingly systematic deviation between the observed tracer time course and model fit (see earlier). Indeed, the residual fraction of labeled C correlated with both $T_{\text{soil}}$ and PPFD on the day before harvest ($P \leq 0.012$, $R^2 \geq 0.13$; Table S5). Similar relationships did not exist for the other periods (data not shown).

Discussion

Model realism

The present analysis supports a two-pool model, with a well-mixed metabolic and a nonmixed (first-in, first-out) nonmetabolic pool, as a meaningful and largely unbiased abstraction of the $^{13}$C tracer incorporation kinetics in whole-shoot biomass of several co-dominant plant species from different functional groups in the same grassland community.

Steady state

That the plant systems were reasonably close to a steady state at the day-by-day time scale of the analysis was supported by the relative constancy of the thermal conditions within the experimental periods, the absence of drought stress as a result of daily watering and the constancy of C mass in total aboveground green biomass in both experimental periods in 2007 (which suggested that the flux of new tissue was balanced by a corresponding senescence flux at the whole-community level). Furthermore, the C mass of individual shoots was constant during the whole labeling duration for all species in these two experimental periods (data not shown), according with the notion that the composite size of plant C pools was constant in the OTCs. Although daily PPFD was markedly reduced on a few days (as a result of periodic cloudiness, a regular attribute of weather conditions at the site), the predictions of pool kinetics and their effect on tracer incorporation, pool turnover and allocation. In addition, the choice of continuous rather than pulse labeling may have promoted the buffering of short-term environmental fluctuations in tracer incorporation and kinetics. Nevertheless, in May 2007, some bias in the prediction of tracer content was found, which was related to cloudiness and deviant $T_{\text{soil}}$ on the day before sampling. Yet, the elimination of data with the most strongly aberrant $T_{\text{soil}}$ or PPFD conditions (30% of the data) had no significant effect on the predicted pool kinetics (data not shown).

Table 3 Model comparison by Akaike information criterion (AIC), expressed as relative model performance: $\Delta AIC = AIC$ of a tested model – AIC of the ‘proposed’ model (cf. Fig. 1)

<table>
<thead>
<tr>
<th>Exp. period</th>
<th>Species</th>
<th>One-pool models</th>
<th>Two-pool models</th>
<th>Three-pool model</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 2006</td>
<td>L. perenne</td>
<td>14</td>
<td>35</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>P. pratensis</td>
<td>1.0</td>
<td>12</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>T. officinalis</td>
<td>20</td>
<td>25</td>
<td>-0.1</td>
</tr>
<tr>
<td></td>
<td>T. repens</td>
<td>-1.4</td>
<td>13</td>
<td>-0.4</td>
</tr>
<tr>
<td>May 2007</td>
<td>L. perenne</td>
<td>-3.8</td>
<td>14</td>
<td>-2.2</td>
</tr>
<tr>
<td></td>
<td>P. pratensis</td>
<td>10</td>
<td>32</td>
<td>-0.4</td>
</tr>
<tr>
<td></td>
<td>T. officinalis</td>
<td>-0.8</td>
<td>28</td>
<td>-2.2</td>
</tr>
<tr>
<td></td>
<td>T. repens</td>
<td>-2.8</td>
<td>10</td>
<td>-2.9</td>
</tr>
<tr>
<td>September 2007</td>
<td>L. perenne</td>
<td>-2.8</td>
<td>10</td>
<td>-2.9</td>
</tr>
<tr>
<td></td>
<td>P. pratensis</td>
<td>0.0</td>
<td>13</td>
<td>-0.1</td>
</tr>
<tr>
<td></td>
<td>T. officinalis</td>
<td>0.0</td>
<td>13</td>
<td>-0.1</td>
</tr>
<tr>
<td></td>
<td>T. repens</td>
<td>0.0</td>
<td>13</td>
<td>-0.1</td>
</tr>
</tbody>
</table>
Model configuration  In most cases, the proposed model (Fig. 1) performed better than either simpler or more complex models. Never, with one exception (T. officinale in September 2006, for which \(\tau_{\text{metab}}\) was probably overestimated), did it perform significantly inferiorly to any other tested model. The first-in, first-out mechanism implemented in the nonmetabolic pool (i.e. its non-mixed nature) was supported by the absence of tracer in senesced tissue, even at the end of the labeling period. Indeed, if C incorporation in the nonmetabolic pool stops at the end of secondary cell wall synthesis, a few days after full expansion of leaf tissue (MacAdam & Nelson, 2002), one should not expect any significant tracer appearance in senesced leaf tissue within < 20 d of labeling (cf. Schleip et al., 2013). In addition, the first-in, first-out principle agrees well with observations and mechanistic models of leaf production and senescence in grassland plants (e.g. Thornley, 1998; Hennessy et al., 2008).

