Rate of Belowground Carbon Allocation Differs with Successional Habit of Two Afromontane Trees

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Abstract

**Background:** Anthropogenic disturbance of old-growth tropical forests increases the abundance of early successional tree species at the cost of late successional ones. Quantifying differences in terms of carbon allocation and the proportion of recently fixed carbon in soil CO2 efflux is crucial for addressing the carbon footprint of creeping degradation.

**Methodology:** We compared the carbon allocation pattern of the late successional gymnosperm *Podocarpus falcatus* (Thunb.) Mirb. and the early successional (gap filling) angiosperm *Croton macrostachyus* Hochst. es Del. in an Ethiopian Afromontane forest by whole tree 13CO2 pulse labeling. Over a one-year period we monitored the temporal resolution of the label in the foliage, the phloem sap, the arbuscular mycorrhiza, and in soil-derived CO2. Further, we quantified the overall losses of assimilated 13C with soil CO2 efflux.

**Principal Findings:** 13C in leaves of *C. macrostachyus* declined more rapidly with a larger size of a fast pool (64% vs. 50% of the assimilated carbon), having a shorter mean residence time (14 h vs. 55 h) as in leaves of *P. falcatus*. Phloem sap velocity was about 4 times higher for *C. macrostachyus*. Likewise, the label appeared earlier in the arbuscular mycorrhiza of *C. macrostachyus* and in the soil CO2 efflux as in case of *P. falcatus* (24 h vs. 72 h). Within one year soil CO2 efflux amounted to a loss of 32% of assimilated carbon for the gap filling tree and to 15% for the late successional one.

**Conclusions:** Our results showed clear differences in carbon allocation patterns between tree species, although we caution that this experiment was unreplicated. A shift in tree species composition of tropical montane forests (e.g., by degradation) accelerates carbon allocation belowground and increases respiratory carbon losses by the autotrophic community. If ongoing disturbance keeps early successional species in dominance, the larger allocation to fast cycling compartments may deplete soil organic carbon in the long run.

Introduction

The residence time of the assimilated carbon in an ecosystem is a function of its allocation in the plant-soil system [1]. While carbon allocated in fast-cycling tissues and compounds is quickly returned to atmosphere by respiration, carbon incorporated into structural compounds of the plant or transformed to slow-cycling soil organic matter has a much longer lifetime and, by this, determines the long-term carbon sequestration [2,3]. Consequently, modification in the carbon allocation pattern by compositional and structural changes of the forest vegetation affects carbon pools and turnover and may influence the carbon balance at ecosystem scale [4,5,6].

Isotope tracer techniques give the opportunity to follow carbon fluxes in the plant-soil system in situ. During the last few years good progress has been made with 13C and 14C pulse labeling experiments of whole trees to measure the carbon allocation along with the contribution of recent photosynthates to soil CO2 efflux, e.g., see reviews of Brüggemann et al. [7] and Epron et al. [8]. Studies in boreal [9,10,11] and temperate [12,13,14] forests provided convincing evidence that soil respiration is closely linked to photosynthesis and that the contribution of recently assimilated carbon to soil CO2 efflux is large. The transfer of assimilated carbon belowground may account for up to 25–63% of gross primary production [15]. Once belowground, it becomes rapidly available for metabolic processes in the autotrophic continuum, i.e., roots and soil biota closely associated with roots [3,16,17]. Recently gained carbon can be rapidly transferred belowground, influencing soil CO2 efflux rates on short-time scales (from hours to days) [1,3,18,19]. The rate of carbon transfer depends both on environmental factors as well as on the tree species. Transfer rates are reported to slow down with decreasing temperature and soil moisture [14,20,21], tentatively due to changes in the turgor.
pressure gradients between the source and sink organs [8]. Pulse labeling experiments also showed broadleaved tree species characterized by much higher carbon transfer rates than coniferous species [8,21]. However, as emphasized by Epron et al. [8], hitherto pulse labeling studies have been restricted to temperate and boreal forests, while trees from tropical ecosystems have not yet been assessed. Given the importance of tropical forests in the climate change debate, this is a serious shortcoming, since prolonged vegetation period and higher biomass production suggest pronounced differences to temperate forests.

Though tropical ecosystems, and particularly African forests, have been recognized as a globally relevant carbon sink [22,23,24], there is still great uncertainty on magnitude and variability of the African carbon stocks and fluxes [25,26]. Controversially, evaluations of African carbon stocks have found the carbon balance of African ecosystems to range from a sink of about 0.3 Pg C yr\(^{-1}\) to a small source, mainly depending on whether the rate of deforestation and forest degradation had been taken into account [23,27]. For sub-Saharan Africa, Gaïs et el. [27] estimated the CO\(_2\) emission induced by forest degradation and deforestation to be about 0.24 Pg C yr\(^{-1}\). In addition, scant information on the effects of anthropogenic pressure on representative African forest ecosystems makes the regional estimates and biogeochemical models of carbon cycle highly uncertain [23,24]. In addition, there is little information concerning the effects of anthropogenic pressure on representative African forest ecosystems, which makes the regional estimates and biogeochemical models of carbon cycle highly uncertain [23,24].

In contrast to tropical lowlands, Afromontane ecosystems are often highly productive in agricultural terms and thus densely populated. Consequently, there deforestation as well as forest degradation is much more pronounced than in the lowlands. As a consequence, deforestation as well as forest degradation is much more pronounced than in the lowland areas. The often fertile soils in east African mountains areas also have larger organic carbon stocks than their counterparts in the lowlands [28]. This makes them an important potential source of greenhouse gases due to deforestation and forest degradation [29]. The latter aspect is also important as the Afromontane forest belt covers a relatively large area extending at altitudes above 2000 m from Sierra Leone in the west to Somalia in the east and from the Sudan Republic in the north to the Cape Peninsula in the south [30]. Among them the Ethiopian highlands contribute to more than 50% by area of the tropical Afromontane vegetation [31]. Ethiopia is representative for the ongoing processes of forest degradation as a result of anthropogenic disturbance such as timber extraction, firewood collection, and in particular forest grazing. In contrast to deforestation, where one type of vegetation is replaced by another with easily recognizable effects on carbon stocks and fluxes, the impact of forest degradation on carbon cycling is considerably more subtle. The Afromontane forests in central and southern Ethiopia have been described as Podocarpus mixed forests characterized by a mixture of evergreen and deciduous tree species with dominance of Podocarpus falcatus (Thunb.) Mirb. (Podocarpaceae) and Croton macrostachyus Hochst. ex Del. (Euphorbiaceae) in the upper canopy [32]. These two coexisting tree species represent two different functional types [33]. While gymnosperm coniferous P. falcatus is a late successional shade tolerant tree, the angiosperm C. macrostachyus is an early successional light demanding tree (i.e., gap filler). In Ethiopia as in whole Eastern Africa P. falcatus is among the tree species that are locally most threatened by illegal cutting and encroachment [34]. These activities create gaps that favor the abundance of early successional tree species like C. macrostachyus and cause problems in natural regeneration of P. falcatus if occurring too frequently [35,36]. Along with the drastic decline of P. falcatus, C. macrostachyus appears to become the most abundant indigenous tree species of Ethiopian highlands [37].

