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BAND 29

Yonas Yohannes Shikur

IMPACT OF TREE SPECIES AND FOREST MANAGEMENT ON CARBON CYCLING IN THE PLANT-SOIL-ATMOSPHERE SYSTEM

INSTITUT FÜR BODENKUNDE DER LEIBNIZ UNIVERSITÄT HANNOVER

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Dedicated to Dr. Demel Teketay Fanta

I am always inspired by your intellect, kindness and positive thinking.

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AM	Arbuscular Mycorrhiza
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	ANalysis Of Variance
BS	Base Saturation
^{13}C	Stable carbon isotopes with atom mass 13
C	Element Carbon
CAR	Continuous AutoRegressive
CEC	Cation Exchange Capacity
CDM	Clean Development Mechanisms
Cm	*Croton macrostachys*
CV	Coefficient of Variation
DBH	Diameter at Breast Height
DFG	Deutsche Forschungsgemeinschaft
EA	Elemental Analyzer
EA-IRMS	Elemental Analyzer- Isotopic Ratio Mass Spectrometer
FAO	Food and Agricultural Organization
GC	Gas Chromatography
GC-C-IRMS	Gas Chromatography-Combustion- Isotopic Ratio Mass Spectrometer
Gt	Giga tone
HPLC	High-performance Liquid Chromatography
IRMS	Isotopic Ratio Mass Spectrometer
LME	Linear Mixed Effect
MRT	Mean Residence Time
N	Element Nitrogen
NLFA	Neutral Lipid Fatty Acid
OC	Organic Carbon

OMMI	d-1-O-Methyl-Muco-Inositol
PAR	Photosynthetically Active Radiation
Pf	*Podocarpus falcatus*
Pg	Peta gram
PLFA	Phospho Lipid Fatty Acid
PVC	Poly Vinyl Chloride
REDD	Reducing Emissions from avoided Deforestation and forest Degradation
RMSE	Root Mean Square Error
SM	Soil Moisture
SOC	Soil organic carbon
SR	Soil Respiration
ST	Soil Temperature
TN	Total Nitrogen
UNCED	United Nations Conference on Environment and Development
UNFCCC	United Nations Framework Convention on Climate Change
UNPF	United Nations Population Fund
VPDB	Vienna Pee Dee Belemnite

Abstract

Forests play a decisive role in the global carbon cycle. At present, climate change, anthropogenic disturbances and management intervention change the size of carbon pools and affect the flow of carbon between them. Soil CO_2 efflux tends to dominate total respiration in many ecosystems, and their measurement can contribute much to our understanding of carbon flow through forest ecosystem. The aims of this study was to assess the influence of tree species, soil moisture and soil temperature on seasonal variability of soil CO_2 efflux rate in the natural forest stand comprising three individual plots of *Croton macrostachys*, *Podocarpus falcatus* and *Prunus africana*. In addition, two stands of *Cupressus lusitanica* plantation growing in two contrasting management intervention were studied with the objective to assess the impact of forest management on soil CO_2 efflux rates and variability. The other aim of this work was to determine the temporal resolution of recently fixed carbon as it is flows from tree canopy to soil-atmosphere continuum from two indigenous tree species (angiosperm pioneer *C. macrostachys* and gymnosperm late successional *P. falcatus*). Stable isotope ^{13}C was used to trace C from the canopy to the belowground and to the soil CO_2 efflux.

In the natural forest stand soil CO_2 efflux rate ranged between 2.0 and 7.0 μmol $m^{-2}s^{-1}$ and it was being larger under *P. falcatus* trees followed by *P. africana* and *C. macrostachys*. In the plantation stand, soil CO_2 efflux ranged between 1.3 to 6.9 μmol $m^{-2}s^{-1}$ and it was being larger under the managed stand. Seasonal variations in soil CO_2 efflux rates was mainly related to changes in soil moisture, being lower during dry periods and higher during wet periods. Soil moisture alone in both forest stand explained about 50% of the seasonal variability. Unlike soil moisture, soil temperature had weak relation with soil CO_2 efflux but relatively better explained at the plantation forest stand. Cumulative annual soil CO_2 efflux was largest in the natural forest stand with values in decreasing order were 160 mol m^{-2} (*P. falcatus*), 154 mol m^{-2} (*P. africana*), 149 mol m^{-2} (C. *macrostachys*), 123 mol m^{-2} (managed Cupressus), and 99 mole m^{-2} (unmanaged Cupressus), respectively

The two indigenous trees differed on speed and allocation of recently assimilated C between tree canopy and to arbuscular mycorrhiza (AM) and soil CO_2 efflux. Phloem sap velocity was about 4 times faster for *C. macrostachys*, where the label also appeared earlier in the AM. The newly fixed carbon in *C. macrostachys* was initially detected in soil CO_2 efflux after 24 h pulse labeling and after 72 h in *P. falcatus*. Within the one year chasing period 32% of the

assimilated carbon appeared in soil CO_2 efflux under the pioneer angiosperm as compared to 15% in case of the late-successional gymnosperm.

Despite the higher CO_2 losses by soil respiration, the natural forest soil contained more organic C, total N and microbial biomass estimated as phospolipid fatty acid concentration, suggested that the natural forest stand could stock more long-term soil organic carbon than the plantation forest. On the other hand, differences in soil organic C, total N and microbial biomass between the two plantations stand may indicate positive effects of silvicultural manipulation on the carbon balance of the stand. This study showed the importance of photosynthesis, phloem transport of sugars to belowground, and root and mycorrhizal fungi respiration as a quick channel of carbon from the canopy to soil and back to the atmosphere. Larger C allocation and rapid recovery of recently assimilated C in soil CO_2 efflux in case of the pioneer may have a negative impact on carbon accumulation in vegetation and soil in the long term. I assume that any change in relative abundance of tree species in the natural forest stand and ongoing climate change, typically change in precipitation pattern, may likely alter the future ecosystem carbon balance of the Munessa forest by their impact on C input, allocation and soil respiration.

Keywords: *Carbon flux, Munessa forest, Soil CO_2 efflux*

ZUSAMMENFASSUNG

Wälder haben eine entscheidende Rolle im globalen Kohlenstoffkreislauf. Gegenwärtig werden durch Klimawandel, Eingriffe in die Forstnutzung und weitere anthropogene Störungen sowohl die Höhe der C-Pools als auch der Fluss zwischen den einzelnen Kompartimenten des C-Pools beeinflusst. Derzeit erscheint in vielen Ökosystemen die Abgabe von CO_2 aus dem Boden die Assimilation zu übertreffen. Es wird angenommen, dass dies maßgeblich zur C-Fluss in Waldökosystemen beiträgt.

Ziel der Arbeit war es, den Einfluss von drei verschiedenen Baumarten, der Bodenfeuchte und –temperatur auf die jahreszeitliche Variabilität der CO_2-Freisetzungsrate aus dem Boden für einen natürlichen Wald zu untersuchen. Hier wurden jeweils drei Flächen mit *C. macrostachys*, *P. falcatus* and *Prunus africana* ausgewählt. Zusätzlich wurden zwei Bestände einer *Cupressus lusitanica* Plantage mit unterschiedlichen Bewirtschaftungsstrategien einbezogen, um den Einfluss der Bewirtschaftung auf die Variabilität der Abgabe von CO_2 aus dem Boden zu untersuchen. Ein weiteres Ziel dieser Arbeit war es, den Fluss von im Kronenraum kurzfristig fixiertem Kohlenstoff zum Wurzel-Boden Kontinuum zeitlich aufgelöst zu untersuchen. Diese Dynamik wurde für zwei einheimische Baumarten, die angiosperme Pionierart *Croton macrostachys* und die gymnosperme, am Ende der Sukzession stehende Art *Podocarpus falcatus* untersucht. Das stabile Isotop ^{13}C wurde genutzt, um den Weg von C von der Baumkrone in den Boden und den Anteil an der CO_2-Freisetzungsrate aus dem Boden zu untersuchen.

Im natürlichen Wald betrug die CO_2 Freisetzungsrate aus dem Boden zwischen 2 und 7 µmol $m^{-2}s^{-1}$. Höchste Werte wurden für die Art *P. falcatus* gefolgt von *P. africana* und *C. macrostachys erhalten*. In der Plantage betrug die CO_2–Freisetzung aus dem Boden zwischen 1.3 bis 6.9 µmol $m^{-2}s^{-1}$ mit höchsten Werten für den bewirtschafteten Bereich. Jahreszeitliche Variationen in der CO_2–Freisetzung stehen hauptsächlich in Beziehung zur Bodenfeuchte, mit niedrigeren Raten in Trockenperioden und höheren in regenreichen Perioden. Für beide Forsttypen erklärte allein die Bodenfeuchte 50% der jahreszeitlichen Variabilität. Anders als die Bodenfeuchte ist die Beziehung der Bodentemperatur zur Freisetzung von CO_2 aus dem Boden nur schwach. Am engsten ist diese für den Standort der Plantage. Die kumulative jährliche CO_2–Freisetzung war für den natürlichen Waldbestand am größten. Die Werte nehmen in folgender Reihung ab: 160 mol m^{-2} (*P. falcatus*), 154 mol m^{-2} (*P. africana*), 149

mol m^{-2} (*C. macrostachys*), 123 mol m^{-2} (bewirtschafteter Cupressus) und 99 mol m^{-2} (unbewirtschafteter Cupressus).

Die beiden einheimischen Baumarten unterscheiden sich in der Geschwindigkeit und Aufteilung von kurzfristig aufgenommenen C zwischen dem Kronenraum und Arbuscular Mykorrhizen (AM) sowie auch der Freisetzung von CO_2 aus dem Boden. Die Geschwindigkeit der Phloemflüssigkeit war mehr als viermal so groß für *C. macrostachys*, wo der C-Marker auch früher in AM nachgewiesen werden konnte. Der frisch fixierte C wurde für *C. macrostachys* bereits 24 h nach der Markierung im aus dem Boden freigesetzten CO_2 nachgewiesen, während dies für *P. falcatus* erst nach 72 h gelang. Im einjährigen Beobachtungszeitraum erschienen für die angiosperme Pionierbaumart 32 % des assimilierten C in dem aus dem Boden freigesetzten CO_2 im Vergleich zu 15% für die gymnosperme Baumart der späten Sukzession.

Trotz hoher Abgaben von CO_2 durch Bodenatmung weist der natürliche Waldbestand die höchsten Gesamtgehalte an organischem C und N als auch mikrobieller Biomasse auf. Letztere wurde mit Hilfe der Konzentration an phospholipider Fettsäure abgeschätzt. Aus diesem Befund kann abgeleitet werden, dass der natürliche Waldbestand im Boden größere Vorräte an stabilem organischem Kohlenstoff speichern kann als die Forstplantagen. Andererseits weisen in den beiden Forstplantagen Unterschiede in den Gesamtgehalten an organischen C und N sowie mikrobieller Biomasse darauf hin, dass die Bewirtschaftungsform auch positive Effekte auf das C-Gleichgewicht im Bestand haben können. AM wurde als eine schnelle Wegsamkeit für C aus dem Kronenraum in den Boden und zurück in die Atmosphäre identifiziert. Die für die Pionierbaumart feststellbare starke Aufteilung und schnelle Wiederfindung von kürzlich assimiliertem C in dem aus dem Boden freigesetzten CO_2 ist hinsichtlich der Akkumulation von C in der Vegetation und im Boden negativ zu bewerten. Es kann angenommen werden, dass im natürlichen Waldbestand jede Veränderung in der relativen Häufigkeit der verschiedenen Baumarten sowie die durch den voranschreitenden Klimawandel geänderte Niederschlagsverteilung das C-Gleichgewicht von Munessa Forsten durch ihren Einfluss auf den C-Eintrag, Formen der C-Speicherung und Bodenatmung beeinflussen.

Schlüsselwörter: Kohlenstoffflüsse, Munessa Forst, CO_2 Abgabe aus dem Boden

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EXTENDED SUMMARY

forest soils are arbuscular mycorrhizal fungi (AMF). Arbuscular mycorrhiza fungi obtain their carbon source directly from plant roots in the form of reduced carbon and stored it partly as lipids (Bago *et al.*, 2002). The neutral lipid fatty acid (NLFA 16:1ω5) is the major fatty acid in AM fungi (Madan *et al.*, 2002). A large portion of photosynthate carbon is also allocated to mycorrhizal fungi and the proportion ranges from 5–20% of net photosynthate (Pearson and Jakobsen, 1993), consequently mycorrhizal fungi are intimately involved in the fate of belowground processes of plant derived carbon. The NLFA 16:1ω5 has been used as a specific biochemical marker to track plant-derived carbon fluxes into microbial biomass and this has opened up a window to understanding the flux of carbon through plant-associated microbial communities (Olsson and Johnson, 2005).

Part of assimilated carbon retained by the root is released into the rhizosphere in the form of root exudates such as sugars, amino acids, organic acids, hormones, enzymes etc. (Jones *et al.*, 2004) and these substances are taken up immediately by the rhizomicrobial community. Due to the heterogeneous nature and layering of soils, SOC cycling is concentrated in some hot spots. In forests, one site of high metabolic activity is the rhizosphere with its high availability of easily degradable organic substances derived from roots and dead rhizosphere microorganisms (Kuzyakov and Domansky, 2000). Because of this, in the rhizosphere microbial biomass relative to in the bulk soil is larger (Phillips and Fahey, 2006). Like the plant root, AMF and rhizosphere microbial community utilized parts of retained C for metabolic activities that end up with the release of C in the form of CO_2 which is called respiration.

1.5. Soil respiration

Soil respiration from forest floor is a crucial component of carbon cycles in forest ecosystem. Globally it is the second largest C flux with estimated annual release of 75 Pg CO_2-C to the atmosphere (Raich and Schlesinger, 1992), approximately three times greater than that being emitted by the aboveground terrestrial biosphere (Rustad *et al.*, 2000). Many different methodologies to quantify total soil respiration exist at present. The soil chamber method is a well recognized technical approach to estimate gas fluxes between the soil and the free atmosphere in specific locations, representing the spatial heterogeneity of ecosystem carbon fluxes (Pumpanen *et al.*, 2004). The use of chambers placed over the soil is the most direct way of measuring respiration occurring within the soil and litter layers. Soil respiration is fuelled by two different sources. One origin is complete microbial mineralization of organic substances

(i.e., leaf and root litter, microbial residues, and soil organic matter), termed heterotrophic respiration. The other source stems from respiration resulting from maintenance and growth of root and associated mycorrhizae defined as autotrophic respiration. Both parts of soil respiration respond differently to edaphic and climatic factors, as well as on soil management. Quantifying the two sources, therefore, enables us to better understand mechanisms underlying total soil CO_2 efflux that have a profound implications for the soil and ecosystem carbon balance (Ryan and Law, 2005; Subke *et al.*, 2006). In their review on partitioning methods Hanson *et al.* (2000) reported a mean contribution from autotrophic sources of 48% of the total soil respiration in forest ecosystems.