Certainly, the kinetic description of metabolic C as a single well-mixed pool supplying both respiration and growth is a simplification. Assimilate pools with short \(\tau\) (on the order of hours) (Farrar & Farrar, 1985, 1986) were not considered separately. However, although vitally important, these pools form a small proportion of the total shoot biomass (Lattanzi et al., 2005; Lehmeier et al., 2008), and therefore contribute little to its longer term (> 1 d) labeling kinetics. In addition, such fast pools are not resolved on the day-by-day time scale of labeling in the present study. Further, there is good evidence for an intimate connection between fast pools and storage pools in the supply of substrate to growth and respiration on a daily time scale (Lattanzi et al., 2005; Lehmeier et al., 2008, 2012). The common function in feeding both growth (structural biomass synthesis) and respiration also agrees with the similar \(\tau_{\text{metab}}\) in this study and \(\tau\) of the substrate pool supplying autotrophic respiration of the ecosystem in the same experiment in May 2007 (Gaminzter et al., 2009). Finally, the ‘single’ metabolic pool idea is also supported by previous approaches implemented in mechanistic plant growth models (Thornley, 2011; and references therein) or the ‘single-substrate’ pool model of Ogée et al. (2009).
Co-dominant species exhibited very similar kinetics and size of the metabolic pool

The four species exhibited similar metabolic C content (c. 44% of aboveground biomass), and the tracer kinetics analysis indicated a similar C residence time in this pool (\(\tau_{\text{metab}}\) of c. 6 d) for all species. This concordance existed irrespective of the compartmental analysis of the tracer kinetics as a function of labeling duration, accumulated \(T_{\text{soil}}\) or PPFD. Furthermore, the interspecific similarity was unrelated to a putative systematic bias in the estimate of \(w_{\text{metab}}\), provided that such a bias was equally directed for all species (Fig. S2). The concordance and small variation in \(\tau_{\text{metab}}\) is remarkable, given the different morphologies (two bunchgrasses, a stoloniferous legume and a leaf rosette-forming dicot) and main storage carbohydrates (vacuolar sucrose and fructan in the two grasses and in \(T. officinale\), Kaiser et al., 1982; Pollock & Cairns, 1991; Van den Ende et al., 2000; Lattanzi et al., 2012b; starch in \(T. repens\), Baur-Höch et al., 1990) of the species. To our knowledge, the kinetic characteristics of metabolic C have not been reported for members of different functional groups growing in the same plant community, and so opportunities for discussion are limited. However, Poorter & Bergkotte (1992) observed similar concentrations of soluble and insoluble carbohydrates, organic acids and organic nitrogenous compounds for herbaceous species from different functional groups with similar relative growth rates.

Relationships between turnover time of the nonmetabolic pool, C allocation and leaf life span

Both the residence time of C in the nonmetabolic pool, \(\tau_{\text{nonmetab}}\), and the allocation of C to the nonmetabolic pool, \(A_{\text{nonmetab}}\), exhibited notable variation. Interestingly, these variations were not associated with any obvious difference between species, or between experimental periods. However, the variations were not random, but linked with independent estimates of leaf life span.
Table 4 Influence of the independent variable on carbon (C) turnover, giving $T_{\text{metab}}$ and $T_{\text{nonmetab}}$ from fits of the tracer kinetics vs accumulated $T_{\text{soil}}$ and photosynthetic photon flux density (PPFD), relative to $T_{\text{metab}}$ and $T_{\text{nonmetab}}$ from fits vs labeling duration, for all experimental periods and species.