To assess the direction and magnitude of the impact of forest degradation on carbon cycling, the physiological traits of trees have to be taken into consideration [38,39]. According to Leuschner et al. [40], photosynthetic capacity and maximum leaf conductance seem to be clearly different between gap fillers and late-successional tree species with minor or no overlap between the two groups, although considerable interspecific variation exists. Typical gap fillers are fast growing angiosperms, such as C. macrostachyus. The usually faster growth of angiosperms as compared to gymnosperms (e.g., podocarps) [41] is the result of the evolutionary gained physiological features, including higher stomata conductance and higher specific leaf area and hence higher photosynthetic capacity, rapid accumulation and cycling of nutrients, and low investment of acquired carbon in wood [42,43,44,45]. In general, gymnosperm podocarps have low photosynthetic rates per unit leaf mass compared to angiosperms [46], and thus low leaf-level nutrient productivity [47]. On the other hand, tropical podocarps have longer lifetimes than tropical angiosperms [48]. A few comparative studies between P. falcatus and C. macrostachyus report higher rates of photosynthesis and transpiration, larger specific leaf area and a higher metabolic activity for the latter [49,50,51].

The degraded Munessa-Shashemene forest in southern Ethiopia is a typical Afromontane forest where anthropogenic disturbance causes higher abundance of the gap filler C. macrostachyus at the cost of the late successional P. falcatus [35,36]. The forest provides an excellent opportunity to study in situ differences in the carbon allocation by these two coexisting tree species. For this purpose we exposed individual trees P. falcatus C. and macrostachyus to a stable carbon isotope tracer in order (i) to evaluate the timing of the recently fixed carbon allocation belowground and its respiratory release as CO\(_2\) by the autotrophic continuum in soil and (ii) to quantify the fraction of newly assimilated carbon lost by soil respiration underneath the tree species. We hypothesize that the velocity of recently assimilated carbon translocation from the tree canopy to soil CO\(_2\) efflux is faster in case of the gap filling angiosperm C. macrostachyus. In addition, we propose that compared with the gymnosperm P. falcatus, more carbon is allocated belowground to fuel the autotrophic continuum and is released into the atmosphere as soil CO\(_2\) efflux.

**Materials and Methods**

**Site Description**

The study was conducted in the Munessa-Shashemene forest, which is located at the eastern escarpment of the southern Main Ethiopian Rift Valley, about 250 km south of Addis Ababa (7°26’ N, and 38°32’ E). The climate is sub-humid with a mean annual temperature of about 15°C; and average annual rainfall amounts to 1150 mm [52]. The rainfall pattern has been referred to as bimodal with a minor rainy season from March to May and a major rainy season from July to September [53]. However, meteorological monitoring during the last 9 years revealed that the short dry season in between the minor and the major rainy seasons actually diminished [52]. Site conditions are homogenous [54], with soils developed from volcanic parent material and are rich in clay minerals and iron oxides [55]. According to the World Reference Base of Soil Resources [55], the soils are classified as Mollic Nitisols. There is a mosaic of rudimentary natural forest and forest plantations, which ranges from about 2.000 to 2.700 m
a.s.l. However, the natural forest is strongly degraded by grazing and illegal logging. These disturbances favour the early successional *C. macrostachyus*, concentrating primarily at canopy gaps, at cost of late successional species such as *P. falcatus* and *Prunus africana* (Hook. F.) Kalkman.

**Experimental Setup**

We established the study plot at an elevation of 2300 m a.s.l in a typical patch of the degraded natural forest. Dominant canopy species are *C. macrostachyus* (143 trees ha$^{-1}$) and *P. falcatus* (73 trees ha$^{-1}$), and tree species with less abundance include *P. africana*, *Syzygium guineense* (Wild.) DC., *Celtis africana* Burm. f. and *Pouteria adolfi-friederici* (Engl.) [56].

In July 2008, one pair (experimental and control) of each of *C. macrostachyus* and *P. falcatus* trees were selected at a distance of c. 100 m. The trees in pairs were similar in height but differed in stem diameter and foliage mass (Table 1). The projected areas of the canopies of experimental and control trees varied between 12.7 m$^2$ and 16.7 m$^2$. We assumed these areas to contain the main part of the canopies of each considered tree [57]. About six months prior starting the experiment, the roots were trenched around the corresponding areas to 50 cm depth. A net was also installed underneath the canopies to prevent the potential input of labeled leaves onto the soil.

Five permanent PVC collars (20 cm diameter) were randomly installed on the forest floor under the canopy of each candidate tree for subsequent measurements of soil CO$_2$ efflux and gas sampling. Within the collars, the ground vegetation was gently removed to avoid its contribution to soil CO$_2$ efflux.

**Pulse Labeling**

Short-term $^{13}$CO$_2$ pulse labeling of *C. macrostachyus* and *P. falcatus* trees was carried out on two consecutive cloudless days (November, 12 and November, 14, 2008) on the offset of the main rainy season. For the whole tree $^{13}$CO$_2$ labeling in the remote field experiments. During the labeling period the air temperature inside the chambers increased to a maximum of 29.4°C and 27.3°C, respectively, being 5°C higher than the ambient temperature. Relative air humidity increased from 50% to 79% within the *C. macrostachyus* chamber and from 56% to 82% within the *P. falcatus* chamber, when the ambient air humidity ranged from 38 to 67% and 36 to 69% during the labeling period of the two consecutive days. Photosynthetically active radiation (PAR) within the chambers averaged 1070 ± 317 μmol m$^{-2}$ s$^{-1}$ for *C. macrostachyus* and *P. falcatus* during labeling, respectively.

**Sample Collection**

Leaves, phloem sap, soil CO$_2$ efflux, and the 0–10 cm soil depth increment were sampled over a one year period at 1, 2, 3, 4, 8, 16, 32, 64, 120, and 365 days after the labeling. In addition, within the first 24 hours after labeling, leaves and phloem sap were sampled in 4 hour intervals, while soil CO$_2$ efflux was collected 12 hours after the labeling.