1.6. Belowground carbon cycling as affected by vegetation and management

The balance between photosynthesis and respiration, and hence the carbon storage, varies with many ecological parameters. On seasonal to interannual timescales, the phenology of plants and the supply of decomposable litter and root exsudates, besides weather conditions, are most important variables (Borken *et al.*, 2006; Scott-Denton *et al.*, 2006). On decadal and longer time scales, changes in composition, age, and structure of vegetation, and the associated changes in soil physical or chemical conditions are important driving factors controlling belowground carbon fluxes and storage (Trumbore, 2006). Hence, the type of vegetation and its management are important factors in the belowground carbon cycling.

Tree species strongly differ in the characteristics of the input of organic residues to the soil in terms of quantity, vertical distribution and substrate quality (Ayres *et al.*, 2009). On the one hand this refers to the different litter production and quality (Binkley, 1996; Bernhard-Reversat, 1998). On the other hand there is also profound evidence that processes in the rhizosphere are different between different tree species and silvicultural management. These peculiarities include the proportion of photosynthates allocated to above-ground and below-ground biomass for different tree species (Grayston *et al.*, 1996), root architecture and fine root distribution (Fritzsche *et al.*, 2006), and characteristic mycorrhizal associations (Philipps and Fahey, 2006). Different plant species can selectively stimulate the growth of particular microbial species in the rhizosphere by modifying the nature and rate of organic carbon release into soil (Kourtev *et al.*, 2003). The input pattern of organic substances to soil, may also influence the carbon flow between different functional soil pools and, thereby, finally the autotrophic and heterotrophic respiration. In mature forests, carbon storage in soil approaches a steady-state where the

outputs nearly equal the input, with some seasonal or year-to-year variation. Transformation of mature forests to forest cultivation often involves accelerated rates of decomposition and a change in organic carbon stocks (van Cleve and Powers, 1995). Ashagrie *et al.* (2003; 2005) observed a significant decrease in organic carbon and nitrogen storage for soils of the Munessa forest, after clearing of the natural forest and transformation to forest plantations. Most of it occurred in the organic layer and the particulate organic matter fraction of the mineral soil. A proper management, however, can reduce losses after harvesting and reforestation to about 10% (Turner and Lambert, 2000; Zinn *et al.*, 2002).

1.7 Aims

The general aim of this study was to elucidate effects of tree species and forest management practice on processes of the carbon cycling in the plant-soil-mycorrhizal system. The specific objectives of the studies were:

- *To analyze seasonal variation in soil CO_2 efflux rate as a function of soil temperature and soil moisture* (Manuscripts I and II)

  Many studies have been conducted in the last two decades or so, elucidating abiotic factors controlling soil CO_2 efflux rates of temperate and boreal forests. There, seasonal variations in soil CO_2 efflux rate are often considered to be controlled primarily by soil temperature (Malhi *et al.*, 1999; Shibistova *et al.*, 2002). However, information on temporal and spatial heterogeneity of soil CO_2 efflux rates and their abiotic drivers in African montane forest is scarce.

- *To investigate the effect of tree species on soil CO_2 efflux rate* (Manuscript I)

  Many studies in the past have focused on the influence of abiotic factors on the temporal variability of soil CO_2 efflux rate but only few have studied the effects of biotic factor on spatial variability of soil CO_2 efflux (e.g., Bréchet *et al.*, 2009; Oscar 2007). Trees differ in a variety of physiological and phenotypical traits that have species-specific effects on soil properties (Ayres *et al.*, 2009) and thereby soil CO_2 efflux rate.

- *To investigate the effect of thinning treatment on microbial biomass and soil CO_2 efflux rate* (Manuscript II)

The removal of trees from a stand could cause change in the microclimate, aboveground and belowground plant biomass (Tian *et al.*, 2009) which could also bring change in the population of soil microbes. There is an increasing need on how different management intervention will influence the carbon balance of a forest. So far there is no consensus on the effect of forest thinning treatment on soil CO_2 efflux variability. Soil CO_2 efflux has decreased (Sullivan *et al.*, 2008), increased (Selmants *et al.*, 2008), or not changed Masyagina *et al.* (2010) following thinning treatment and this calls for more investigation.

- *To trace the fate of newly fixed carbon as it flowed from leaves to phloem, root and associated arbuscular mycorrhiza fungi and back to the atmosphere via soil respiration* (Manuscript III)

  Much of our current understanding on the fluxes of carbon in trees is based on seedling and sapling studies, during the past few years however, good progress has been made of *in situ* ^{13}C labeling of young trees to understand the carbon fluxes on plant-soil-atmosphere continuum (e.g., Högberg *et al*, 2008; Plain *et al.*, 2009; Kuptz *et al.*, 2011). Some of the recent findings were localized in temperate and boreal ecosystems but for trees in a natural setting of tropical forest ecosystem the timing and fluxes of C is unknown. The use of C isotopes techniques together with PLFA analysis reveals information to directly link *in situ* plant-microbial interaction on the C cycling (Boschker *et al.*, 1998).

These aims have been approached by comparing three different functional native tree species and monospecific even aged exotic *Cupressus lusitanica* plantation. While in the heterogenous natural forest stand, studies were carried out focusing on three different tree species differing in their functional traits, in the plantation forest the impact of thinning treatment in two stands being in close proximity to one another was of interest. Soil CO_2 efflux rates were measured under the canopies of each selected trees on weekly basis for two years using a closed chamber technique. Thinning treatment in one of the plantation stands was carried out one and half years before the start of soil CO_2 efflux measurement. This thesis also made use of canopy chamber ^{13}C pulse labeling techniques to chase the fate of recently assimilated carbon on two of the indigenous trees for one year period since labeling initiated.

2. Study site and experimental approach

2.1. Study site

The study area is located at Munessa-Shashemene Forest found in Oromia Regional State, West Arsi Zone, Ethiopia. The forest has an estimated area of 23,000 ha of both natural and plantation forest. The forest is divided into three blocks; namely Gambo, Sole and Degaga. For the present study, the experimental sites were established in the Degaga block (Figure S.1) of the natural forest stand at 'Kuke' (07^0 25' 16'' N and 038^0 51' 49'' E) and adjacent plantation forest stand at 'Petros' (07^0 25' 416'' N and 038^0 51' 05'' E).

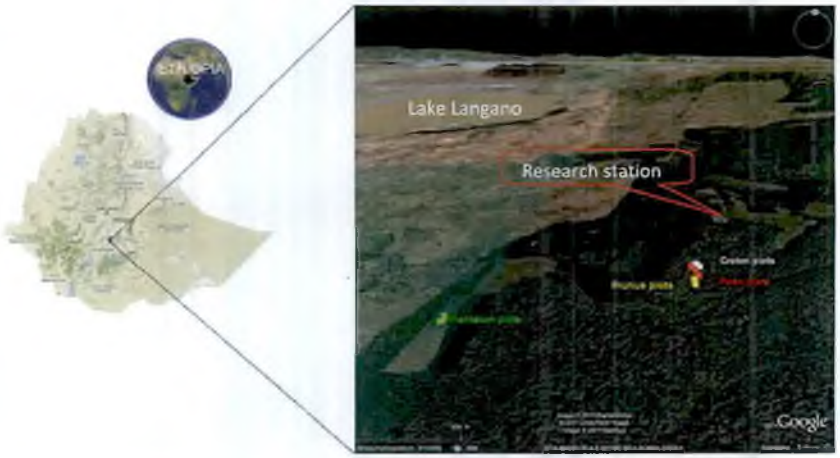

Fig. S.1: 3D2 image of the study area

The elevation at the study stand in the natural forest is between 2266 to 2279 m and at the plantation stand is 2126 m above see level. The mean annual rainfall is about 1500 mm, with a minor rainy season occurring from March to May and a major rainy season from July to November (Griffits, 1972). Mean annual air temperature is about 15.3 °C. Vegetation of the natural forest is dominated by the canopy species *Podocarpus falcatus* (Thunb.) and *Croton macrostachys* Hochst. Other plant species with relatively less abundance include *Prunus africana* (Hook. F.) Kalkman, *Syzygium guineense* (Wild.) DC., *Celtis africana* Burm. f. and *Pouteria adolfi-friederici* (Engl.). The plantation stand (*Cupressus lusitanica*) was established in 2003 as part of commercial forestry activity and administered by the regional government under Arsi Forest Development Enterprise.

2.2. Stand and experimental trees selection

In the present study, "stand" refers to the natural forest stand as well as the managed stand and unmanaged stand within the plantation forest. Within each experimental forest stand "plots" were established in replicates under the canopies of individual trees, where e.g. the ^{13}C soil efflux measurements have been carried out. Three indigenous tree species namely *C. macrostachys*, *P. africana*, *P. falcatus*, and one exotic tree species *C. lusitanica* were selected for the soil CO_2 efflux measurements. In the natural forest, three similar trees of each of *C. macrostachys*, *P. africana*, *P. falcatus* of the third height class at the distance of *c.* 100 m were chosen (Tesfaye *et al.*, 2010). The three different tree species in the natural forest have been chosen, because they represent different functional types (Whitmore, 1989). In the plantation forest four 6-years old individual *C. lusitanica* were randomly selected within each of the managed and unmanaged stand, respectively. The three indigenous trees species were selected because of their relative abundance, ecological and/or economical importance. *Cupressus lusitanica* was selected because of its economic value and it is also the dominant planted tree species in the Munessa forest. For the labeling experiment in natural forest, pairs (control and experimental) of *C. macrostachys* and *P. falcatus* trees were considered. The tree species were similar in height but differed in stem diameter and foliage dry mass (Table S.1).

Table S.1: Main characteristics of the experimental trees

Natural forest stand				*Plantation forest stand*			
Plot	Status*	Height	DBH	Plot	Code$^{\$}$	Height	DBH
		m	cm			m	cm
Croton	I	8.3	7.2	*Cupressus*	M	5.6	9.3
Croton	C	5.1	4.5	*Cupressus*	M	5.9	5.5
Croton	L	5.6	4.1	*Cupressus*	M	7.2	7.9
Podocarpus	I	4.8	6.7	*Cupressus*	M	5.8	9.3
Podocarpus	C	5.6	9.4	*Cupressus*	U	5.1	6.0
Podocarpus	L	6.2	11.5	*Cupressus*	U	6.1	6.8
Prunus	I	5.0	7.2	*Cupressus*	U	6.0	6.8
Prunus	2	6.1	9.8	*Cupressus*	U	6.0	8.1
Prunus	3	6.0	8.5				

Status* C, control trees; L, labeled trees $^{\$}$Code M, managed stand; U, unmanaged stand

2.3. Meteorological data

Meteorological parameters were continuously recorded by two weather stations located (1) at the field station "Kuke" about 0.6 km in distance from the natural forest experimental stand and (2) within the natural forest stand at a representative place close to the experimental plots. Soil temperature and volumetric soil water contents were measured in the upper 0.1 m using 8-bit

temperature sensor and ECH2O soil moisture probe respectively (Onset Computer Corporation, Bourne, Massachusetts). Air temperature were measured using μMETOS SMT 160-30 sensor, Pessl Instruments GmbH, Werksweg, Germany. In the natural forest, bulk precipitations were determined on a weekly basis using five randomly distributed polyethylene funnels with a 120 mm upper diameter. According to Fig. S.2., the rainfall pattern was not bimodal, rather for the years 2009 and 2010 there was almost rainfall throughout the year. However, the period from December-February was relatively dry. Although the pattern of rainfall showed large annual variation, the total rainfall between the study years was comparable. Monthly minimum and maximum air temperature during the study periods ranged from 13.3 °C to 18.9 °C with a yearly averaged ranged from 14.7 °C to 16.2 °C.

2.4. Soil Chamber and soil CO_2 efflux measurement

In June 2008 (natural forest) and July 2009 (plantation forest), 5 PVC soil collars (20 cm in diameter and 5 cm long) were inserted ca. 1-2 cm into the soil under the canopy of each selected individual tree for soil CO_2 efflux measurement. The insertion depth was kept 1-2 cm to minimize severing of roots and the mycorrhizal system, while ensuring that no leaks occurred in the chamber–soil system. Collars were fixed securely in place and the ring between the PVC collar and soil around the collars was sealed with fine sand to create a diffusion barrier. Collars were installed randomly around the selected tree at a distance of about 0.5 to 1.3 m from the bole. Soil collars were remained in place throughout the measurement periods. Soil CO_2 efflux from the forest floor was measured using an Infrared Gas Analyzer Li-8100 supplied by LI-8100-103 soil survey chamber (LI-COR, Lincoln, NE, USA). Soil temperature ($^{\circ}$C) adjacent to each PVC collar at a depth of 0.1 m was also simultaneously measured with soil CO_2 efflux measurements using a thermocouple probe (Li-8100-201) connected to the Li-8100. The volumetric soil water content at 0.06 m depth was measured adjacent to each PVC collar with a handheld theta probe (ML2, Delta-T Device Ltd, Cambridge, UK) at three replicates around the collars immediately following the soil CO_2 efflux measurement.

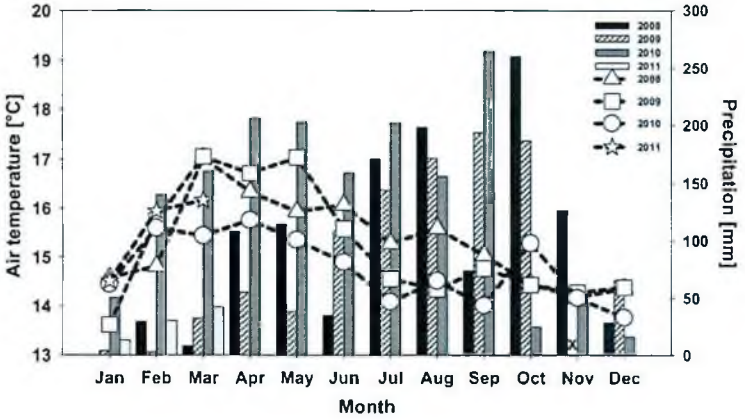

Fig. S.2: Monthly total precipitation (bar graph) and mean monthly air temperature (line graph) from January 2008 to March 2011 measured at the Kuke field station (data from Strobl *et al.*, unpublished). Data for 2011 measurements are up to March. x shows missed data in November 2009 due to rain gauge failure.

2.5. Canopy chamber and ^{13}C labeling

The labeling was carried out in November 2008. Before, in October 2008 rectangular frames made from eucalyptus poles were constructed around the canopy of *C. macrostachys* and *P. falcatus* trees selected for the labeling experiment. Greenhouse plastic cover (UVA Clear, Ginegar Plastics Products Ltd, Israel: 120 micron, 88% light transmission in PAR) was pulled over the frame and tightly sealed before the labeling event within 30 min. The chambers were sealed with the plastic film from the bottom side at 1.60 m distance from the forest floor in order to avoid diffusion of the labeled material into the soil during labeling. ^{13}C-labeled CO_2 inside the chambers was generated by injecting diluted sulfuric acid into a flask containing isotopically enriched $Na_2{}^{13}CO_3$ solution (99 atom% ^{13}C; Campro Scientific, Berlin, Germany). Five electric fans (12V, 0.21A) circulated the air in the chambers while labeling, and CO_2 concentration, air temperature, and relative humidity were monitored continuously using a LI-8100 infrared gas analyzer (LI-COR Inc., Lincoln, NE, USA).