<table>
<thead>
<tr>
<th>Experimental period</th>
<th>Species</th>
<th>$T_{\text{soil}}$</th>
<th>PPFD</th>
<th>$T_{\text{soil}}$</th>
<th>PPFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 2006</td>
<td><em>Lolium perenne</em></td>
<td>0.90</td>
<td>0.84</td>
<td>1.20</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td><em>Poa pratensis</em></td>
<td>0.90</td>
<td>0.83</td>
<td>1.21</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td><em>Taraxacum officinale</em></td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>May 2007</td>
<td><em>L. perenne</em></td>
<td>1.02</td>
<td>1.00</td>
<td>0.99</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td><em>P. pratensis</em></td>
<td>1.01</td>
<td>0.95</td>
<td>0.98</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td><em>T. officinale</em></td>
<td>1.01</td>
<td>0.93</td>
<td>1.02</td>
<td>1.29</td>
</tr>
<tr>
<td>September 2007</td>
<td><em>L. perenne</em></td>
<td>1.02</td>
<td>1.15</td>
<td>0.98</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td><em>P. pratensis</em></td>
<td>1.02</td>
<td>1.14</td>
<td>0.99</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td><em>T. officinale</em></td>
<td>1.02</td>
<td>1.12</td>
<td>0.98</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td><em>T. repens</em></td>
<td>1.02</td>
<td>1.12</td>
<td>0.98</td>
<td>0.91</td>
</tr>
</tbody>
</table>

A value of 1 means that $t$ calculated for $T_{\text{soil}}$ or PPFD data (see respective column) is identical to that calculated from the labeling duration. na, not available. In these cases, the fitting routine did not converge.

Table 5 Correlation ($P$ values and $R^2$) of the residual fraction of labeled carbon (C; observed $T_{\text{soil}}$ minus model fit) with soil temperature ($T_{\text{soil}}$) on the day before harvest and photosynthetic photon flux density (PPFD) on the day before harvest for the individual species in May 2007.

<table>
<thead>
<tr>
<th>Species</th>
<th>$T_{\text{soil}}$</th>
<th>PPFD</th>
<th>$P$</th>
<th>$R^2$</th>
<th>$P$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lolium perenne</em></td>
<td>0.000</td>
<td>0.32</td>
<td>0.003</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Poa pratensis</em></td>
<td>0.011</td>
<td>0.13</td>
<td>0.009</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Taraxacum officinale</em></td>
<td>0.012</td>
<td>0.13</td>
<td>0.001</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trifolium repens</em></td>
<td>0.000</td>
<td>0.40</td>
<td>0.000</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 6 Relationship between $T_{\text{nonmetab}}$ and leaf life span (upper panel) and $A_{\text{nonmetab}}$ and leaf life span (lower panel). Leaf life span data were taken from Schleip et al. (2013) and report the time from leaf appearance to the beginning of senescence (defined as <5% senescence-related nongreen leaf area per leaf, $t_{\text{c,5}}$ in the article). Black squares, *Lolium perenne*; red circles, *Poa pratensis*; green triangles, *Taraxacum officinale*; open blue triangles, *Trifolium repens*. The numbers indicate the different experimental periods (1, September 2006; 2, May 2007; 3, September 2007). Error bars indicate ± SE. Black line, 1 : 1 line.

20% for eight of 12 species × experimental period combinations) or connected with strong increases in uncertainty (four of 12 cases).

The opposite relationships of $T_{\text{nonmetab}}$ and $A_{\text{nonmetab}}$ with leaf life span existed across species and experimental periods. Thus, the inverse relationship of $T_{\text{nonmetab}}$ and $A_{\text{nonmetab}}$ dictated for each species in each experimental period by the steady-state condition of the pool model, seemed to be a dominant feature. This opposing response of $T_{\text{nonmetab}}$ and $A_{\text{nonmetab}}$ at the species level to mechanisms determining leaf traits would stabilize the size of the nonmetabolic pool (which was mainly composed of leaf structural biomass), as increased allocation to this pool is associated with decreased $t_c$ and vice versa. Furthermore, the absence of a systematic species-related pattern in both $T_{\text{nonmetab}}$ and $A_{\text{nonmetab}}$ is expected to scale up to a lesser variability in nonmetabolic C content at the community scale. In this regard, it is interesting that the relative photosynthesis rates of the different species were rather similar in the different experimental periods, particularly in September 2006 and 2007, supporting the idea of a stable co-dominance pattern of these species at the time scale of the studies.
Implications for plant, community and ecosystem C relations