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**Table 1. Main characteristics of the experimental trees.**

| Characteristic       | Croton macrostachyus | Podocarpus falcatus |
|----------------------|----------------------|---------------------|
| Functional group     | Gymnosperm           | Angiosperm          |
| Life strategy        | Early successional   | Late successional   |
| Shade tolerance      | Light demanding      | Shade tolerant      |
| Leaf habit           | Facultative shedding, deciduous | Evergreen, coniferous |
| Leaf life span [51]  | Often <1 year, depending on weather conditions | >2 years |
| Leaf flush [51]      | During moist periods | Throughout the whole year with maxima during moist periods |
| Mycorrhiza type [79] | Arbuscular mycorrhiza | Arbuscular mycorrhiza |
| Height, m            | 5.1', 5.6'          | 6.2', 5.6'         |
| DBH, cm              | 4.5', 4.1'          | 11.5', 9.4'        |
| Foliage mass, g$_{ow}$ | 780*                | 4910*              |

* labeled tree; ¹ control tree.

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Leaves were randomly sampled from 4 opposite directions from the top, middle and bottom part of the trees crown, both for labeled and control trees. *Cotoneaster macrostachys* lost part of its leaves at the end of a dry period in February 2009. In the following moist period, new leaves have been produced and thereafter leaf samples were represented by a mixture of mature and young leaves. All foliage samples were dried for 48 h at 65 °C and ground by a ball mill. Phloem soluble sugars were extracted according to the phloem exudation method described by Gessler et al. [59]. Briefly, pieces of phloem tissues (about 1 × 1 cm) were removed with a scalpel from the stems at 1.3 and 0.5 m height and were immediately transferred to glass vials, containing 2 ml of 15 mM polyphosphate buffer (Sigma, München, Germany). After 5 hours of extraction the supernatant was decanted and kept frozen.

Soil cores were taken from 0–10 cm depth. Within four hours after the soil samples were taken, they were sieved <2 mm, and the ‘adhering soil method’ [60] was employed to separate the rhizosphere soil from the bulk soil. Immediately thereafter the soil samples were frozen until analysis of the neutral lipid fatty acid (NLFA) 16:1ω5, a marker for arbuscular mycorrhiza (AM) [61].

Soil CO2 efflux was measured at each of the PVC collars placed underneath the canopy of the study trees. A 8100–103 Survey Chamber (LI-COR Inc., Lincoln, NE) was tightly fitted on the collars, and soil CO2 efflux was recorded by a LI-8100 infrared gas analyzer [54]. The LI-8100 system was also used to collect gas samples for analyzing the 13C signature of the CO2 emitted from the survey chamber to establish Keeling plots [62].

### Stable Carbon Isotope Analysis

The stable carbon isotope composition was analyzed for leaves, water soluble phloem sugars, NLFA 16:1ω5 extracted from adhering and bulk soil, and soil-derived CO2.

To determine the stable carbon isotope ratio in leaves, c. 1 mg of finely ground material was analyzed on a Thermo Finnigan MAT DELTAplus Advantage isotope ratio mass spectrometer (Thermo Electron Corporation, Waltham, USA) coupled to an Euro EA 1110 C/N analyzer (Elementar Analyser System GmbH, Hanau, Germany).

For compound-specific analysis of the phloem sugars, an HPLC system ( Dionex Corporation, Sunnyvale, CA, USA) was coupled to a Finnigan Delta V Advantage Mass Spectrometer by a Finnigan LC IsoLink Interface. Briefly, sample compounds were first separated by the HPLC system. Then the individual compounds were oxidized to CO2 in the Finnigan LC IsoLink Interface, excess O2 was removed oxidizing elemental copper, and the O2-free gas stream was transferred to the mass spectrometer for stable isotopic analysis. Standards were referenced with EA/IRMS (Euro EA 1110 CN analyzer coupled to a Finnigan MAT DELTAplus IRMS) as pure chemicals before preparing the solutions. Details of the analytical procedure including the correction of the HPLC/IRMS data can be obtained in [63]. Based on the individual concentrations and isotope composition of the sugars glucose, fructose and sucrose a weighted average of the isotope composition of the mono- and disaccharides in the phloem sap was calculated.

### Calculations

Isotope values are expressed in δ notation (‰), relative to the Vienna Pee Dee Belemnite (VPDB) standard. All δ13C values (‰) were converted to the absolute isotope ratio (13C/12C) of the sample (Rsample):

\[
R_{\text{sample}} = \left(\frac{1}{R_{\text{standard}}} + 1\right) \times R_{\text{standard}}
\]

where \( R_{\text{standard}} \) is the standard value for isotope ratio of VPDB. The fractional abundance (A) of 13C relative to 12C+13C was then related to R by equation:

\[
A = R_{\text{sample}} / (R_{\text{sample}} + 1)
\]

The excess 13C in the foliage of the labeled trees (13CFL, g) was calculated by multiplication of the difference in the fractional abundances in leaves of the labeled trees and the control trees with the leaf biomass carbon of the labeled trees as following:

\[
13C_{FL} = (A_{FL} - A_{FC}) \times DW_{F} \times C_{F}
\]

where \( A_{FL} \) and \( A_{FC} \) is 13C fractional abundance of leaf samples from labeled and control trees respectively, \( DW_{F} \) is total dry foliage biomass of labeled trees in g and \( C_{F} \) is foliage carbon concentration of the labeled trees (437 mg g\(^{-1}\) for *C. macrostachyus* and 462 mg g\(^{-1}\) for *P. falcatus*).

The isotopic signature of the soil CO2 efflux collected with the closed chamber was estimated by calculation of the intercept a of the Keeling plot relationship.

\[
\delta^{13}C_{\text{soil CO2 efflux}} = \delta^{13}C_{\text{in CO2 sample}} + a \times (1/[CO_2\text{sample}])
\]

at each sampling time [62]. Keeling plots with R² < 0.9 were discarded. No correction was carried out for physical isotopic fractionation and mixing processes of δ13C in soil-respired CO2.
However, the consequences of these non unambiguous data should be negligible as the label effect on soil respiration is nearly two orders of magnitude larger than the isotopic effect of diffusion. To calculate the excess $^{13}$C in soil CO$_2$ efflux under the labeled trees as compared to that under the control trees at each sampling point, the fractional abundance $A$ (equation 2) and the measured soil CO$_2$ efflux rate were used to calculate the amount of $^{13}$C evolved from soil ($^{13}$C$_{SRL}$, g $^{-1}$ C m$^{-2}$ h$^{-1}$) under the labeled and control trees:

$$^{13}C_{SRL} = (A_{SRL} - A_{SRC}) \times E_{SRL}$$  (5)

where $A_{SRL}$ and $A_{SRC}$ is $^{13}$C fractional abundance of soil CO$_2$ efflux under labeled and control trees, respectively; and $E_{SRL}$ is the soil CO$_2$ efflux rate under labeled trees (mg m$^{-2}$ h$^{-1}$) recorded during the correspondent sampling time. To minimize the temporal variability in isotopic ratio values due to changes in environmental parameters we used the difference between the values of the individual collars from labeled and control trees.