2.6. Plant material, soil and soil CO_2 gas sampling

Before and after exposure to $^{13}CO_2$, the stable carbon isotope composition of leaves, phloem sap, bulk and rhizosphere soil and soil CO_2 was examined from labeled and control trees. With the exception for foliage, phloem and soil CO_2 efflux sampling started 24 h after the labeling

procedure. Leaves, phloem sap, soil CO_2 efflux, and the 0-10 cm and 10-25 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 with 4 hours interval, while soil CO_2 efflux was collected 12 hours after the labeling.

Leaf samples were collected from three heights within the crown at bottom, middle and top position of the crown and from four expositions. Composite samples from each crown part were separately put into paper bag and air dried. Phloem samples were collected at breast height (1.3 m) and at a height of 0.5 m. A small piece of bark (~1 cm x 1 cm) was incised down to cambium using a sharp blade. The samples were placed in 20 ml glass vial containing 2 ml of 15-mM polyphosphate buffer solution and left for 5 h. Then phloem exudates were frozen until being analyzed.

Soil samples were collected using soil cores (equipped with a detachable cylindrical steel core, 4.0 cm diameter, 40 cm length) in triplicate from 0–10 and 10–25 cm depths under the canopy of each experimental tree. Soil samples collected under the canopies of same tree species were bulked and passed through a 2-mm sieve. Adhering soil was carefully separated manually. Samples of adhering and bulk soil were transferred into separate glass vials and kept frozen until analyzed. The remaining soil samples were air dried and stored until analyzed.

A LI-8100 infrared gas analyzer supplied with 8100-103 Survey Chamber (LI-COR Inc., Lincoln, NE) was used for soil CO_2 efflux measurements and air sampling. CO_2 gas samples evolved from soil was sampled from all 5 PVC collars. In addition, five gas samples were taken at an interval of every 100 ppm increment for Keeling plot analysis at each plot and each sampling time. About 15 ml gas volume was injected into 12 ml Exetainer glass vials (Labco, High Wycombe, Buckinghamshire, UK) that had been evacuated to less than 30 Pa.

3. Laboratory methods

All soil samples were air-dried and sieved to <2 mm, except for those used for extraction of lipid fatty acids which were stored below 0 °C.

3.1. Carbon and nitrogen

Leaf samples were finely grinded with a steel ball mill (Mixer Mill, Retsch MM 200, Haan, Germany) and oven dried at 65 °C for 48 h. Five to ten milligrams of dried powder was packaged in tin capsules and analyzed for total carbon and nitrogen using a Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Germany).

Soil samples were finely grinded with the same procedures as described above but they were oven dried overnight at 105 °C. Forty to fifty milligram of dried samples was then packaged in tin capsules and analyzed for total carbon and nitrogen using the same instrument as for leaf samples. As the soils did not contain carbonates, total carbon was assumed to represent organic carbon.

3.2. Lipid Biomarkers

Lipids were extracted following the method described by Bligh and Dyer (1959). Thereafter, lipids were separated into neutral, glyco-, and phospolipids using solid phase extraction with a silicic acid column (Bond Elut LRC-Si, Varian). The lipids were subjected to a mild-alkali methanolysis, and the resulting fatty acid methyl esters were separated by gas chromatography using an Agilent 7890A gas chromatograph (Varian Agilent Technologies, Santa Clara, CA). Selected phospholipid fatty acids (PLFAs) were used as bacterial and fungal marker. The neutral lipid fatty acid (NLFA 16:1ω5) was used as a marker fatty acid for AM fungi (Olsson and Johansen, 2000; Madan *et al.*, 2002). Biomarker phospholipids and neutral fatty acid concentrations (nmol g^{-1} dry soil) were calculated by comparing peak areas to an analytical standard peak (19:0, Sigma Chemical Co., St. Louis, MO, USA).

3.3. Stable carbon isotope analysis

Leaf and **Soil** samples were finely grinded with a steel ball mill (Mixer Mill, Retsch MM 200, Haan, Germany). Leaves were oven dried at 65 °C for 48 h and soils were oven dried overnight at 105 °C. Dried leaves and soil of samples were then packed separately in tin capsule and were analyzed using either on a Thermo Finnigan MAT DELTA^{plus} Advantage isotope ratio mass spectrometer coupled to an Euro EA C/N analyzer (IRMS from Thermo Electron Corporation, Waltham, USA) or on an Elementar IsoPrime 100 IRMS coupled to an Elementar vario MICRO cube EA C/N analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

Compound-specific isotope analysis of **phloem sugars** were analyzed by coupling an HPLC system (Dionex Corporation, Sunnyvale, CA, USA) to a Finnigan Delta V Advantage Mass Spectrometer (Thermo Electron Corporation, Waltham, USA) by a Finnigan LC IsoLink Interface. The HPLC-IRMS measurements were carried out at the Department of Chemical Ecology and Ecosystem Research, University of Vienna, and followed the method of Wild *et al.* (2010).

The **stable carbon isotopic composition of the lipid fraction (PLFA and NLFA)** was determined by an Agilent 7890A gas chromatograph (Varian Agilent Technologies, Santa Clara, CA) coupled to an Elementar IsoPrime 100 IRMS (Elementar Analysensysteme GmbH, Hanau, Germany) via a combustion interface (GC-C-IRMS). The $\delta^{13}C$ value of lipid was corrected for the methyl group added during methanolysis (Goodman and Brenna, 1992).

Gas samples were analyzed at the Department of Chemical Ecology and Ecosystem Research, University of Vienna, by continuous-flow isotope-ratio mass spectrometry on a Thermo Finnigan Delta V Advantage Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled to a Finnigan GasBench.

4. Results and Discussion

4.1. Soil chemical, physical and biological properties (Study I and II)

Soils were quite homogenous and represented Mollic Nitisols (Fritzsche *et al.*, 2007). In natural forest the soil texture was the same under all plots (Table A.1), indicating that the soils developed from the same parent material. As for other ecosystem parameters such as precipitation, slope inclination and exposition, this suggests that the research plots are comparable. Soils under the plantation forest are also quite homogenous and comparable with their counter parts under the natural forest (Table A.1).

Soils of the natural forest stand were characterized by high SOC and total nitrogen (TN) concentrations, being on average 111 ± 29.7 g C kg^{-1} soil and 10.4 ± 2.3 g N kg^{-1} soil. Such high concentrations have been reported previously (Ashagrie *et al.*, 2003), and are assigned due to high rates of litter input in these productive stands and large contents of reactive minerals (i.e., Fe- and Al-(hydroxides), stabilizing SOC by interactions with minerals (Fritzsche *et al.*, 2007). There was no significant difference in soil pH as well as base saturation (BS) under natural and plantation forest trees (p>0.05), but soil pH tend to be slightly more

acidic under the plantation forest trees (Table A.1). There was a difference in cation exchange capacity (CEC) being significantly ($p<0.05$) larger under *C. macrostachys* driven primarily by differences in exchangeable Ca^{++}. Since leaf litter enters the soil surface and root biomass is largest in the topsoil (Abate, 2004), from 0-10 to 10-25 cm soil depth the SOC concentration decreased significantly ($p<0.05$). Concurrently to the decline in SOC with soil depth, the CEC decreased as well, showing the impact of soil organic matter on exchangeable nutrients in these forest soils.

The results of one-way ANOVA showed significantly ($p<0.05$) larger SOC concentrations in the 0-10 cm soil layer under *C. macrostachys* or *P. falcatus* than in the unmanaged *C. lusitanica* stand (Table A.1). At a soil depth of 10-25 cm, SOC and TN was significantly different between natural and unmanaged plantation forest stand ($p<0.001$) where the natural forest soil had higher concentrations than samples from the plantation forest stand.

The quality and quantity of input by net primary production and its decomposition rate are the two most important factors influencing the amount of SOC storage (Lutzow *et al.*, 2006). The higher concentration of SOC and TN in the natural forest could have resulted from higher litter mass accumulation (Abate, 2004). Further, root litter input might be different. Up to the depth of 1m soil layer, Abate (2004) reported that live fine root biomass of *P. falcatus* was largest compared to *C. lusitanica*. Finally, the history of the plantation site must be considered. In this particular stand, the soils have been used for agriculture for about three years before reforested. Agricultural land use is known to deplete soil organic matter as has been also shown in the same studied fores (Lemenih *et al.*, 2005).

Soil organic C and TN also differ significantly between the managed and unmanaged stand ($p<0.001$). Compared to the unmanaged stand, the soil attributes under managed stand of *C. lusitanica* showed an overall change towards the direction of the soil attributes under the natural forest stand (Table A.1). The plantation stands are located close to each other and trees were initially planted at the same time and with the same stocking and hence differences in belowground properties could be possibly as the result of management intervention (thinning). This notion emanates from the observation that larger above ground biomass from individual potential crop trees was found (Nenninger, in preparation). In the same stands, Asaye (2011) identified significantly higher annual fine root biomass production in the thinned stand (554-603 g m^{-2}) as compared to the unthinned stand (327-364 g m^{-2}). Thinning treatment allowed the

remaining trees rapidly filled in the canopy gaps with increased leaf area (Medhurst and Beadle, 2000) and freed from competition for the available moisture and nutrients, which significantly increased their stem growth (Haggar and Ewel, 1995) and this may stimulate growth that resulting in increased living root activity (Ma *et al.*, 2004). As both, above and belowground fine litter are a major component of biomass (Clark *et al.*, 2001) larger input of the former materials under the experimental plots in thinned stand was also expected and this could probably contribute to larger carbon input. Two years after thinning treatment, Hwang and Son (2006) found out mean SOC concentrations (g kg^{-1}) for pitch pine and Japanese larch plantations were 39.6 and 58.8 for the control, 44.0 and 63.4 for thinned plot.

Owing to difference in the genetic makeup of the host plant and differences in the intimacy of root inhabiting microbes, differences in abundance of microbial community are likely under the rhizosphere of different trees species (Ayres *et al.*, 2009). As a biological indicator, PLFAs can be used as to estimate viable microbial biomass (Arias *et al.*, 2005). In general PLFA concentration followed the patterns of soil organic C and TN content, and decreased from 0-10 to 10-25 cm soil depth. At 0-10 cm soil depth, PLFA concentration tended to be larger in soil under Podocarpus plots (161.0 ± 14.6 nmol PLFA g^{-1} dry soil) followed by soil under managed Cupressus plots (119.7 ± 22.6 nmol PLFA g^{-1} dry soil), and in soil under Croton plots (113.2 ± 15.0 nmol PLFA g^{-1} dry soil). The soil under unmanaged Cupressus exhibited smallest concentration (71.7 ± 20.1 nmol PLFA g^{-1} dry soil). From the depth 10-25 cm, PLFAs concentration under the natural forest stand had always contained larger concentration than the plantation plots (95.8 ± 25.0, Podocarpus plots; 84.8 ± 4.1, Croton plots; 60.3 ± 11.5, Cupressus managed, and 33.6 ± 4.1 nmol PLFA g^{-1} dry soil, Cupressus unmanaged).

At a depth of 0-10 cm soil layer, biomass of fungal marker indicator estimated as sum of PLFA concentration 18:2ω6 and 18:1ω9 (Frostegård and Bååth, 1996; Zelles, 1997) were tended to be also larger in soil under *P. falcatus* (21.2 nmol fungal PLFA g^{-1} dry soil) followed by managed Cupressus (15.8 nmol fungal PLFA g^{-1} dry soil), *C. macrostachys* (14.5 nmol fungal PLFA g^{-1} dry soil) and unmanaged Cupressus (11 nmol fungal PLFA g^{-1} dry soil). At the same soil depth, biomass of bacterial marker indicator estimated from PLFA concentration i15:0, a15:0, i16:0, 18:1ω7c and cy19:0 (Zelles, 1999) were found to be larger in soil under the natural forest trees with that values in decreasing order were 104 nmol bacterial PLFA g^{-1} dry soil (*P. falcatus*), 66.5 nmol bacterial PLFA g^{-1} dry soil (*C. macrostachys*), 66 nmol bacterial PLFA g^{-1} dry soil

(managed Cupressus) and 39.5 nmol bacterial PLFA g^{-1} dry soil (unmanaged Cupressus) respectively. From the depth 10-25 cm soil layer, biomass of bacterial or fungal marker indicators PLFAs concentration in soils under the natural forest were tended to be larger than in soil under the plantation forest. The Declining PLFAs concentration with soil depth is the result of accompanied decline in organic matter and is a common pattern across many ecosystems (Feng *et al.*, 2003). It is clear that, there must be a positive relationship between the substrate and those who are using the substrate (e.g., Wardle, 1992; Lee and Jose, 2003). Solomon *et al.* (2002) found for the same forests significantly higher production of microbial metabolites below the natural forest, indicated by higher ratios of microbial to plant-derived sugars.

Relative abundance of individual fatty acids profiles extracted from soils samples under the experimental plots is shown in Fig. S.3.

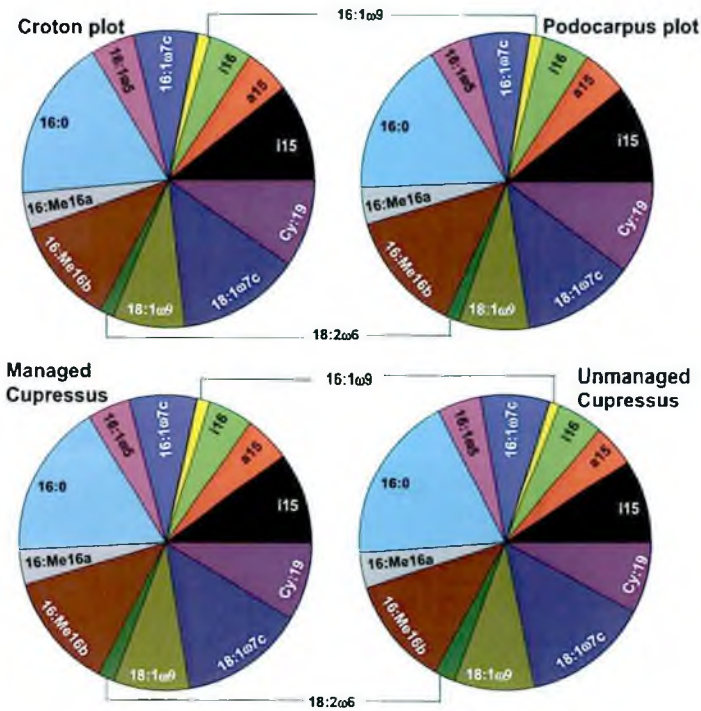

Fig. S.3: Proportion of individual fatty acids under the different experimental stand/plot

The normal saturates 16:0 and monosaturate 18:1ω17c, methyl branched 10:Me16b and branched chain saturate i15 were the most abundant PLFAs, accounting for 54% of the total concentration (Table A.2). Monounsaturate 16:1ω7, 18:1ω9, and Cyclopropyl fatty acid cy19:0 were formed the second most predominant group (24%). Branched-chain saturates (a15 and i16), monosaturate 16:1ω5 and methyl branched 10:Me16a were 19% of the total concentration. 18:2ω6 and 16:1ω9 were 3% the total PLFA concentration. There is no major differences in mole percentage of individual PLFAs between the forest stand, which indicating the lack of noticeable effects of soil origin.