This analysis presents evidence for a remarkable similarity of metabolic pool turnover and size in co-dominant members of different functional groups of grassland species in the same community. It is suggested that the mechanisms controlling the size and kinetics of metabolic C in the different species are connected to similar C demands in the buffering capacities of stores to satisfy the needs of growth and maintenance processes of these fast-growing species. In addition, we observed notable variation in \( \tau_{\text{nonmetab}} \) and \( A_{\text{nonmetab}} \), which was directly connected with the leaf life span. To our knowledge, the (signaling and physiological) mechanism underlying the opposite variation of \( \tau_{\text{nonmetab}} \) and \( A_{\text{nonmetab}} \) is presently unknown, but may be connected with the mechanisms controlling C allocation between shoot and root nonstructural pools or the rhizosphere. Again, the species did not differ systematically in \( \tau_{\text{nonmetab}} \) across the three experimental periods. This similarity was related, at least in part, to the fact that the leaf life span of the present species was near-constant in terms of growing degree days (Schleip et al., 2013), the grassland community was largely composed of vegetative plants and thermal conditions were similar in the different periods.

The close interrelationships between leaf life span, residence time of C in the aboveground nonmetabolic pool and allocation of C to that pool (and – by difference, see Fig. 1 – allocation to respiration plus root and rhizosphere) adds new meaning to the leaf life span as a functional trait in the leaf and plant economics spectrum (Reich et al., 1992; Wright et al., 2004; Funk & Cornel, 2013; Poorter et al., 2014; Reich, 2014; Reichstein et al., 2014) and its implication for C cycle studies in grassland systems (as shown here) and forest systems (Reich et al., 2014). We suggest that the extension of similar studies of C allocation and pool turnover to belowground biomass in grassland and other ecosystems would further benefit the understanding of tissue life span as a functional trait in C cycle studies.

Overall, the present observations support the view that the compartmental model identified here is useful, at least for intensively managed grassland communities, and that it reflects basic mechanisms of C allocation and pool turnover in the shoots of the co-dominant species. In addition, the model provides predictions of the delay between C incorporation in aboveground plant biomass and transfer to the litter fraction, a major substrate for the heterotrophic food chain. Together with a knowledge of root life span, such information is useful for the separation of functional components (autotrophic and heterotrophic) of ecosystem respiration (Gamnitzer et al., 2009). As the four co-dominant species accounted for c. 80% of the total shoot biomass of the entire grassland community, we should also expect that the observed similarities in pool kinetics and allocation will scale up to similar relationships at the level of the whole plant community.

Acknowledgements

We thank Tom Gottfried, Iris Köhler and Claudia Landinger for help during field sampling, Richard Wenzel for technical assistance with the labeling system, Monika Michler, Angela Ernst-Schwärzli and Anja Schmidt for help with sample preparation and Rudi Schäufele for elemental and isotope analysis. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 607).

Author contributions

U.O., I.S. and H.S. planned and designed the research. U.O. and I.S. performed the experiments. U.O., I.S. and F.A.L. analyzed the data. U.O. performed the compartmental modeling. U.O., I.S., F.A.L. and H.S. discussed the results and implications. U.O., I.S. and H.S. wrote the manuscript.

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Thornley JHM. 2011. Plant growth and respiration re-visited: maintenance respiration defined – it is an emergent property of, not a separate process within, the system – and why the respiration: photosynthesis ratio is conservative. Annals of Botany 108: 1365–1380.


Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Environmental conditions during the labeling experiments, labeling durations and sampling times.

Fig. S2 Sensitivity of carbon (C) residence time in the metabolic and nonmetabolic pools to the metabolic C content.

Fig. S3 Fraction of labeled carbon (C) ($f_{labeled}$) in live (green) shoot biomass vs accumulated soil temperature $T_{soil}$ (growing degree days) and photosynthetic photon flux density (PPFD).

Table S1 Variation between species and experimental periods in carbon (C) residence time and allocation

Methods S1 Derivation of equation describing the tracer kinetics in aboveground biomass.

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