The cumulative excess of $^{13}$C in soil CO$_2$ efflux between two points in time ($\sum_{t}^{t+1} \frac{^{13}C_{SRL}}{}$ g $^{-1}$ C m$^{-2}$ time period$^{-1}$) was calculated according to:

$$\sum_{t}^{t+1} \frac{^{13}C_{SRL}}{} = \left(\frac{\left(\frac{^{13}C_{SRL}}{} + \frac{^{13}C_{SRL}+1}{2}\right)}{2} \times \Delta t \right)$$  (6)

where $^{13}C_{SRL}$ and $^{13}C_{SRL+1}$ is the excess of $^{13}$C in soil CO$_2$ efflux (g $^{-1}$ C m$^{-2}$ h$^{-1}$) at two consecutive points in time, and $\Delta t$ defines the time interval (h) between the two points in time. The cumulative excess $^{13}$C in soil CO$_2$ efflux during the 365 days chasing period ($\sum_{t}^{t+1} \frac{^{13}C_{SRL}}{}$) was obtained by summarizing the cumulative excess of $^{13}$C for the different time intervals.

The mean residence time (MRT) and half-life of the label in foliage and the soil $^{13}$CO$_2$ efflux were calculated by fitting exponential functions to the cumulative excess of $^{13}$C in the foliage and of $^{13}$C in soil CO$_2$ efflux in the one-year time course. Mean residence times ($1/K$) and half-lives ($ln(2)/K$) are expressed in h.

The rate of $^{13}$C assimilation by the trees could not be analyzed directly, because non-dispersive infrared gas analyzer underestimates the true values of CO$_2$ concentration in $^{13}$CO$_2$ enriched atmosphere due to the shift in the absorption spectrum of $^{13}$CO$_2$ relative to that of $^{12}$CO$_2$ [65,66]. Therefore, the $^{13}$C assimilation was estimated by two independent indirect ways. Approach 1 was based on the known atom percent of $^{12}$CO$_2$ and $^{13}$CO$_2$ in the chamber directly after adding the defined amount of label [67]. Thus the measured decline of CO$_2$ inside the chamber following the labeling was the sum of the real $^{12}$CO$_2$ uptake and the apparent $^{13}$CO$_2$ uptake. Considering an isotopic discrimination of $^{13}$C against $^{12}$C during assimilation of 0.973 [68], the real $^{12}$CO$_2$ uptake and the apparent $^{13}$CO$_2$ uptake during the labeling period could be distinguished. The real $^{13}$C concentration was then estimated by multiplying the apparent $^{13}$CO$_2$ uptake by a factor of 4.62, which was obtained by comparing the measured increase in the CO$_2$ concentration in the chamber and the expected increase based on the amount of $^{13}$CO$_2$ added.

Approach 2 was based on the measured $^{13}$C enrichment in leaves immediately after the labeling (time 0) related to the foliage biomass of the labeled trees, which was estimated by an allometric approach [57,69]. Fifteen $C. macrostachyus$ and 15 $P. falcatus$ trees of comparable tree height and diameter at breast height as the study trees were selected. From each tree, 15 randomly selected branches were cut and the basal branch diameter of each branch was measured. The leaves from each branch were harvested and dried (65°C for 48 h). The dry weight of the foliage was related to the basal branch diameter of the corresponding branch by using a double exponential decay function (Table 2), suggesting two pools with different decay rates.

### Results

#### $^{13}$C Assimilation and Recovery in Foliage

Carbon dioxide concentration declined inside the chambers during the labeling, indicating a photosynthetic uptake by the trees (Fig. 1). The calculated rate of carbon uptake of $C. macrostachyus$ was higher (12.7±2.2 μmol m$^{-2}$ chamber basal area s$^{-1}$) in comparison to that of $P. falcatus$ (5.5±3.1 μmol m$^{-2}$ chamber basal area s$^{-1}$). Overall, $C. macrostachyus$ and $P. falcatus$ assimilated $0.4$ and $0.5$ mol, or 5.2 and 6.5 g $^{13}$C, respectively.

Before labeling, the experimental and control trees did not differ in the $^{13}$C natural abundance in the foliage (Fig. 2a, b). Immediately after the chambers were opened (sampling time 0), the $\delta^{13}$C of the foliage of both labeled trees was strongly elevated as compared to the control trees, with a $\delta^{13}$C value of 1557±871‰ for $C. macrostachyus$ and of 248±50.5‰ for $P. falcatus$.

The greater net assimilation rate of $C. macrostachyus$ was offset by the larger foliar biomass of the $P. falcatus$, thus resulting in only minor differences in the total amount of $^{13}$C in the foliage of the two species. Excess $^{13}$C in the foliage, i.e. the $^{13}$C assimilation calculated with approach 2, was 5.9±0.3 g for $C. macrostachyus$ and 6.9±1.3 g for $P. falcatus$ (Fig. 3). This result fits well to the $^{13}$C assimilation as estimated by the decline in the CO$_2$ concentration within the chamber, and indicates a pulse labeling efficiency of 61% for $C. macrostachyus$ and 59% for $P. falcatus$.

With time, labeled leaves of both species became $^{13}$C-depleted, and approached the isotopic signature of the leaves in the reference trees within one year (Fig. 2a, b). The tree species differed in $^{13}$C recovery kinetics (Fig. 3). In case of $C. macrostachyus$ already after 24 hours only about 50% of the total assimilated $^{13}$C were found in the leaves, whereas for $P. falcatus$ foliage a 50% decrease took more than 2 days. The time course of the $^{13}$C recovery in leaves was best fitted with a double exponential decay function (Table 2), suggesting two pools with different decay rates.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Change in the apparent amount of CO$_2$ (mol) in chambers of $C. macrostachyus$ and $P. falcatus$ during the $^{13}$CO$_2$-labeling period. The decline in the amount of CO$_2$ reflects the predominance of photosynthesis over leaf and stem respiration. The arrow shows the release of 12.3 mmol $^{13}$CO$_2$ m$^{-3}$ chamber volume.

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Figure 2. Time course of the $^{13}$C label in plant-soil compartments of *Croton macrostachyus* and *Podocarpus falcatus* during the one year chasing period. Shown are (a) leaves of *C. macrostachyus*, (b) leaves of *P. falcatus*, (c) weighed sum of soluble mono and disaccharides in tree phloem sap at 1.3 m and 0.5 m above ground of *C. macrostachyus*, (d) weighed sum of soluble mono and disaccharides in tree phloem sap at 1.3 m.
and 0.5 m above ground of *P. falcatus*, (e) 16:1o5 NLFA in adhering and bulk soil under *C. macrostachyus*, (f) 16:1o5 NLFA in adhering and bulk soil under *P. falcatus*, (g) soil CO2 efflux under *C. macrostachyus*, (h) soil CO2 efflux under *P. falcatus*. For the sake of clarity of the figure, we omitted to show the δ13C values for the control of soluble sugars in tree phloem sap and the 16:1o5 NLFA in adhering and bulk soil. The former was −25.53±0.85% for *C. macrostachyus* and −25.36±0.33% for *P. falcatus*, and the latter was −27.7±1.4% for *C. macrostachyus* and −29.3±1.1% for *P. falcatus*. For leaves and soil CO2 efflux data are means ± standard deviation (n=5). No replicates were taken for phloem sap extraction and soil cores for analysis of 16:1o5 NLFA in adhering and bulk soil to impact of destructive sampling to the plant-soil system to a minimum. doi:10.1371/journal.pone.0045540.g002

constants. The size of the fast pool was larger for *C. macrostachyus* as compared to *P. falcatus*, and the MRT of the label within this pool was almost 4 times shorter.