4.2. Soil respiration (Study I and II)

Soil CO_2 efflux rates were larger in the natural forest than the rates in the plantation forest. Across the parallel measurement periods between June 2009 and September 2010, soil CO_2 efflux rates in the natural forest plots were ranged from 2.39 to 6.38 μmol $m^{-2}s^{-1}$ under *C. macrostachys*, 2.37 to 6.92 μmol $m^{-2}s^{-1}$ under *P. africana*, and from 2.24 to 7.23 μmol $m^{-2}s^{-1}$ under *P. falcatus* (Table A.3). Corresponding values at the plantation stand ranged from 2.26 to 5.20 μmol $m^{-2}s^{-1}$ unmanaged Cupressus, and from 2.37 to 6.92 μmol $m^{-2}s^{-1}$ under managed Cupressus. The higher rates of soil CO_2 efflux in the natural forest stand may be associated with different biological activity and larger organic C concentration that could accelerate both the plant root and microbial activities. Our result showed that microbial biomass (as estimated by the PLFA concentration) was greater in the natural forest stand than those in the plantation forest stand. Soil microbial concentration represents an important labile pool of nutrients and carbon (Guggenberger and Haider, 2002). The differences in soil CO_2 efflux between the plantation forest stand could probably originated mainly from the autotrophic respiration. Larger biomass production requires more nutrient acquisition by the trees, thus leading to larger fine root biomass and larger colonization by arbuscular mycorrhiza, as it can be concluded from the larger concentrations of 16:1ω5 NLFA in the thinned stand soils. Hence, both components of the autotrophic continuum may have enhanced soil respiration at the thinned stand due to higher energy demand, thus increasing the autotrophic respiration (Ma *et al.*, 2004). In addition, higher total microbial biomass (all PLFAs), bacteria (i15:0, a15:0, i16:0, 18:1ω7c and cy19:0 PLFAs) and fungi (18:2ω6 and 18:1ω9 PLFAs) at the thinned stand suggesting fuelling of heterotrophic activity by higher substrate supply, i.e. by rhizodeposition.

Soil CO_2 efflux data from October 2009 to September 2010 (water year period of the study area) was chosen to serve as a benchmark in order to estimate and compare cumulative annual soil CO_2 efflux. Where there were missing data due to rainfall incidence or failure of instrument, soil CO_2 efflux was estimated based on the Gaussian relation of soil moisture with soil CO_2 efflux shown in Fig. S.4. Based on that, cumulative annual soil CO_2 efflux was highest for *P. falcatus* with 160 mol m^{-2}. Respective values with decreasing orders were 154 mol m^{-2} (*P. africana*), 149 mol m^{-2} (C. *macrostachys*), 123 mol m^{-2} (managed Cupressus stand), and 99 mole m^{-2} (unmanaged Cupressus stand) respectively. With that the cumulative annual soil CO_2 efflux falls within the range of other published estimates of secondary tropical forests in eastern Amazon (150 mol CO_2 m^{-2} $year^{-1}$; Davidson *et al.*, 2000), tropical monsoon forests in Thailand (213 mol CO_2 m^{-2} $year^{-1}$; Hashimoto *et al.*, 2004), and monospecifc forest plantation in Rwanda (112 mol CO_2 m^{-2} $year^{-1}$; Nsabimana *et al.*, 2009).

Soil CO_2 efflux rates followed changes in the precipitation and the resulting changes in the volumetric soil water content. Consequently, in both forest stands the relationships between soil respiration and soil environmental variables was more influenced by soil moisture than soil temperature. Using the Gaussian Equation depicted in Fig. S.4, soil moisture alone in both forest stand explained more than 48% of the seasonal variability. In all experimental plots soil respiration rates steadily increased with increasing volumetric soil water content up to a certain threshold level, after exceeding this threshold values soil respiration declined (Fig. S.4). It is interesting to note that the optimal volumetric soil moisture at which soil CO_2 efflux was highest, was greater for the plantation stand (31%) than the natural forest with that it was 27.2% (*C. macrostachys*), 30% (*P. africana*), and 30.6% (*P. falcatus*) respectively (Fig. S.4). This result indicated that *C. lusitanica* was more tolerant than the other studied trees, which could probably related with the shallow root system with large fine root biomass that enable it to utilize the water from the topsoil effectively (Fritzsche *et al.*, 2006).

The strong positive relationship between soil respiration and soil moisture content is in agreement with previous studies in tropical forests where a majority of biological processes coincides with moisture dynamics (e.g., Epron *et al.*, 2006; Hashimoto *et al.*, 2004). There is a wealth of information that microbial activity is restricted when soils contain too much or too little moisture (Herron *et al.*, 2009 and references cited therein). Excess soil moisture may negatively affect CO_2 efflux rates by reducing soil aeration and thus CO_2 diffusivity (Janssens

and Pilegaard, 2003). Oxygen deficit as result of too high soil moisture decreases activity of plant roots (Adachi *et al.*, 2006) and the heterotrophic decomposition of soil organic matter (Linn and Doran, 1984). This may be particularly the case in the clayey soils under study.

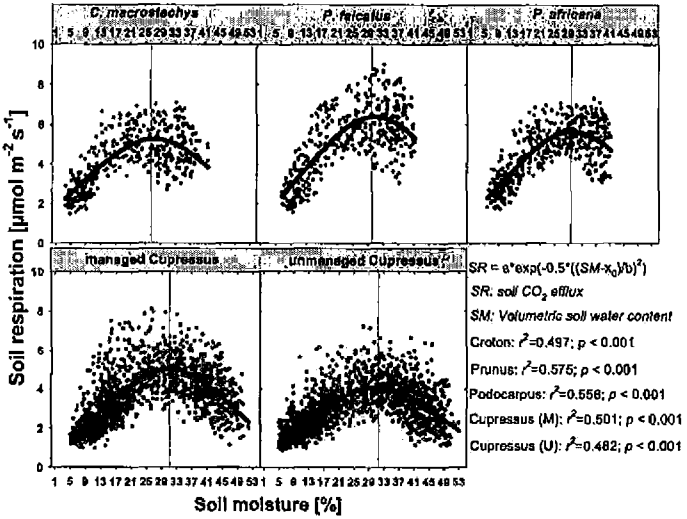

Fig. S.4: Relationship between soil CO_2 efflux rate and volumetric soil water content at 0.06 m soil depth. The vertical dashed line in the graphs indicates the cutoff value where soil CO_2 efflux rate start to declined.

The influence of soil temperature on the overall variation of soil CO_2 efflux was smaller as compared with that of soil moisture. Comparing the two forest type, a minor positive exponential relationship between soil respiration and soil temperature was observed under the plantation stand (r^2 = 0.17; p<0.01 vs. r^2 = 0.10; p<0.01 in the managed vs. unmanaged *C. lusitanica* stands, respectively). Generally, weak correlation of soil temperature with soil respiration is also a feature widely reported in other tropical forest ecosystem studies where soil temperature is relatively constant within the year (e.g. Davidson *et al.*, 2000; Hashimoto *et al.*, 2004). In other biomes such as boreal and temperate forests, there is a wide range of seasonal temperatures that leads to seasonal variation in soil respiration rate (e.g., Malhi *et al.*, 1999; Shibistova *et al.*, 2002).

4.3. Labeling efficiency and ^{13}C recovery in aboveground plant pool (Study III)

A crown labeling chamber approach suggested by Simard *et al.* (1997) was used to in the present study to track the fate of recently assimilated carbon in plant soil-atmosphere system. This approach has the advantages to prevent diffusion of $^{13}CO_2$ to soil pores and also avoids a labeling of understory vegetation. It thus increases the accuracy of $^{13}CO_2$ efflux measurements (Plain *et al.*, 2009). About 12.5 mM of $^{13}CO_2$ per 1 m^3 was introduced into each chamber. Immediately after opening the chamber (t_0) the $\delta^{13}C$ of the foliage of the labeled *C. macrostachys* and *P. falcatus* trees was significantly enriched as compared to control ($p<0.001$). Based on excess ^{13}C of the foliage at t_0, approximately 5.9 g ^{13}C was assimilated by *C. macrostachys* and 6.9 g ^{13}C by *P. falcatus*. The labeling efficiency of 61.3% for *C. macrostachys* and 59.4% for *P. falcatus* was relatively larger than in a study by Högberg *et al.* (2008), who reported that 40% of the tracer was taken at $^{13}CO_2$ pulse labeled experiment on 4 m tall boreal *Pinus sylvestris* forest. Losses of ^{13}C from leaves were fast, and about 50% of the labeled material was lost from the leaves within the first 24 hours in case of *C. macrostachys* and within 72 hours in case of *P. falcatus*. These values were in accordance with the findings of Högberg *et al.* (2008) who reported that within 48 h after pulse initiation more than 50% of the tracer was lost from the needles. The shape of the ^{13}C recovery in the foliage was fitted with a double exponential decay function (fast and slow decay components). The size of the fast pool was larger for *C. macrostachys* compared to *P. falcatus* with a mean residence time (MRT) of the label in this pool almost 4 times shorter in case of the former.

The composition of the water soluble non-structural carbohydrates in the phloem sap differed between the two tree species. In *C. macrostachys* sucrose represented the major soluble product, accounting up to 70%. In addition to soluble di- and monosaccharides, the phloem sap of *P. falcatus* contained the cyclic sugar alcohol D-1-*O*-methyl-*muco*-inositol (OMMI), representing about 42% of the overall sugars found. The natural abundance of phloem sap sugars was -25.53±0.87‰ for *C. macrostachys* and -25.36±0.33‰ for *P. falcatus*. The time between labeling and the appearance of ^{13}C-labeled compounds in phloem sap at a height 1.3 m (diameter at breast height, DBH) was found to be shorter for *C. macrostachys* (4 to 8 h after labeling) than for *P. falcatus* and peaked (2250‰) in the following 40 hours. For *P. falcatus*, the tracer in phloem sap at DBH appeared 12 h after pulse labeling and peaked between 72 and 96 h (965‰) after the labeling. Such time lag reported of about 8-12 h was found within the range of other studies (Zimmermann and Braun, 1971; Högberg *et al.*, 2008). Usually, because

of highly differentiated sieve tube, phloem transport rate in angiosperms is faster than in conifers trees (Kozlowski, 1992).

4.4. ^{13}C recovery in AMF and soil CO_2 efflux (Study III)

With phloem sap the ^{13}C label was transported to the roots, from where it was partly delivered to the fungal symbiont. Biomarker NLFA 16:1ω5 was considered to trace the flux of the label through AM fungi (Table A.2). The natural abundance of biomarker NLFA 16:1ω5 from the reference trees was -28.96±0.64‰ for *C. macrostachys* and -27.70±0.72‰ for *P. falcatus*. For *C. macrostachys* the label was first found in AMF fatty acid biomarkers 48 h after pulse labelling and peaked in the next 8 days. For *P. falcatus*, the labeled material appeared in biomarker NLFA 16:1ω5 initially 8 days after pulse labeling. In both cases adhering soil showed larger ^{13}C enrichment than bulk soil samples.

Enrichment of ^{13}C in soil CO_2 efflux was strongly temporally synchronized with phloem transport. Under *C. macrostachys*, ^{13}C enrichment was initially observed in soil CO_2 efflux after a time lag of 2 days following the labeling. Under *P. falcatus* the first evidence of the tracer occurred around 3 days after the labeling. There was also variation in the maximum values and timing of ^{13}C enrichment between the two tree species. The labeled material peaked in soil CO_2 efflux 3 days after pulse labeling in case of *C. macrostachys* (117.2‰±11.96), whereas it took 8 days in case of *P. falcatus* (-3.9±1.4‰). The temporal pattern of soil respiration showed that most of the ^{13}C lost from the plant-soil system as soil CO_2 efflux occurred during the first 3 to 8 days after pulse labeling, and roughly followed the time course of ^{13}C in the AM marker at both tree species. In both trees, the $\delta^{13}C$ values in soil CO_2 efflux exponentially depleted with time ($r^2 = 0.95$ for *C. macrostachys* and $r^2 = 0.93$ for *P. falcatus*), being faster *for C. macrostachys*. The isotope ratio of CO_2 samples under both labeled trees almost returned to natural abundance values after one year of chasing periods (-26.9±0.6‰ and -27.4±0.7‰ for *C. macrostachys* and *P. falcatus*, respectively). After one year, about twice the amount of the assimilated labeled were found in soil CO_2 efflux under *C. macrostachys* as compared to *P. falcatus* (32% versus 15%) (Fig. S.5).

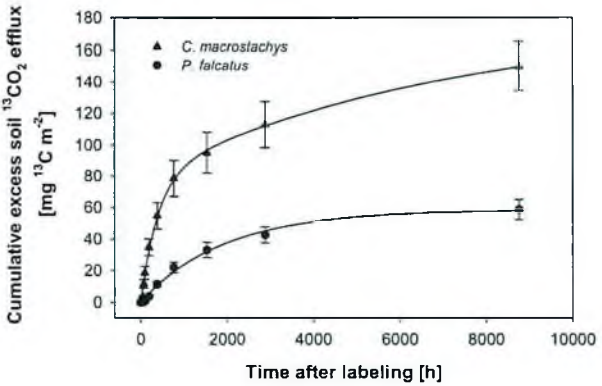

Fig. S.5 : Time course of cumulative excess of ^{13}C in soil CO_2 efflux under C*roton macrostachys* and *Podocarpus falcatus* during the one year chasing period. Data are means ± standard deviation (n=5). The curves are fitted with a double exponential function for *C. macrostachys* and a single exponential function for *P. falcatus*. Parameters are shown in Table 3.2.

As was shown before for trees of boreal (Ekblad and Högberg, 2001; Högberg *et al.*, 2008; Subke *et al.*, 2009) temperate forests (Plain *et al.*, 2009; Kuptz *et al.*, 2011), also for the two investigated trees in the Ethiopian Afromontane forest, there is 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. With that the study supports recent findings showing a close temporal coupling between tree canopy photosynthesis and belowground activity. Further, the different functional traits of the two tree species are also reflected in their carbon allocation. The transport of recently assimilated carbon from the tree canopy to soil CO_2 efflux in case of the angiosperm pioneer *C. macrostachys* was faster as for the gymnosperm late-successional *P. falcatus*. The larger loss of recently assimilated carbon as soil CO_2 efflux in case of pioneer is probably due to the large energy investment into the AMF to acquire nutrients. In consequence, more carbon is pumped belowground that is turned over faster in soil. Conversely, in case of *P. falcatus* more of the recently assimilated carbon stays in the plant-soil system, presumably as structural carbon components in above and below-ground biomass and more slowly turning over soil organic matter.

5. Conclusions

The two years of data discussed in this study indicated that, soil CO_2 efflux from soil to the atmosphere in the Munessa forest is primarily controlled by soil moisture and thus, the rainfall pattern. This in turn will have an impact on the whole carbon balance of this ecosystem. This study pointed out that forest ecosystem dynamics and forest management practice are also an important factor determining soil CO_2 efflux magnitude and variability.

This study also demonstrated that coupling of *in situ* ^{13}C pulse labeling and PLFA analysis is a useful tool to obtaining information on the carbon flow in plant-soil-atmosphere system. The study has shown clearly that the carbon allocation pattern reflects differences between the tree species. As the continuing forest degradation in the Munessa forest leads to the formation of more canopy gaps, the shift in relative abundance towards the angiosperm is proceeding. This more likely leads to decrease of tree biomass accumulation and acceleration of the carbon exchange in the tree-soil-atmosphere system.

Overall a subtle picture emerges on how the belowground carbon cycling is controlled by interactive biotic and abiotic factors. Hence, both direct human impact and climate change may possibly impact soil CO_2 efflux and with that the whole carbon balance of this ecosystem. In the native forest, both the continuing degradation together with warmer temperatures and more erratic precipitation pattern may favor the pioneer species *C. macrostachys*. Here, my data showed that such shift in tree species composition may accelerate processes of carbon allocation belowground and soil respiration with a loss of organic matter on the long term. On the other hand, a more positive picture emerged from the forest plantation. Though the investigated management intervention, designed to increase timber production, has led to increased carbon losses from soil as CO_2, this appeared to be more than offset by larger carbon input to the soil due to higher productivity.

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IMPACT OF TREE SPECIES AND FOREST MANAGEMENT ON CARBON CYCLING IN THE PLANT- SOIL-ATMOSPHERE SYSTEM

CUMULATIVE STUDIES

Study 1:

Soil CO_2 efflux in an Afromontane forest of Ethiopia as driven by seasonality and tree species

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Participation in the conception of the study, performance of the field work, performance of the laboratory work, leadership in data analysis and writing.

Abstract

Variability of soil CO_2 efflux strongly depends on soil temperature, soil moisture and plant phenology. Separating the effects of these factors is critical to understand the belowground carbon dynamics of forest ecosystem. In Ethiopia with its unreliable seasonal rainfall, variability of soil CO_2 efflux may be particularly associated with seasonal variation. In this study, soil respiration was measured in nine plots under the canopies of three indigenous trees (*Croton macrostachys*, *Podocarpus falcatus* and *Prunus africana*) growing in an Afromontane forest of south-eastern Ethiopia. Our objectives were to investigate seasonal and diurnal variation in soil CO_2 flux rate as a function of soil temperature and soil moisture, and to investigate the impact of tree species composition on soil respiration. Results showed that soil respiration displayed strong seasonal patterns, being lower during dry periods and higher during wet periods. The dependence of soil respiration on soil moisture under the three tree species explained about 50% of the seasonal variability. The relation followed a Gaussian function, and indicated a decrease in soil respiration at soil volumetric water contents exceeding a threshold of about 30%. Under more moist conditions soil respiration is tentatively limited by low oxygen supply. On a diurnal basis temperature dependency was observed, but not during dry periods when plant and soil microbial activities were restrained by moisture deficiency. Tree species influenced soil respiration, and there was a significant interaction effect of tree species and soil moisture on soil CO_2 efflux variability. During wet (and cloudy) period, when shade tolerant late successional *P. falcatus* is having a physiological advantage, soil respiration under this tree species exceeded that under the other two species. In contrast, soil CO_2 efflux rates under light demanding pioneer *C. macrostachys* appeared to be least sensitive to dry (but sunny) conditions. This is probably related to the relatively higher carbon assimilation rates and associated root respiration. We conclude that besides the anticipated changes in precipitation pattern in Ethiopia any anthropogenic disturbance fostering the pioneer species may alter the future ecosystem carbon balance by its impact on soil respiration.

Keywords: *Croton macrostachys*, Ethiopia, *Podocarpus falcatus*, *Prunus africana*, soil CO_2 efflux, soil moisture, soil respiration, soil temperature, tropical forest

1. Introduction

Despite of the general concern that tropical forests, and particularly intact African forests may play an important role as a carbon sink in the global terrestrial carbon cycle (Ciais et al., 2009; Lewis et al., 2009; Stephens et al., 2007), the size of the carbon stocks and the carbon fluxes in these ecosystems still remain highly uncertain, especially with respect to potential effects of climate change and anthropogenic disturbances.

Soil CO_2 efflux is one of the largest components in the terrestrial carbon budget (Raich and Schlesinger, 1992), with the global magnitude in the order of 98 Pg C per year (Bond-Lamberty and Thomson, 2010). Contributing more than 50% to the ecosystem respiration across variety of biomes (Janssens et al., 2001; Schlesinger, 1997; Shibistova et al., 2002), soil respiration determines the ecosystem carbon balance and thus an ecosystem sink or source activity (Valentini et al., 2000). Yet to date there has only been a limited amount of data available on soil efflux and its abiotic and biotic determinants for Eastern Africa (Werner et al., 2007) and, likewise, comparative studies across major ecosystems are scarce (Bahn et al., 2010).

Among the climatic conditions controlling soil respiration in different ecosystems on hourly, weekly and seasonally scale, soil temperature and soil moisture are considered to be the two most influential parameters (Davidson et al., 1998; Reichstein et al., 2003; Rustad et al., 2000). While the relationship between soil CO_2 efflux and soil temperature often is described by a simple exponential function (Davidson et al., 2000; Lloyd and Taylor, 1994; Qi et al., 2002), there is no common agreement on the type of relation between soil moisture and soil respiration. For example, working in an old-growth neotropical forest in Costa Rica, Schwendenmann et al. (2003) found a parabolic relation, while Kosugi et al. (2007), working in a Southeast Asian lowland rain forest, identified a linear dependence of soil CO_2 efflux on soil moisture. Epron et al. (2004) used an exponential regression to fit a relation between soil water content and the seasonal variability of soil respiration. Strong effect of soil moisture on temporal pattern of soil respiration has been shown in some tropical forests and plantations (e.g., Epron et al., 2004; Hashimoto et al., 2004; Kosugi et al., 2007, Ohashi et al., 2008), but just a weak (Salimon et al., 2004) or even no correlation (Adachi et al, 2006) between soil temperature and soil respiration in primary and secondary forests has been reported. In a tropical plantation of New French Guiana, Bréchet et al. (2009) showed no significant relationship between soil respiration and both, soil temperature and soil moisture.

Spatial variability of soil CO_2 efflux is primarily affected by biotic factors. So Bréchet et al. (2009) stated that variability of soil respiration was mainly explained by leaf litterfall. Oscar (2007) emphasised the role of different tree species and noted that their different fine root production explained much of the spatial variability in soil CO_2 efflux. Leaf and total aboveground litter (leaf, bark and woody debris) have also been reported to have an effect on soil respiration (Epron et al., 2004).

In this context, long term measurements of soil CO_2 efflux with concurrent climatic record are needed to understand the influence of abiotic and biotic drivers on the magnitude of soil CO_2 efflux. Here, we report on seasonal and diurnal pattern of soil respiration measured underneath a natural mixed evergreen-deciduous Afromontane forest, growing in Ethiopia, East Africa. The Munessa Shashemene forest is one of the largest Afromontane forests in the country. The soil organic carbon pool (0-60 cm depth) of the forest is appreciably high (134 Mg ha^{-1}) and is within the range of similar ecosystems (Lemenih and Itanna, 2004). We carried out our studies under three different tree species: *Croton macrostachys* Hochst, *Podocarpus falcatus* (Thumb.) R.BR.ex and *Prunus africana* (Hook. F.) Kalkman. These tree species differ in a variety of traits, such as crown architecture (Bekele-Tessema et al., 1993), depth and distribution of roots (Fritzsche et al., 2006), and CO_2 assimilation rate and water relation (Fetene and Beck, 2004; Lüttge et al., 2003). The tree species also represent different functional types according to Whitmore (1989). *Croton macrostachys* is a pioneer deciduous tree, while *P. falcatus* and *P. africana* are late successional evergreen trees, the first being coniferous and the second broadleaf. *Podocarpus falcatus* and *P. africana* are among tree species that are locally threatened by extinction as a result of illegal cutting and encroachment. Such activities created gaps that favour the abundance of the pioneer tree species *C. macrostachys* and diverse shrubs at the expense of climax species. This anthropogenically driven change in relative abundance of tree species may bring modifications in the stand-level carbon balance.

The objective of the present study was, first, to analyze seasonal and diurnal variation in soil CO_2 flux rate as a function of soil temperature and soil moisture. Since Ethiopia is characterized by a strong seasonality of precipitation, we assume soil moisture as the major abiotic driving factor. As a second objective, we were addressing the impact of different functional-type forest tree species on soil CO_2 efflux. Here, we hypothesised that soil CO_2

efflux under deciduous pioneer *C. macrostachys* shows a larger seasonal variability than that under the late successional tree species.

2. Materials and Methods

2.1. Study site

The Munessa-Shashemene forest is located in Oromia Regional State on the eastern escarpment of the southern Main Ethiopian Rift Valley, about 240 km south of Addis Ababa. The forest area, 23.000 ha in size, is divided into three blocks; namely Gambo, Sole and Degaga. For the present study, the experimental plots were established in the Degaga block (07° 25' 51'' N and 038° 51' 52'' E). The elevation is at 2266 to 2279 m above sea level. Mean annual temperature is 15°C and mean annual precipitation is about 1200 (data from Ethiopian Meteorological Agency at Degaga town, 07° 26' 00'' N and 038° 50' 26'' E). The study site is located at the central area of the country, with a minor rainy season occurring from March to May and a major rainy season from July to November (Griffits, 1972). Own meteorological records since 2001 show that 80% of the annual precipitation fell in the major rainy season from July to November, and no clear indication of a minor rainy season can be made (Strobl et al., unpublished). Further, the monthly precipitation pattern outside the major rain season varied considerably e.g. in the years 2008 and 2009 (Fig. 1.1). Total annual precipitation in both years was similar, with 1133 mm in 2008 and 1036 mm in 2009. Mean annual air temperature corresponded well to the average value, with 14.7°C in 2008 and 15.3°C in 2009.

Soils of the study area are rich in clay evolved from volcanic parent material (Fritzsche et al., 2007). At the experimental plots they were classified as Mollic Nitisols according to the WRB system (FAO, 1998). Vegetation of the natural forest is dominated by the canopy species *P. falcatus* and *C. macrostachys*. Other plant species with relatively less abundance include *P. africana, Syzygium guineense* (Wild.) DC., *Celtis africana* Burm. f. and *Pouteria adolfi-friederici* (Engl.). The forest is strongly degraded by grazing and illegal logging activities leading to its transformation from a primary to secondary forest.

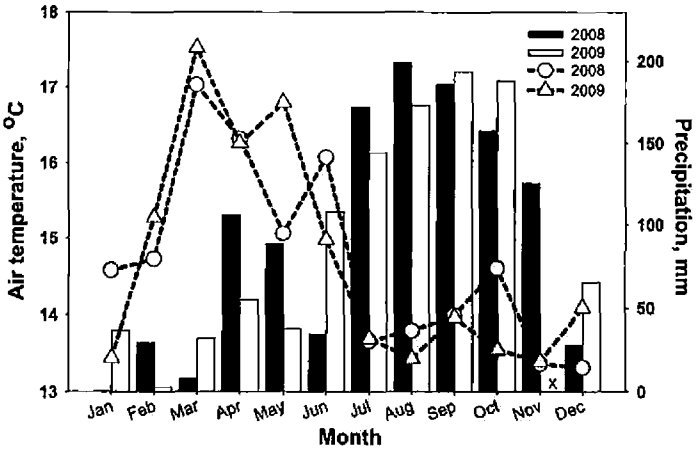

Fig. 1.1: Monthly total precipitation (*bar graph*) and mean monthly air temperature (*line graph*) for the year 2008 and 2009 measured at the Kuke field station being adjacent to the experimental plots (data from Strobl et al., unpublished). x shows missed data in November 2009 due to rain gauge failure.

2.2. Experimental setup and soil CO_2 efflux measurement

Since a major goal of our study was to assess the temporal and spatial variability of the soil CO_2 efflux with respect to the interrelationship of tree species with abiotic factors, the study was carried out on an individual tree basis. In June 2008, for each of the three tree species, *C. macrostachys, P falcatus* and *P. africana,* three individual juvenile trees of the third height class (Tesfaye et al., 2010) were selected. In total, nine experimental plots were established, with three replicates for each tree species within a distance of about 100 m. For characterization of the soils underneath the canopies, soil samples were collected from each plot before the start of soil CO_2 efflux measurement campaign. Core soil samples (cylindrical steel core with 4.0 cm diameter) were obtained separately in triplicate from 0–10 and 10–25 cm depths under the canopy of each experimental tree. Soil samples collected under the canopies of same tree species were bulked. According to Table 1.1, the soil under *C. macrostachys* tends to have a higher pH, appears to be richer in organic carbon and nitrogen, and has a larger cation exchange capacity and more available phosphorus than that under the other tree species. The better soil nutritional status under *C. macrostachys* is likely due to the larger nutrients content in its leaves as compared to the other two tree species (Zech, unpublished). With litter fall, the nutrients are returned to the topsoil.

Table 1.1: Basic characteristic of the soils under the studied trees

Tree/ Depth[1]	Sand*	Silt*	Clay*	C‡	N‡	pH§	CEC¶	BS[2]	P_{avail}††
	—— g kg^{-1} ——			– g kg^{-1} –			$mmol_{(+)}$	%	mg kg^{-1}
C. macrostachys									
0-10	211±6	379±20	410±26	138±20	11.7±0.	6.7±0.0	732±24	100	26±8.5
10-25	219±2	301±24	480±25	64±8	5.9±0.5	6.5±0.4	498±63	100	9.5±5.1
P. falcatus									
0-10	180±3	409±20	411±17	116±11	11.5±0.	6.5±0.1	565±55	100	15±2.4
10-25	214±4	304±20	482±24	66±13	6.5±0.8	6.2±0.2	376±42	100	8.6±2.4
P. africana									
0-10	195±5	405±37	400±32	122±9	12.2±0.	6.6±0.1	640±45	100	16±3.0
10-25	222±8	319±27	459±36	80±3	8.2±0.3	6.5±0.1	497±49	100	8.6±0.3

[1] in cm; * measured by the pipette method (Gee and Bauder, 1986); ‡ total carbon and nitrogen measured by dry combustion (Elementar Vario EL, Hanau, Germany); § analyzed potentiometrically in 1 M KCl [1:2.5 (m/v)]; ¶ cation exchange capacity determined with the $BaCl_2$ compulsive exchange method (Gillman and Sumpter, 1986); [2] base saturation; †† available phosphorus measured after extraction with Bray I (Bray and Kurtz, 1945). Values are mean and standard deviation of three replicated samples.