### 13C Translocation Belowground

The soluble phloem sugars of *C. macrostachyus* were mostly mono- and disaccharides with predominance of sucrose (up to 70%). The phloem sap of *P. falcatus* contained in addition the indirect product of photosynthesis 1-O-methyl-muco-insitol (OMMI), with a proportion of about 42% of the transported sugars and polysols. The velocity of translocation of recent photoassimilates to the root system via phloem transport differed between the tree species. Soluble sugars in phloem sap of *C. macrostachyus* at 1.3 m stem height were already enriched in 13C 4 to 8 hours after labeling, and the tracer peaked in the following 40 hours at 1.3 and 0.5 m height (Fig. 2c). For *P. falcatus* the first evidence of the 13C label in phloem soluble sugars appeared 4 hours later, and δ13C reached a peak at 1.3 m and 0.5 m height between 72 and 96 hours after the labeling event (Fig. 2d). During the chasing period in both trees the mono- and disaccharides in the phloem sap exponentially became 13C depleted (R2=0.93, P<0.0001). In contrast, OMMI in the *P. falcatus* phloem sap showed a relatively constant enrichment of about 2–10% above the control throughout the period of sampling (data not shown).

The adhering soil (0–10 cm depth) underneath *C. macrostachyus* had a larger concentration of NLFA 16:1o5 than that under *P. falcatus* (18.1±4.6 versus 10.4±4.8 mmol g of dry soil−1). The same was true for the bulk soils (11.1±1.9 vs 6.1±2.7 mmol g of dry soil−1). The enrichment of the AM fungal biomarker with 13C appeared synchronized with the transport of the label within the phloem sap (Fig. 2e, f). Under *C. macrostachyus* the 13C in NLFA 16:1o5 was elevated already within 40 hours and peaked 4 to 8 days after the labeling. For *P. falcatus* highest 13C enrichment of the AM fungal biomarker in adhering soil occurred later (8 to 16 days after labeling) and was less pronounced. The temporal pattern of the tracer in the AM NLFA in bulk soils was similar in shape but the biomarker was less enriched in 13C than that in the adhering soils.

### 13C Recovery in Soil CO2 Efflux

At the beginning of the chasing period the soil CO2 efflux rate was 4.8±0.3 μmol m−2 s−1 under *C. macrostachyus* and 4.6±0.5 μmol m−2 s−1 under *P. falcatus* (n=5). With decreasing soil moisture during the first four months after labeling, the soil CO2 efflux rates decreased concurrently to 3.1±0.3 μmol m−2 s−1 under the deciduous tree and to 2.8±0.6 μmol m−2 s−1 under the conifer.

The temporal course of the tracer’s occurrence in CO2 soil efflux mirrored that in the phloem sap and the NLFA 16:1o5. Beneath *C. macrostachyus* soil CO2 efflux from all 5 collars coincidently showed a remarkable increase in δ13C (99.3±4.6%) already 48 hours after the labeling, reaching a maximum within the next 24 hours (Fig. 2g). Under *P. falcatus* the first evidence of the label in soil CO2 efflux occurred around 72 hours after the labeling, and the maximum enrichment (δ13C of 3.9±1.4%) was recorded at day 8 of the chasing period (Fig. 2 h). The time lag between 13C photosynthetic uptake and release in soil CO2 efflux, as calculated by fitting a quadratic function to the relationships between δ13C and the time after labeling [21], were 29 and 51 hours for *C. macrostachyus* and *P. falcatus*, respectively. For both trees, the δ13C values of soil CO2 efflux decreased exponentially with time (R2=0.90 for *C. macrostachyus* and 0.86 for *P. falcatus*), being faster for *C. macrostachyus*. At the end of the chasing period the δ13C values approached natural abundance under both tree species (Fig. 2g, h).

Despite the comparable values of total 13C assimilated by the trees during labeling, Fig. 4 shows that the label recovered in the cumulative soil CO2 efflux within one year for *P. falcatus* was (14.9±2.4%), which was half that of *C. macrostachyus* (32.2±3.3%). In the case of *C. macrostachyus* the label recovery in soil CO2 efflux followed a double-exponential function, indicating that 17% of the overall assimilated carbon was released as fast pool within 17 days (Table 2). In contrast, the kinetics of the label recovery in cumulative soil CO2 efflux under *P. falcatus* did not allow one to distinguish between the two sources of soil respiration.

### Discussion

**Labeling**

We used two independent approaches to calculate the total amount of assimilated 13C by the trees. The first approach was based on the average rate of 13CO2 uptake as a function of the concentration over the labeling time, and the second estimated the 13C excess in leaves immediately after the labeling. In the first approach, uncertainties arose from assumptions in the pressure application for the Ideal Gas Law and the correction of the absorption spectra of the infrared gas analyser, while in the second approach the foliage biomass estimation was quite rough. However, the two independent methods resulted in comparable 13CO2 uptake by the trees and gave reasonable estimates of the labeling efficiency [70].
Accordingly, \( \text{Lu} \text{t} \text{t} \text{g} \text{e} \) falcatus. macrostachyus

The rate of photoassimilation of the early successional C. macrostachyus was higher in comparison to the late successional P. falcatus. Accordingly, Litte \( \text{e} \) et al [49], Fetene and Beck [50], and Scyun et al. [51] showed that C. macrostachyus had much higher photosynthesis and transpiration rates than P. falcatus. This fits well to the general differences in the functional traits of early and late successional, or angio- and gymnosperm tree species [71,72]. Further, it is widely accepted that species with a longer leaf lifespan (i.e., gymnosperms, like P. falcatus) have a lower photosynthetic capacity per mass unit as well as per surface unit [73]. In the following section, we will address how this relates to the carbon allocation belowground.