To assess the seasonal and diurnal variation of soil CO_2 efflux, five permanent soil collars (20 cm in diameter and 5 cm long) made from PVC were inserted in the soil at randomly selected positions underneath the canopy of each individual tree. To minimize any influence of mechanical disturbance of soil surface on diffusion rates, and to avoid cutting of fine roots, the soil collars were inserted into the soil not more than 2 cm. The collars were sealed at the outside with fine sand. Herbaceous understory vegetation was avoided during collar set-up. However, when, in the following two years period, any vegetation grew inside the collars, it was clipped back. Once inserted, soil collars were left in place throughout the measurement period.

Total CO_2 from the forest floor was measured using an Infrared Gas Analyzer (Li-8100, LI-COR, Lincoln, NE, USA) supplied by a LI-8100-103 Soil Survey Chamber. For each measurement, the soil respiration chamber was placed on each collar, and CO_2 flux rate was automatically calculated from exponential regression of increasing CO_2 concentration over the 2–3 min following chamber equilibration. For each of the tree species mean CO_2 efflux rates were calculated from the 15 chamber measurements obtained during individual sampling events. The measurements began on July 11, 2008 and have been carried out on a weekly basis

between 12:00 h and 15:00 h until July 24, 2010 with the exception of October-November, 2009 due to an instrument failure. No measurements were also taken during or immediately following rainfall. Diurnal soil CO_2 efflux measurements were performed over a 24 h period at a 4 h interval. Three days representing different soil moisture categories were selected: 03 July 2008 (wet season); 06 December 2008 (transition from wet to dry season) and 15 March 2009 (dry season).

Soil temperature (°C) at a depth of 0.1 m was monitored within 10–20 cm distance of each collar.simultaneously with soil CO_2 efflux measurements using a thermocouple probe (Li-8100-201) connected to the Li-8100. The volumetric soil water content at 0.06 m depth was measured adjacent to each PVC collar with a theta probe (ML2, Delta-T Device Ltd, Cambridge, UK) at three replicates around the collars (the data is available from. May 30, 2009).

At a representative place between the plots air temperature underneath the forest canopy, canopy precipitation and soil moisture data were obtained at a weather station. Temperature was measured continuously using 8-bit temperature sensor (Onset Computer Corporation, Bourne, Massachusetts), while bulk precipitation was measured on weekly basis using five randomly distributed polyethylene funnels with a 120 mm upper diameter. Volumetric soil water content was recorded using frequency-domain reflectometer (ECHO probe, Decagon Devices Inc., Pullman, WA) probe installed at 0.1 m depth.

2.3. Data analysis

We separated dry and wet periods following the approaches of Gibbs and Maher (1967), where the distribution of precipitation events over a long-term record is divided into sections for each ten percent of the distribution. Such rainfall deciles were calculated from ten years historical rainfall data (1998 to 2007) obtained from a nearby metrological station (Degaga town 07° 26' 00'' N and 038° 50' 26'' E). By definition, the fifth decile is the median, and it is near normal classification of wet and dry periods. According to Gibbs and Maher (1967), we used the fifth decile range as a cut-off for dry and wet period classification. Based on that months with more than 55.2 mm rainfall were defined as wet periods and the drier periods are months that received less than 55.2 mm rainfall. This categorized December 2008 to May 2009, December 2009 and January 2010, and March 2010 as dry months while the other months belonged to the wet seasons.

To account for changes in the environmental variables, soil moisture and soil temperature and their interactions with the tree species over the course of the study periods were analysed with regression approaches to analysis of variance (ANOVA). A general linear model was fitted with soil CO_2 efflux rate as response, and tree species and season (wet *vs.* dry) as categorical explanatory variables. Soil moisture and soil temperature were fitted into the model as numerical covariates. The model terms were fitted sequentially and ANOVA (Type I sums of squares) was computed leading to F-tests for the main effects and interactions. We fitted the covariates sequentially in the order soil moisture then soil temperature. Homoscedastic residuals were obtained with untransformed values. Statistical differences were significant if the probability of type I error was less than 0.05. On the other hand, to analyze the interannual soil CO_2 variability among and between the tree species, we averaged soil CO_2 efflux data over the wet and dry periods for both years of observations. Then the data set was analyzed using a three-way ANOVA, with factor effects (Tree species, years of observation (first year *vs.* second year), and season (wet *vs.* dry). When ANOVA results indicated significant difference, Tukey's HSD test was performed.

The data for all measurements with both, soil moisture and soil temperature values available from 30 May 2009 to the end of the experiment, were analysed using nonlinear regression. The objective of the analysis was to describe the dependence of soil respiration on soil moisture alone and jointly with soil temperature under the three tree species separately. Models were fit using non linear least squares method in R 2-11.0 (R Development Core Team, 2009). The performances of the equation were evaluated by goodness-of-fit measures (Root Mean Square Error (RMSE), and r^2). Since in preliminary graphical analysis, the non-linear relation between soil respiration and soil moisture was prominent, while a potential dependency of soil respiration on soil temperature was only weak, we initially modelled the dependency of respiration on soil moisture by different nonlinear models. The Gaussian model was fitting best for all three species both in terms of maximal r^2 values and fitting the downturn of soil respiration rates for high soil moisture values. After fitting the first part, different models for including the effect of soil temperature were added: a linear regression, a linear regression including an interaction term for temperature and soil moisture. After that the reduction of the residual sums of squares (at costs of additional parameters) were tested using F-tests.

$$SR = a*\exp(-0.5*((SM-x_0)/b)^2) \quad \text{(Equation 1)}$$

$$SR = a*\exp(-0.5*((SM-x_0)/b)^2) + c*ST \quad \text{(Equation 2)}$$

$$SR = a*\exp(-0.5*((SM-x_0)/b)^2) + c*ST + d*ST*SM \quad \text{(Equation 3)}$$

where SR is soil respiration (μmol m^{-2} s^{-1}), SM is soil moisture (%), ST is soil temperature (°C) and a, b, c, d, and x_0 are fitted parameters.

Annual soil CO_2 efflux was estimated by extrapolating each weekly measurement to a 7-day period. Where there were missing data due to rainfall incidence or failure of instrument, soil CO_2 efflux was estimated based on the Gaussian relation of soil moisture and soil temperature with soil CO_2 efflux for the different tree species shown in Table 1.3. Since the linear relation between the volumetric soil water content measured at the weather station and underneath the individual tree species was close ($r^2 = 0.94$ for *C. macrostachys*; $r^2 = 0.93$ for *P. falcatus*; $r^2 = 0.93$ for *P. africana*), the former was used to simulate the latter in cases of gaps. All graphing and statistical analysis was performed using R 2-11.0 or SigmaPlot version 11 (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Seasonal variability of soil CO_2 efflux

Soil CO_2 efflux rates varied between 2 and 7 μmol m^{-2} s^{-1} (Fig.1.2). In general, the respiration rates followed changes in the precipitation and the resulting changes in the volumetric soil water content, which varied considerably between 8% and 39%, being on average 16% and 28% for dry and wet months, respectively. So during rainy periods from July to November 2008, from July to November 2009 and from May to July 2010 (end of observation period) soil CO_2 efflux rates mostly exceeded 4 μmol m^{-2} s^{-1}. Drying out of the soil with the onset of dry periods in December 2008 and December 2009 resulted in a continuous decline in the soil respiration rates. Remarkable is the pronounced response of the soil CO_2 efflux on individual rain events during or at the end of a dry period, e.g. in January or April 2009. Table 1.2 displays the net effect of tree species, season, the covariates soil moisture and soil temperature, and their interaction on the dependent variable soil CO_2 efflux. The ANOVA shows that seasonal changes exerted the strongest influence on soil CO_2 efflux in the Munessa forest, followed by the covariate soil moisture. Seasonality accounted for 38% of variability, with F

(1, 89) = 354.98, $p < 0.001$, indicating significant difference in the response variable soil CO_2 efflux in wet and dry periods. Soil moisture explained 22% of the overall variances with F (1, 89) = 198.93, $p < 0.001$. There was also a significant interaction effect of soil moisture and seasonal variability on soil CO_2 efflux with F (1, 89) = 73.62, $p < 0.001$.

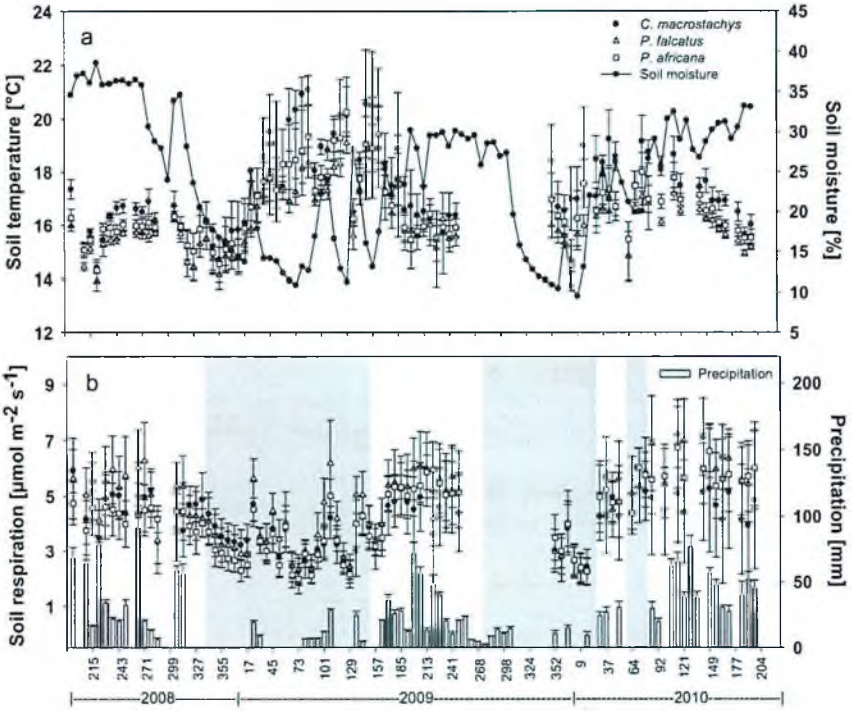

Fig. 1.2: Seasonal courses of daily mean volumetric soil water content and soil temperature under the canopy (a), weekly canopy precipitation and soil CO_2 efflux rate measured under three tree species (b). Each data point for soil respiration and soil temperature is a mean of fifteen measurements and for precipitation is a mean of five measurements. *Error bars* indicate standard deviation. Data gaps are due to rain events or instrument failure. Periods with light grey background indicate dry periods as calculated by the approaches of Gibbs and Maher (1967).

Table 1.2: Analysis of variance for the net effect of explanatory variable tree species, season, and covariates (soil moisture and soil temperature), and their interaction

Effect	df	SS	(% SS)*	F	*p*-value
Tree	2	12.970	8.35	38.516	< 0.001
Season	1	59.767	38.49	354.982	< 0.001
SM	1	33.494	21.57	198.937	< 0.001
ST	1	6.531	4.21	38.792	< 0.001
Tree:Season	1	1.844	1.18	5.476	0.005
*Tree*SM*	2	5.488	3.53	16.298	< 0.001
*Tree*ST*	2	0.332	0.21	0.984	0.3776
Season:SM	1	0.332	7.98	73.622	< 0.001
Season:ST	1	12.395	3.49	32.387	< 0.001
*SM*ST*	1	2.017	1.29	11.981	< 0.001
Residual	89	14.985			

* % SS indicate increases in multiple r^2 (explained variance) due to the addition of this term. df, degree of freedom; SS, sum of square; *SM*, soil moisture; *ST*, soil temperature.

Using the data set available from 30 May 2009, we assessed the dependence of soil CO_2 efflux rate on soil moisture individually under the three different tree species. Under all three tree species soil respiration rates steadily increased with increasing volumetric soil water content threshold level (Fig. 1.3).

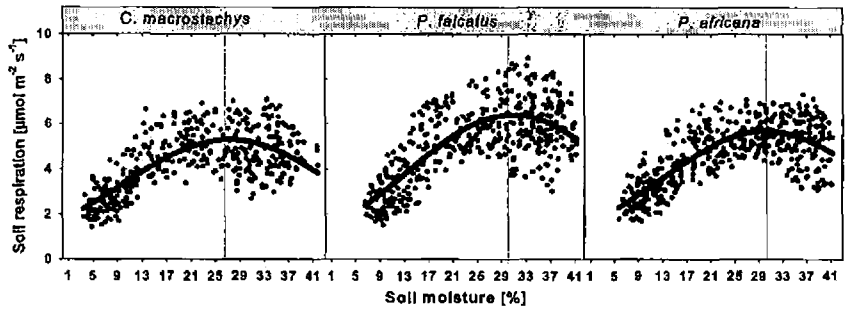

Fig. 1.3: Relationship between soil CO_2 efflux rate (*SR*) and volumetric soil water content (*SM*) at 0.06 m soil depth. The parameters of the Gaussian function (Equation 1) are shown in Table 1.3.

After exceeding the threshold, soil respiration decreased with increasing volumetric soil water content. The Gaussian Equation (1) shows that the effect of soil moisture had a significant ($p <$

0.05) relationship with soil CO_2 efflux that explained about 50% for *C. macrostachys*, 56% for *P. falcatus*, and 58% for *P. africana* (Table 1.3).

In contrast to soil moisture, soil temperature at 0.1 m soil depth did not vary much, ranging from about 15°C to 20°C. The higher soil temperatures were recorded during the dry periods (Fig.1.2). The influence of soil temperature on the overall variation of soil CO_2 efflux was smaller as compared with that of soil moisture and tree species. Considering soil temperature as covariate and incorporated in the general linear model, it explains only 4% of the variability of soil CO_2 efflux (Table 1.2). This is also mirrored by Fig. 1.4, showing a high degree of scatter between soil temperature and soil respiration.

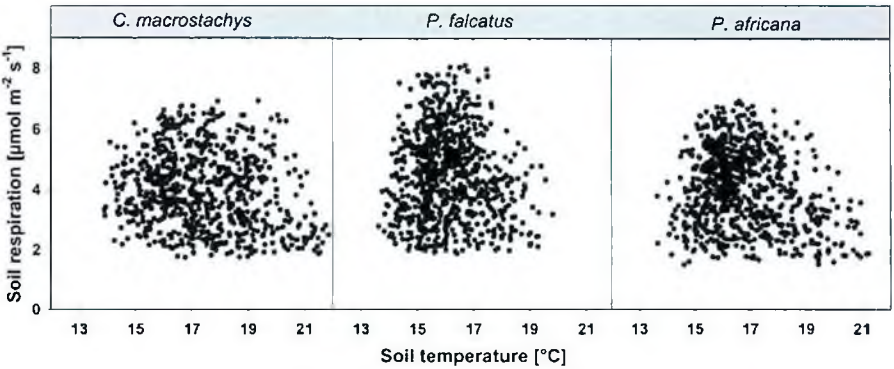

Fig. 1.4: Relationship between soil CO_2 efflux rate (*SR*) and soil temperature (*ST*) at 0.1 m soil depth.

We also used the same data set to show the joint effects of soil moisture and soil temperature on soil CO_2 efflux rate. The inclusion of the soil temperature in Equation (1) increased the r^2 values only weakly and slightly reduced the RMSE (Table 1.3).

Table 1.3: Comparative values of regression coefficients, r^2 and RMSE using the Gaussian and a linear regression, a linear regression including an interaction term for soil temperature and soil moisture

Parameter	*C. macrostachys*			*P. falcatus*			*P. africana*		
	Eq. 1	Eq. 2	Eq. 3	Eq. 1	Eq. 2	Eq. 3	Eq. 1	Eq. 2	Eq. 3
a	5.29	4.84	2.34	6.42	7.32	5.82	5.67	4.91	3.23
b	18.14	11.71	9.88	17.27	18.81	17.90	18.26	16.48	14.31
c	-	0.02	0.03	-	-0.05	-0.137	-	0.05	0.012
d	-	-	0.006	-	-	0.006	-	-	0.0042
x_o	27.27	27.45	18.32	30.60	30.65	24.33	30.28	30.44	24.29
pC	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
pLinear	-	0.3245	-	-	0.1683	-	-	0.0811	
pLT_T:M	-	-	< 0.0001	-	-	0.056	-	-	0.002
pInteraction	-	-	< 0.0001	-	-	0.050	-	-	0.002
r^2	0.4975	0.4975	0.5317	0.556	0.558	0.562	0.574	0.577	0.585
RMSE	0.931	0.900	0.900	1.137	1.136	1.132	0.866	0.864	0.856

Eq. 1:SR = a*exp(-0.5*((SM-x_0)/b)^2), Eq. 2:SR = a*exp(-0.5*((SM- x_0)/b)^2) + c*ST and Eq. 3: SR = a*exp(-0.5*((SM-x_0)/b)^2) + c*ST + d*ST*SM, where a, b, c, d and x_0 are the fitted parameters; r^2 is the correlation coefficient, SM is volumetric soil moisture content in %, SR is soil respiration in µmol m^{-2} s^{-1} and ST is soil temperature in °C, pC (p value of complete model), pLinear (test which compares the additional effect of the linear regression of ST relative to the previous model including the Gaussian model for SM. pLT_T:M (p-value for adding a linear trend and a linear interaction for ST and SM to the Gaussian model fitted with SM. pInteraction (p-value for adding just a linear interaction term ST:SM to the Gaussian model with linear regression for SM.

The intercepts of the models with the linear trend of soil temperature terms were slightly lower than those with just soil moisture (Table 1.3). However, the *p*Linear test, which compares the additional effects of the linear regression (soil temperature) relative to the previous Gaussian model (soil moisture term in Equation (1), was not significant (Table 1.3). Inclusion of the joint effects of the linear trend depended on soil temperature and the interaction of soil moisture and soil temperature in Equation (1) brought differences for *C. macrostachys* ($p < 0.001$) and *P. africana* ($p = 0.002$). The small difference in r^2 and the RMSE brought by adding a soil temperature and soil moisture interaction (*ST*SM*) term in Equation (2), indicates that the interaction term was more influential than soil temperature alone.

3.2. Diurnal variability of soil CO_2 efflux

The volumetric soil water contents in the days representing wet, transition and dry conditions were 26%, 19% and 11% respectively. The diurnal pattern of the soil temperature was similar for the different seasons, with minima in early morning and maxima in afternoon (not shown). The lowest mean daily soil temperature was recorded under all trees in December, and there were no significant differences in soil temperature for *P. falcatus* and *P. africana* plots between dry and wet seasons (Fig. 1.5).

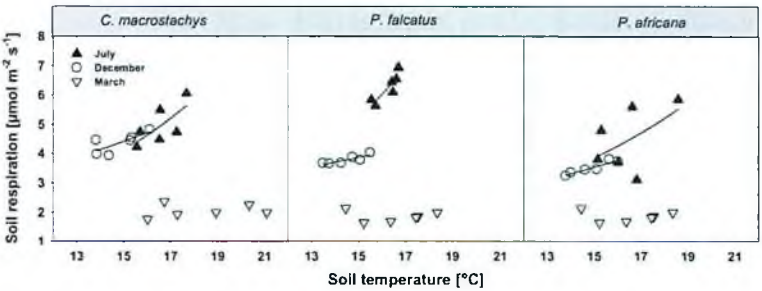

Fig. 1.5: Diurnal dependence of soil CO_2 efflux rate (*SR*) on soil temperature (*ST*) at 0.1 m soil depth. Each data point is the mean of fifteen measurements made per sampling hour. For July 03, 2008 and December 06, 2008 measurements, a single exponential function with $SR = a*\exp^{b*ST}$ was used to describe the relationships between both parameters; a and b are fitted parameters.

Under *C. macrostachys*, the soil temperatures were higher as compared to other plots due to the less dense canopy and showed maxima during the dry and sunny March term. The season was exerting a strong influence on the soil CO_2 efflux rates. Largest values were obtained in the July measurement, representing the wet period and optimal soil moisture, while in March

the smallest diurnal CO_2 efflux rates were obtained. The impact of the season on temperature dependency of the diurnal CO_2 efflux is also reflected by the Q_{10} values calculated from the exponential function in Figure 1.5 (i.e., $\exp^{10b}$). Q_{10} values of 3.2 for *C. macrostachys*, 4.5 for *P. falcatus*, and 2.7 for *P. africana* during the wet season indicated a higher temperature sensitivity of soil respiration than respective Q_{10} values of 2.0, 1.6, and 2.0 in the transition season. In contrast to wet and transition seasons where the pattern of the soil CO_2 efflux rates followed the diurnal soil temperature fluctuations, no diurnal temperature variation in soil CO_2 efflux rates was observed for all the plots in the dry period.

3.3. Tree species variability in soil CO_2 efflux

It has been shown that the tree species was the third significant factor affecting soil CO_2 efflux variability $F(2, 89) = 38.51$, $p < 0.001$ (Table 1.2). Tree species contributed with 8% to the overall variance. Further, the significant interaction effects of tree species and soil moisture ($p < 0.001$) indicates that the change in soil CO_2 efflux rate as a function of soil moisture was not uniform under the different tree species. This is also obvious from the Gaussian functions (Table 1.3). The threshold level when higher soil moisture leads to a decline of the soil CO_2 efflux decreased in the order *P. falcatus* (volumetric soil water content 31%), *P. africana* (30%), *C. macrostachys* (27%). Likewise, absolute largest soil respiration rates at optimum soil moisture was observed under *P. falcatus* (Fig. 1.3). In contrast, it appears that the decrease in soil CO_2 efflux with decreasing soil moisture was least for *C. macrostachys* followed by *P. africana*, while *P. falcatus* exhibited the steepest decline in soil respiration with drying out of the soil. This result is mirrored by the three-way ANOVA showing that soil respiration did vary significantly ($p < 0.05$) between the tree species during wet periods (Fig. 1.6). In both years under study, the mean soil respiration rate during the wet period was highest under *P. falcatus*. In contrast, there were no significant differences ($p > 0.05$) in the soil CO_2 efflux rate under the three tree species during the dry season.

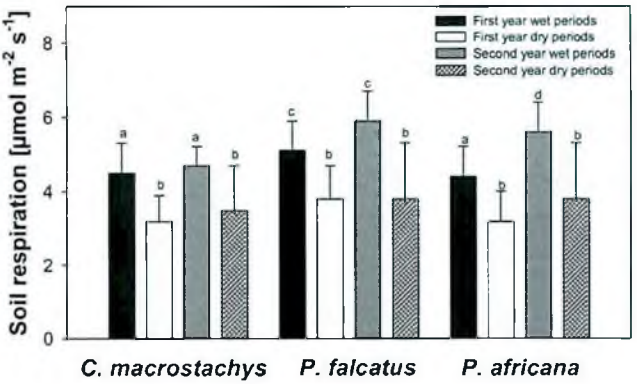

Fig. 1.6: Comparison of soil CO_2 efflux between wet and dry periods for both years of observation (July 11, 2008 to July 10, 2009 and July 11, 2009 to July 24, 2010. *Bar graphs* following a different letter are significantly different (Tukey's HSD, $p < 0.05$). *Error bars* indicate standard deviation.

4. Discussion

4.1. Variability of soil respiration related to soil moisture

Soil respiration under the canopy of all three tree species showed pronounced seasonal variation. It is evident that these seasonal changes are generally driven by the precipitation pattern and its response variable volumetric soil water content. The strong positive relationship between soil moisture and soil respiration is in agreement with previous studies in tropical forest ecosystems where a majority of biological processes coincides with moisture dynamics (e.g. Epron et al., 2004; 2006; Hashimoto et al., 2004; Nsabimana et al., 2009; Salimon, 2004; Werner et al., 2007). In general, the highest soil respiration rate during the wet periods may have resulted from the high physiological activity of both plants and microorganisms (Lee et al., 2002) in not limiting soil moisture conditions. Besides this general effect of soil moisture on soil respiration rates, rewetting of the soil after dry periods causes a short-lived but strong increase in soil CO_2 efflux. This observation is known as ''Birch effect'' and is the result of burst mineralization of labile soil organic matter that has been accumulated during the dry period and is available to microorganisms after re-wetting of the soil (Birch, 1964; Jarvis et al., 2007).

At our study, volumetric soil water content was positively related to soil CO_2 efflux rates only when it was below around 30%, at higher values there appeared a negative relation. Since volumetric soil water contents of up to 42% were measured, soil respiration rates were below the maximum values under these wet conditions. Excess soil moisture may negatively affect CO_2 efflux rates by reducing soil aeration and thus CO_2 diffusivity (Janssens and Pilegaard, 2003). Oxygen deficit as result of too high soil moisture decreases activity of plant roots (Adachi et al., 2006) and the heterotrophic decomposition of soil organic matter (Linn and Doran, 1984). This may be particularly the case in the clayey soils under study. An alternative explanation is based on the photosynthetic activity of the trees. The periods with the most amount of precipitation are associated with conditions of low photosynthetic active radiation in the Munessa forest with *c.* 1-4 mol m^{-2} leaf area d^{-1} (Seyoum et al., in preparation). This translates to small carbon assimilation rates and, consequently, to small rates of autotrophic soil respiration.

4.2. Variability of soil respiration related to soil temperature

We examined possible seasonal effects of soil temperature on soil respiration and found little relation for the investigated forest ecosystem. The apparent weak contribution of soil temperature unlike to soil moisture is partly due to the relatively small temperature fluctuations in this ecosystem being not sufficient enough to drive seasonal variations in soil respiration. Further, soil temperature tended to be higher during the dry periods when soil CO_2 efflux was restrained by low soil moisture. Similar observations have been found in other tropical forest ecosystem where soil temperature is relatively constant within the year and poorly correlated with soil respiration (e.g. Davidson et al., 2000; Hashimoto et al., 2004; Kiese and Butterbach-Bahl, 2002; Nsabimana et al., 2009). In other biomes such as boreal and temperate forests, there is a wide range of seasonal temperatures that leads to seasonal variation in soil respiration rate (Davidson et al., 1998; Malhi et al., 1999; Shibistova et al., 2002).

Over diurnal pattern, when soil moisture is assumed to be almost constant, soil temperature is considered to be a major control of soil CO_2 efflux (e.g., Chang et al., 2008). Also we found a positive relation between soil temperature and soil respiration, as long as soil moisture is not limiting as is the case at the July and December measurements. The Q_{10} values suggest that the more favorable is the soil moisture the more prominent is the temperature dependency of the diurnal soil CO_2 efflux. This may be attributed to increasing soil organic matter mineralization rates with increasing temperature. But according to Tang et al. (2005), largest

soil respiration rates in the afternoon rather suggest that biotic processes, coupled to photosynthesis, carbon allocation to roots and autotrophic may control diurnal CO_2 efflux.

4.3. Effects of trees on soil CO_2 efflux

Our analysis demonstrated that different tree species affected soil respiration. At optimum soil moisture, the soil CO_2 efflux under *P. falcatus* exceeded that under the other two tree species (Fig. 1.3). Several biotic factors such as assimilation rate, root density, phenological differences, soil microbial activity and diversity (Epron et al., 2006; Raich and Tufekcioglu, 2000; Vanhala, 2002) might explain this observation. During the wet season with dominantly clouded sky, the shade tolerant *P. falcatus* is having a physiological advantage as compared with the light demanding *C. macrostachys* in terms of carbon assimilation (Seyoum et al., in preparation). So it seems reasonable that the higher soil respiration rate during the wet period under the canopy of *P. falcatus* than under the other two tree species is associated with the cumulative metabolic activities of the plant.

The situation changes when the dry season is progressing. With decreasing soil moisture, soil respiration decreased under all three tree species, however, the change in magnitude of soil CO_2 efflux rate under *P. falcatus* was larger than under *C. macrostachys* and *P. africana*. The least soil moisture sensitivity was observed for *C. macrostachys*. Probably, this reflects the different sensitivity of the three tree species on soil moisture. Seyoum et al. (in preparation) analyzed particularly larger values of carbon assimilation and transpiration for *C. macrostachys* than for the other two tree species during the dry season. This pronounced photosynthetic activity of *C. macrostachys* during the dry period probably results in a relative large root respiration, thus causing a less pronounced decline in CO_2 efflux during conditions of low soil moisture than for the other two tree species.

4.4. Annual soil CO_2 efflux

Cumulative annual soil CO_2 efflux was highest under *P. falcatus* (144 mol m^{-2} in the first year and 162 mol m^{-2} in the second year of observation). Respective values for *C. macrostachys* were 131 mol m^{-2} and 135 mol m^{-2}, and for *P. africana* 140 mol m^{-2} and 156 mol m^{-2}. With that the cumulative annual soil CO_2 efflux falls within the range of other published estimates of secondary tropical forests in eastern Amazon (150 mol CO_2 m^{-2} $year^{-1}$; Davidson et al., 2000), tropical monsoon forests in Thailand (213 mol CO_2 m^{-2} $year^{-1}$; Hashimoto et al., 2004), monospecific forest plantation in Rwanda (112 mol CO_2 m^{-2} $year^{-1}$, Nsabimana et al., 2009), and moist tropical lowland forest in Panama (128 mol CO_2 m^{-2} $year^{-1}$, Sayer et al., 2007). In

most studies soil collars were installed randomly over the whole experimental plots. In the present study collars were installed randomly but underneath the canopy of our experimental trees. Wiseman and Seiler (2004) showed that measurement position had significant effect on soil respiration, and soil CO_2 efflux rates were consistently near the trees. This suggested that the estimated annual soil respiration probably reflects a comparatively higher CO_2 efflux rate than at a completely randomized design at the forest ecosystem level.