Kinetics of Label Recovery and Carbon Allocation

\( \text{C} \text{o} \text{r} \text{t} \text{o} \text{n} \text{ } \text{m} \text{a} \text{c} \text{r} \text{o} \text{s} \text{t} \text{a} \text{c} \text{h} \text{y} \) lost part of its leaves at the end of the dry season and a flush of new leaves occurred thereafter. However, since most of the label was translocated before the partially shedding of the leaves in February 2009, the effect of this bias on excess of \( ^{13} \text{C} \) in leaves can be considered small. The pattern of the recently fixed \( ^{13} \text{C} \) allocation belowground differed between C. macrostachyus and P. falcatus in time and magnitude. The gap filling angiosperm had a larger fast pool of the label in foliage which was also having a shorter MRT than the one of the late successional gymnosperm. The MRT of the fast pool of P. falcatus was similar as the MRT of 32 hours for the recently fixed carbon in the fast pool of needles of young Scots pine trees, as reported by Hogberg et al. [10]. A part of the assimilated carbon remained in leaves for longer time, most likely as reserve compounds, such as starch. Generally, leaves with a longer lifespan as those of P. falcatus tend to accumulate secondary compounds (i.e., cellulose and phenolics), while those with a shorter lifespan contain larger amounts of proteins [74].

The time lag between the \( ^{13} \text{C} \) uptake and the appearance of \( ^{13} \text{C} \) enriched sugars in phloem sap was about twice as long for P. falcatus as for C. macrostachyus. The velocity of the phloem transport in the two study trees was approximately 0.4 m h\(^{-1}\) (C. macrostachyus) and 0.1 m h\(^{-1}\) (P. falcatus). Due to coarse time resolution of phloem sap sampling (4 hours interval during the first day and then daily for the next 4 days), these values might have some uncertainties, but they are in accordance with other reports of most recently assimilated carbon reaching the phloem within hours to days [10,13,75,76]. The measured carbon phloem transport velocities also agree well with the known differences between deciduous and evergreen species [11,70,76]. The different time resolution of belowground transport between gymnosperm P. falcatus and angiosperm C. macrostachyus could be attributed to the general advances in leaf vein branching, transport of the newly fixed carbon from the sites of CO\(_2\) fixation to the sieve elements and phloem loading mechanism, developed during the evolution from gymnosperms to angiosperms [42,43,70]. The differences in the non-structural carbohydrate composition between the tree species also reflect the evolutionary traits of the phloem with its uniformity in assimilatory metabolites, which are mainly represented by sucrose in angiosperms [42]. In contrast, with OMNI being present in the phloem sap of P. falcatus, also other cyclic polyols are ubiquitous in gymnosperms. As they are no direct products of the primary metabolism, they behave more conservatively. Hence, OMNI became less enriched than mono- and disaccharides but contained the label over the whole period of observation.

In line with the different velocities of the \( ^{13} \text{C} \) flux in the phloem sap, the time lag between the \( ^{13} \text{C} \) assimilation by the foliage and the release

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**Table 2.** Results of the fit of exponential functions on the excess of \( ^{13} \text{C} \) in leaves and the cumulative excess of \( ^{13} \text{C} \) in soil CO\(_2\) efflux related to time after labeling.

| Carbon pool               | \( ^{13} \text{C} \) in leaves | \( ^{13} \text{C} \) in soil CO\(_2\) efflux |
|---------------------------|---------------------------------|------------------------------------------|
|                           | Size \% | MRT h       | Half life h   | \( R^2 \) | Size \% | MRT h       | Half life h   | \( R^2 \) |
| Excess of \( ^{13} \text{C} \) in leaves |                   |                |            |          |                   |                |            |          |
| Fast pool                 | 65      | 14          | 10           | 0.97      | 45      | 55          | 38           | 0.94      |
| Slow pool                 | 30      | 2000        | 1386         | 0.97      | 38      | 1111        | 770          | 0.97      |
| Cumulative excess of \( ^{13} \text{C} \) in soil CO\(_2\) efflux |                   |                |            |          |                   |                |            |          |
| Fast pool                 | 17      | 417         | 289          | 0.99      | 15      | 2000        | 1386         | 0.97      |
| Slow pool                 | 23      | 10000       | 6930         | 0.99      |         |             |              |           |

Shown are the amount of labeled carbon that was recovered in a given compartment as parameters \( a \) and \( c \), being expressed as the relative size of a fast and a slow pool, the mean residence time (MRT) and the half life of both pools, and the coefficient of determination \( (R^2) \). Please note that the size of the pools refer to the percentage of the overall assimilated \( ^{13} \text{C} \).

1No separation between fast and slow pool could be made.

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**Figure 4.** Time course of cumulative excess of \( ^{13} \text{C} \) in soil CO\(_2\) efflux under Crotton macrostachyus and Podocarpus falcatus during the one year chasing period. Data are means ± standard deviation (n=5). The curves are fitted with a double exponential function for C. macrostachyus and a single exponential function for P. falcatus. Parameters are shown in Table 2. doi:10.1371/journal.pone.0045540.g004
of the tracer by soil CO$_2$ efflux was almost 2 times longer for gymnosperm *P. falcatus* than for angiosperm *C. macrostachyus*. For the angiosperm this result is in accordance with 8-m tall European beech trees, for which a time lag of 2 to 3 days was reported [13]. The gymnosperm values also compare to those of other reports, e.g., Andrews et al. [77] identified a time lag of 7 days for a *Pinus taeda* forest labeled in a FACE experiment. Although the total soil CO$_2$ efflux rate was comparable throughout the chasing period (see also [54]), the cumulative amount of the label recovered in soil CO$_2$ efflux was twice as large for the early successional angiosperm as for the late successional gymnosperm.

In the case of *C. macrostachyus* the temporal resolution of the cumulative label recovered in soil CO$_2$ efflux suggests that two different carbon pools with different MRT contributed to soil respiration. A first pool with a MRT of 17 days is closely connected to photosynthesis and reflects the direct use of recent assimilates by the autotrophic continuum. This includes root respiration, transfer to fungal symbionts with subsequent mycorrhizal respiration, and exudation into the rhizosphere, again with subsequent respiration by rhizosphere bacteria [3,78]. With 17% of the overall assimilated $^{13}$C, this first pool represented an equal share of the label recovery in the soil CO$_2$ efflux as under *P. falcatus* during the whole year of observation. For *P. falcatus* no differentiation of a fast and a slow pool could be calculated. This might be due to the longer storage of assimilates in leaves or metabolites in phloem and their allocation to roots during the whole chasing period. This more conservative flow of photoassimilates to the autotrophic continuum in soil might have contributed to the lack of clear distinction of different pools in soil CO$_2$ efflux. Nevertheless it can be assumed also for *P. falcatus* that soil CO$_2$ efflux is primarily driven by autotrophic respiration during the first few days [10].

One member of the autotrophic continuum is mycorrhizal fungi. Both tree species are associated with AM, with *C. macrostachyus* being more intensively colonized [79]. This is also shown by larger NLFA 16:1$\omega_5$ concentrations in soil under *C. macrostachyus*. Such larger mycorrhizal colonization has been reported to increase the proportion of plant carbon allocated below ground [90], and to be associated with higher rates of root respiration [81] and soil CO$_2$ release [92]. In fact, there was a close temporal coupling between the label peaks of phloem sap and 16:1$\omega_5$ NLFA. This confirms the suggestion of Hogberg et al. [10] of a very pronounced carbon transfer from the plant to the fungal symbiont (though AM in the present case). Hence, a considerable part of the carbon flux in soil occurred through mycorrhiza, which is thought to account for up to one quarter of the carbon assimilated by the tree [43]. Higher rates of respiration by the autotrophic community in AM-colonized plants can be related to increased nutrient uptake. An increased demand for respiratory products (i.e. ATP, NADH) is necessary at each of the four stages of nutrient uptake by an AM-colonized plant, from ion uptake by the external fungal hyphae via ion transport within the fungus, ion export by the internal hyphae to ion uptake by plant root cells [83]. Thus, higher nutrient demand should result in higher carbon investments into the mycelia net, fostering nutrient acquisition. In fact, early successional angiosperms are characterized by much larger nutrient concentrations and turnover rates as late successional gymnosperms [84]. This also corresponds to the higher demand for photoassimilates in case of the former. Accordingly, the earlier and much more pronounced $^{13}$C enrichment observed by a steeper decline under *C. macrostachyus* than under *P. falcatus* implies the more direct supply of carbon to the fungal symbiont by the early successional tree.

The second pool describes the soil $^{13}$CO$_2$ efflux released by heterotrophic decomposition of structural organic matter [85]. Since leaf litter was collected over the chasing period and did not contribute to the pool of the labeled structural compounds, the heterotrophic efflux must be primarily driven by decomposition of root litter, mycorrhizal hyphae and other rhizobiota. No data are available about the root turnover of the trees under investigation. But assuming that root lifetimes in broadleaf tropical forests (annual precipitation >1000 mm) range from 0.4 to 3.2 years [86], and that the root life span is longer for slow growing than for fast growing tree species [57], we suggest a higher longevity of roots of slow growing *P. falcatus* as compared with the fast growing *C. macrostachyus*. Hence, probably a smaller share of the $^{13}$C label incorporated into structural components of the root-mycorrhiza system has been mineralized to CO$_2$ in case of *P. falcatus*. In total it appears that in case of the late successional *P. falcatus* more of the recently assimilated carbon stays in the plant-soil system, presumably as structural carbon components in above and belowground biomass [Krepkowski, unpublished] and by producing litter with slow decomposition rates [46].

**Conclusions**

Our study shows a close temporal coupling with a time lag of one to a few days between assimilation of carbon by the tree canopy and the respiratory activity of the autotrophic continuum in a tropical montane forest, although we caution that the experiment was unreplicated because of expense of whole-tree labeling. Nevertheless, this agrees with recent findings with temperate and boreal trees, suggesting that general mechanisms apply across the biomes.

The rate of carbon allocation into soil is determined by the successional habit of the trees. Corresponding to the functional traits, the early successional angiosperm *C. macrostachyus* pumps carbon much faster and at higher quantities belowground than the late successional gymnosperm *P. falcatus*. The more direct and larger carbon translocation belowground probably reflects the larger energy demand by AM-colonized roots in the belowground autotrophic continuum for an enhanced water and nutrient uptake.

As the continuing degradation of the Afromontane forest leads to the formation of more gaps, the shift in abundance towards gap filling tree species is proceeding. In the long run, the shift in carbon allocation pattern driven by forest degradation may affect the forest carbon balance. Larger carbon allocation of gap filling trees into fast cycling belowground pools likely leads to a decrease of tree biomass carbon accumulation and to declining soil organic carbon contents. We propose this negative effect of early successional angiosperms on ecosystem carbon balance as a worldwide phenomenon which merits further investigation.

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**Author Contributions**

Conceived and designed the experiments: OS GG. Performed the experiments: OS YY BW MW GG. Analyzed the data: OS YY JB GG.
Contributed reagents/materials/analysis tools: GG AR. Wrote the paper: OS YJ AR GG.

References

1. Trumbore S (2006) Carbon respired by terrestrial ecosystems - recent progress and challenges. Global Change Biol 12: 141–153.
2. Chambon JG, Hip Tic, NH, Tribouxy ES, Trumbore SE (2001) Carbon sink for a century. Nature 410: 429.
3. Högberg P, Read D (2006) Towards a more plant physiological perspective on carbon allocation. Funct Ecol 20: 736–740.
4. Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogeochim 48: 115–146.
5. Gilford RM (2003) Plant respiration in productivity models: conceptualisation, representation and issues for global terrestrial carbon-cycle research. Funct Plant Biol 30: 171–186.
6. Schulze ED (2006) Biological control of the terrestrial carbon sink. Biogeosci 3: 147–166.
7. Brueggemann N, Goessler A, Kayzer Y, Keel SG, Baudeck F, et al. (2011) Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review. Biogeosci 8: 3457–3489.
8. Epron D, Bahn M, Derrien D, Lattanzia F, Pumpenan J, et al. (2012) Pulse-labeling trees to study carbon allocation dynamics: a review of methods, current knowledge and future prospects. Tree Physiol 32: 776–783.
9. Carbone MS, Czimczik CI, DuMaffe KE, Trumbore SE (2007) Allocation and residence time of photosynthetic products in a boreal forest using a low-level 14C pulse-chase labeling technique. Global Change Biol 13: 466–477.
10. Högberg P, Högberg MN, Gottschalch SG, Betson NR, Keel SG (2006) High temporal resolution tracing of photosynthetic carbon from the tree canopy to forest soil microorganisms. New Phytol 173: 220–228.
11. Pumpenan JS, Heinonalo J, Rasilo T, Hurme K-J, Ilvesniemi H (2009) Carbon balance and allocation of assimilated CO2 in Scots pine, Norway spruce, and Silver birch seedlings determined with gas exchange measurements and 13C pulse labeling. Trees 23: 611–621.
12. Horwarth WA, Pregitzer KS, Paul EA (1994) 14C allocation in tree-soil systems. Tree Physiol 14: 1163–1176.
13. Plain C, Gerant D, Maillard P, Dannoura M, Berveiller D, et al. (2011) Isotope composition on a diurnal timescale? New Phytol 182: 451–460.
14. Liutkev H, Wright J, Reich PB (2003) Photosynthetic differences contribute to competitive advantage of evergreen angiosperm trees over evergreen conifers in productive habitats. New Phytol 159: 329–336.
15. Lusk CH (2011) Conifer-angiosperm interactions: Physiological ecology and life history. Smith Rev 58: 107–222.
16. Canadell JG, Raupach MR, Houghton RA (2009) Anthropogenic CO2 emissions in Africa. Biogeosci 6: 463–468.
17. Tans PP, Hooker BC, Rayner P, Keeling CD, Masarie K (1997) Improved algorithms and data weighting scheme for global atmospheric carbon dioxide measurements from the NOAA/ESRL MD7 gas samples and analyses. J Geophys Res 99: 21117–21132.
18. Canadell JG, Raupach MR, Houghton RA (2009) Anthropogenic CO2 emissions in Africa. Biogeosci 6: 463–468.
19. Tans PP, Hooker BC, Rayner P, Keeling CD, Masarie K (1997) Improved algorithms and data weighting scheme for global atmospheric carbon dioxide measurements from the NOAA/ESRL MD7 gas samples and analyses. J Geophys Res 99: 21117–21132.
20. Barthel M, Hammerle A, Sturm P, Baur T, Gentsch L, et al. (2011) The diel carbon balance of African savannah grassland as driven by seasonality and rainfall events. Global Change Biol 17: 171–186.
21. Dannoura M, Maillard P, Fresneau C, Plain C, Berveiller D, et al. (2011) Tracing of photosynthetic products in a boreal forest using a low-level 14C pulse-chase labeling technique. Global Change Biol 13: 466–477.
63. Wild B, Wanek W, Postl W, Richter A (2010) Contribution of carbon fixed by
62. Keeling CD (1958) The concentration and isotopic abundances of atmospheric
61. Olsson PA (1999) Signature fatty acids provide tools for determination of the
59. Gessler A, Rennenberg H, Keitel C (2004) Stable isotope composition of organic
58. Simard SW, Dorall DM, Jones MD (1997) Carbon allocation and carbon
57. Gessler A, Schulte-Herbrich T, Zech W, Rennenberg H (2000) Carbon
56. Frostegård A, Bååth E, Tunlid A (1993) Shifts in the structure of soil microbial
55. Mordacq L, Mousseau M, Deleens EA (1986) 13C method of estimation of
54. Ehleringer JR (1991) 13C/12C fractionation and its utility in terrestrial plant
53. Svejcar TJ, Boutton TW, Trent JD (1990) Assessment of carbon allocation with
52. Bazzaz FA (1979) Physiological ecology of plant succession. Ann Rev Ecol Syst
51. Larcher W (1969) Effect of environmental and physiological variables on carbon
50. Brodribb TJ, Feld TS, Gregory JJ (2007) Leaf maximum photosynthetic rate and
49. Valiente AJ, Kleinert A (2007) Respiratory responses of arbuscular mycorrhizal
48. Hughes JK, Hodge A, Fitter AH, Atkin OK (2008) Mycorrhizal respiration:
47. Ryan MG, Law BE (2005) Interpreting, measuring and modeling soil
46. Gill RA, Jackson RR (2000) Global patterns of root turnover for terrestrial
45. Eissensat DM, Yanai RD (2002) Root life span, efficiency and turnover. In: 
44. Langley JA, Johnson NC, Koch GW (2005) Mycorrhizal status influences the
43. Andersson JA, Harrison KG, Matamala R, Schlesinger WH (1999) Separation of
42. Valentine AJ, Kleinert A (2007) Respiratory responses of arbuscular mycorrhizal
41. Langley JA, Johnson NC, Koch GW (2005) Mycorrhizal status influences the
40. Bonham CD (1989) Measurements for terrestrial vegetation. New York: John
39. Gessler A, Schulte-Herbrich T, Zech W, Rennenberg H (2000) Carbon
38. Phillips RP, Fahey TJ (2006) Tree species and mycorrhizal associations influence
37. Gindaba J, Olsson M, Itanna F (2004) Nutrient composition and short-term
36. Wubet T, Kottke I, Teketay D, Oberwinkler F (2009) Arbuscular mycorrhizal
35. Kuzakov Y, Gavrichkova O (2010) Time lag between photosynthesis and carbon
34. Kuptz D, Fleischmann F, Matsyssek K, Grams TEE (2011) Seasonal patterns of
33. Bazzaz FA, Grace J, editors. Plant Resource Allocation. San Diego: Academic Press, 
32. Bazzaz FA, Grace J, editors. Plant Resource Allocation. San Diego: Academic Press, 
31. Wright IJ, Reich PB, Westoby Y, Ackerly DD, Baruch Z, et al. (2004) The
30. Ploeg R, Friesen AM, Noy-Meir I, Kremen C, Glunz E, et al. (2003) Global
29. Ehleringer JR (1991) 13C/12C fractionation and its utility in terrestrial plant
28. Brodribb TJ, Feild TS, Gregory JJ (2007) Leaf maximum photosynthetic rate
27. Bonham CD (1989) Measurements for terrestrial vegetation. New York: John
26. Kuptz D, Fleischmann F, Matsyssek K, Grams TEE (2011) Seasonal patterns of
25. Brodribb TJ, Feild TS, Gregory JJ (2007) Leaf maximum photosynthetic rate
24. Ehleringer JR (1991) 13C/12C fractionation and its utility in terrestrial plant
23. Larcher W (1969) Effect of environmental and physiological variables on carbon
22. Brodribb TJ, Feild TS, Gregory JJ (2007) Leaf maximum photosynthetic rate
21. Ehleringer JR (1991) 13C/12C fractionation and its utility in terrestrial plant
20. Bonham CD (1989) Measurements for terrestrial vegetation. New York: John
19. Bonham CD (1989) Measurements for terrestrial vegetation. New York: John
18. Ehleringer JR (1991) 13C/12C fractionation and its utility in terrestrial plant
17. Brodribb TJ, Feild TS, Gregory JJ (2007) Leaf maximum photosynthetic rate
16. Bonham CD (1989) Measurements for terrestrial vegetation. New York: John
15. Bonham CD (1989) Measurements for terrestrial vegetation. New York: John
14. Brodribb TJ, Feild TS, Gregory JJ (2007) Leaf maximum photosynthetic rate
13. Bonham CD (1989) Measurements for terrestrial vegetation. New York: John
12. Ehleringer JR (1991) 13C/12C fractionation and its utility in terrestrial plant
11. Brodribb TJ, Feild TS, Gregory JJ (2007) Leaf maximum photosynthetic rate
10. Langley JA, Johnson NC, Koch GW (2005) Mycorrhizal status influences the
9. Valiente AJ, Kleinert A (2007) Respiratory responses of arbuscular mycorrhizal
8. Hughes JK, Hodge A, Fitter AH, Atkin OK (2008) Mycorrhizal respiration:
7. Ryan MG, Law BE (2005) Interpreting, measuring and modeling soil
6. Gill RA, Jackson RR (2000) Global patterns of root turnover for terrestrial
5. Eissensat DM, Yanai RD (2002) Root life span, efficiency and turnover. In: 
4. Wright IJ, Reich PB, Westoby Y, Ackerly DD, Baruch Z, et al. (2004) The
3. Wright IJ, Reich PB, Westoby Y, Ackerly DD, Baruch Z, et al. (2004) The
2. Ploeg R, Friesen AM, Noy-Meir I, Kremen C, Glunz E, et al. (2003) Global
1. Ploeg R, Friesen AM, Noy-Meir I, Kremen C, Glunz E, et al. (2003) Global