As shown in Table 1.2, the season is having the strongest influence on the soil respiration rates rate. The mean soil CO_2 efflux rate was higher during the wet period than during the dry period, independently of the study tree and the year of observation (Fig. 1.6). When the two study years are compared, there were no differences in the soil CO_2 efflux rates neither for the wet period nor for the dry period (except the second year wet period observation under *P. africana*). During the first year of observation six months were classified as dry periods, whereas at the second year only three months fell into this category because of a more homogenous distribution of the precipitation. Hence, the higher soil CO_2 efflux rates during the second year of observation could be related to fewer periods where soil respiration was restrained due to low soil moisture. This shows that the precipitation pattern is having a strong impact on the soil cumulative soil respiration in the Afromontane forest.

5. Conclusions

The soil CO_2 fluxes from soil to atmosphere in the Munessa forest is primarily controlled by soil moisture, and with that by the precipitation pattern. Therefore, the length of dry and wet seasons is of utmost importance for the soil CO_2 efflux and the whole carbon balance of this ecosystem. In Ethiopia, predicted future changes in precipitation pattern are likely to have considerable direct effects on soil CO_2 efflux over most of the year. However, this study also shows that biological variability is an important factor, because different functional types of trees are responding differently on precipitation pattern with respect to soil CO_2 efflux. Since anthropogenic impact to the forest structure, e.g. by changes in the intensity of grazing-induced disturbance, favors the pioneer *C. macrostachys* at the cost of the late successional tree species, this adds another important driving factor in the seasonal and annual variability of soil respiration at local scales. Interestingly the late successional *P. falcatus* shows a larger variability is soil CO_2 efflux rates than the pioneer *C. macrostachys* that colonizes disturbed niches.

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Study 2

Forest management influence on carbon flux of *Cupressus lusitanica* plantation in the Munessa forest, Ethiopia

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My contribution:

Participation in the conception of the study, performance of part of the field work, performance of part of the laboratory work, leadership in data analysis and writing

Abstract

Development of plantation forests in tropical countries is accelerating to satisfy the increasing demand of forest products and to mitigate the rising CO_2 emissions to atmosphere. The effect of plantation forests on the global carbon balance is controversially discussed in recent times. As soil respiration is a decisive component in the carbon exchange between terrestrial ecosystems and atmosphere, knowledge of consequences of plantation forests management measures (e.g. thinning) in the context of driving parameters of soil CO_2 efflux is a key issue in optimizing carbon friendly land management. In the present study, we report the effects of thinning, soil temperature and soil moisture, and microbial biomass parameters on soil CO_2 efflux rate. We selected an unmanaged and thinned stands within six years old *Cupressus lusitanica* plantation forest. Soil CO_2 efflux rate ranged from 1.47 to 6.92 μmol $m^{-2}s^{-1}$ and 1.31 to 5.20 μmol $m^{-2}s^{-1}$ in thinned and unthinned stand, respectively. Generally higher soil respiration rates were measured during wet than in dry season, whereas the relationship between soil moisture and soil respiration rate followed a parabolic pattern with increase of soil CO_2 efflux rate up to 31% of soil water content. In contrast to soil moisture, soil temperature was a poor predictor of the soil respiration. In general, soil CO_2 efflux rate in the first and second year after thinning was 24% and 14% higher in the thinned stand. Increased soil temperature at the thinned stand contributed minor to the larger soil CO_2 efflux, the more important reason appeared to be the trees' direct response. Higher fine root production together with larger concentrations of PLFA / NLFA biomarkers representing different groups of microbial community infers a higher autotrophic respiration by roots and associated mycorrhizal fungi as well as by heterotrophic respiration. Despite the higher CO_2 losses with soil respiration, the organic C and total N concentrations in soil rather tended to increase, indicating higher organic matter input to soil at the thinned stand.

Keywords: *Cupressus lusitanica*, Fine root biomass, Microbial concentration, Soil CO_2 efflux, Soil moisture, Soil temperature, Thinning

1. Introduction

Recent atmospheric studies have suggested a substantial terrestrial sink for CO_2 could be attributable to the African continent (Stephens *et al.*, 2007, Ciais *et al.*, 2009). However, there is still great uncertainty of the magnitude and variability of the African carbon stocks and fluxes (Williams *et al.*, 2007). The African ecosystems contribute 20% of global net primary production, along with the same proportion on global forest degradation and deforestation induced CO_2 emission (Laporte *et al.*, 2007), of about 0.24 Pg C yr^{-1} for sub-Saharan Africa (Ciais *et al.*, 2011).

One component that needs to be addressed in the terrestrial carbon budget is the efflux of CO_2 from the soil surface to atmosphere as being ultimately attributable to the ecosystem biological activity (Raich and Schlesinger, 1992). Contributing more than 50% to the ecosystem respiration across variety of biomes (Schlesinger, 1997; Janssens *et al.*, 2001; Shibistova *et al.*, 2002) soil respiration determines the ecosystem carbon balance and thus an ecosystem sink or source activity (Valentini *et al.*, 2000). Simulations of the soil CO_2 efflux for biogeochemical models are one of the main challengers due to high temporal variability and spatial heterogeneity of soil respiration at all scales with respect to environmental conditions and long-term site disturbance history. Soil temperature and soil moisture has been recognized to be the two most influential abiotic parameters leading to seasonal and diurnal variations of soil respiration in different ecosystems (Davidson *et al.*, 1998; Rustad *et al.*, 2000; Reichstein *et al.*, 2003; Makhado and Scholes, 2011).

One of the economically viable strategies for sequestering atmospheric carbon and mitigation the climate change scenario in tropics could be development of industrial plantations on suitable land areas (Dabas and Bhatia, 1996). The Global Forest Resource Assessment reported that in 2010 planted forests worldwide accounted 264 million hectare, 7.2 million hectare of which were located in sub-Saharan Africa.

Among other African countries, Ethiopia has one of the longest forest plantation histories (Pohjonen and Pukkala, 1990) with the current total plantation forests area of 511 thousand hectare (FAO, 2010). Forest plantations in Ethiopia are mainly represented by monocultures of fast growing exotic tree species mainly *Eucalyptus* sp. and *Cupressus lusitanica* (Bekele-Tessema *et al.*, 1993). Productivity of plantations needs to be high to meet increasing demand of wood products and to enhance stand quality and ability of effective carbon sequestration in various compartments of a forest ecosystem (Richter *et al.*, 1999).

Forest management practices may determine carbon exchange between the atmosphere and the forest (Maier *et al.*, 2004). One measure to increase the formation in tree biomass, mainly stems, is thinning. This practice affects physiological and hence growth responses of the remaining trees and micro-climatic changes within the modified stand (Della-Bianca and Dils, 1960), influencing the environmental condition beneath the canopy, including the soil microclimate, and root dynamics (Tian *et al.*, 2009). The aboveground productivity and the carbon transfer to soil via litterfall and rhizodeposition are decisive for carbon availability for microbial decomposition processes in soil. Hence, it is likely that changes in aboveground productivity will have consequences for belowground process and *vice versa* (Wardle *et al.*, 2004). All these changes may be reflected in the soil respiration. Most research on thinning treatment on plantation forests, including Ethiopia (e.g., Pukkala and Pohjonen, 1993) has been done in order to identify management guidelines to produce optimal stand growth (e.g., Galloway *et al.*, 2001). Few studies have now emerged that focused on the carbon implications of thinning while targeting volume production (Hoover and Stout, 2007). As principally soil CO_2 flux studies in African intact and plantation forests are scarce (Epron *et al.*, 2004, 2006; Werner *et al.*, 2007; Nsabimana *et al.*, 2009), to our knowledge no study investigated the impact of thinning on soil respiration in east African plantation forests.

The Munessa forest is one of the largest plantation forest in Ethiopia established in the 1970's. It covers about 6973 ha of which *Cupressus lusitanica* (Mill.) is the dominant planted tree species that account for 60% of the total plantation area (Teshome and Petty, 2000). The objectives of this study were (i) to compare the effects of thinning treatment on soil CO_2 efflux rate between intact and thinned stands and (ii) to compare indicators for soil microbial biomass between intact and thinned stand. We hypothesized that thinning treatment would increase soil CO_2 efflux rate by stimulating the productivity of the remaining trees (Balboa-Murias *et al.*, 2006) and changing the soil microclimate conditions and as a result of improved growing conditions of remaining trees and change in soil microclimate, soil microbial biomass will be larger under thinned stand.

2. Materials and Methods

2.1. Study site and silvicultural treatment

The study was conducted in 6-years-old *Cupressus lusitanica* stand established in June 2003 located at the Munessa forest (7°25' 44''N and 38°51' 05''E), West Arsi zone, Oromia regional state, Ethiopia. The slope is gentle and the elevation at the study site is 2126 m above sea level. The soil texture is predominately a slightly acidic and nutrient rich clay-loam that was evolved from volcanic parent material (Fritzsche *et al.*, 2007) (Table 2.1), and was classified as Mollic Nitisols according to the WRB system (FAO *et al.*, 1998).

Table 2.1: Basic characteristic of the soils under each stand

Stand	Soil depth	Sand*	Silt*	Clay*	pH[§]	CEC[¶]	BS[**]
		—— g kg^{-1} ——				$mmol_{(+)}$ kg^{-1}	%
Thinned	0-10 cm	210±15	395±62	395±67	6.0±0.5	638±92	100
	10-25 cm	189±8	371±65	440±70	5.7±0.6	449±69	100
Control	0-10 cm	203±20	376±20	421±20	6.3±0.5	568±24	100
	10-25 cm	190±26	329±7	481±27	5.7±0.7	447±74	100

*measured by the pipette method (Gee and Bauder, 1986); [§] analyzed potentiometrically in 1 M KCl [1:2.5 (m/v)]; [¶] cation exchange capacity determined with the $BaCl_2$ compulsive exchange method (Gillman and Sumpter, 1986); [**] base saturation. Values are mean and standard deviation of three replicated samples.

The study site is located at the central area of the country, with a minor rainy season occurring from March to May and a major rainy season from July to November (Griffits, 1972). Own meteorological records since 2001 show that 80% of the annual precipitation fell in the major rain season from July to November, and no clear indication of a minor rainy season is given (Strobl et al., unpublished). Also the precipitation pattern during the observation time showed pronounced seasonality with a wet period from June to October 2009 and from February to October 2010 showing a monthly precipitation of up to 200 mm (Fig. 2.1). In contrast, November 2009 to January 2010 and the last six months were characterized by dry conditions. Minima of air temperature occurred at the transition of the rainy season to the dry season with an average monthly temperature of *c.* 14°C and increased towards the transition of the dry season to the rainy season peaking in an average monthly temperature of *c.* 19°C.

For the present study, we worked on two fenced stands (40 m x 40 m) located 20 m apart from each other. The experimental stands were selected by a silvicultural research team with the objective to investigate the influence of thinning operation on the growth performance of potential crop trees of *C. lusitanica* (Nenninger *et al.*, submitted). The stand was initially planted at spacing of 2.5 x 2.5 m. In January 2008, selective thinning has been done in one of the fenced areas by removing the competitors trees next to trees assigned as potential crop trees. No thinning operation was carried out on the other stand and this stand was considered as a control. After thinning the number of trees left in the thinned stand was 98 as compared to 144 in the control stand.

2.2. Soil respiration measurement

Before the start of the field campaign, within each stand sixteen randomly spots were selected in order to estimate the number of sampling points required for soil CO_2 efflux measurement. At each selected spot, circular PVC soil collars (20 cm in diameter and 5 cm long) were installed about one year and five months of lag time after the management impact. After sampling point estimation, four individual trees per stand were randomly selected. Collars were placed randomly around the selected tree at a distance of about 0.7 to 1.3 m from the bole. This design was chosen because previous studies had shown higher variability and higher soil respiration rates were observed in collars established at close vicinity to tree stems (Wiseman and Seiler, 2004; Saiz *et al.*, 2006). A sampling design of installing collars close to the vicinity to tree stems gives more reliable estimates of fluxes from a given stand (Butterbach-Bahl *et al.*, 2002). Olsthoorn *et al.* (1999) has shown that such sampling design was also more error free in the assessment of fine root density. An insertion depth of about 1-2 cm into was chosen for soil CO_2 efflux measurement to minimize severing of roots and the mycorrhiza. Finally, the outer wall of the collars was sealed with fine sand to avoid leaking of the chamber-soil system.

The number of individual flux measurements needed for various degrees of precision at various confidence levels was computed following probability equation described by Snedecor and Cochran (1967):

$$n = (t_{\alpha}s/D)^2 \qquad \text{Equation 1}$$

where t_{α} is Student's t with degrees of freedom at the 0.05 probability level, s is the standard deviation with values obtained at this study, and D is the specified error limit. The result in Table 2.2 shows the required number of measurement points in each of the stands under

different degrees of precision. Clearly, large numbers of flux measurements are ideal but we learnt from the 16 collar measurements before the start of the sampling campaign, it was feasible to restrict our measurements on 20 collars per stand. This decision led us our soil CO_2 efflux estimation to fall within an interval of 20% of the population mean at 95% confidence interval (Table 2.2). We installed the 20 collars (five per tree) under the canopy of individual randomly selected trees as described above. Once inserted, soil collars were left in place throughout the measurement period. Herbaceous understory vegetation was avoided during collar set-up. However, when, in the following two year's period, any vegetation grew inside the collars, it was clipped back.

Table 2.2: Number of sampling point required within a precision of ±10 to ±50% of the full population mean and at different confidence interval between 80-99% based on 16 measurements per stand in May 2009.

Interval about the full population mean (%)	99% confidence ($\alpha = 0.05$)		95% confidence ($\alpha = 0.05$)		90% confidence ($\alpha = 0.05$)		80% confidence ($\alpha = 0.05$)	
	Thinned	Control	Thinned	Control	Thinned	Control	Thinned	Control
±10	31.95	34.10	24.27	25.91	20.37	21.74	15.85	16.92
±20	26.85	28.65	20.40	21.77	17.12	18.27	13.32	14.22
±30	22.88	24.41	17.38	18.55	14.59	15.57	11.35	12.11
±40	19.73	21.05	14.99	15.99	12.58	13.42	9.79	10.44
±50	17.18	18.34	13.05	13.93	10.96	11.69	8.52	9.10

Mean and standard deviation of soil CO_2 efflux on the measurement day was 5.34 ± 1.1 and 5.10 ± 0.8 μmol $m^{-2}s^{-1}$ for thinned and control stand respectively.

Soil CO_2 efflux was measured on a weekly basis from May 2009 through May 2011 with the exception of November and December 2009 due to instrument failure. Instantaneous soil respiration was measured using a LI-8100-103 soil survey chamber (LI-COR, Lincoln, NE, USA) connected to an Infrared Gas Analyzer Li-8100 (LI-COR, Lincoln, NE, USA). Soil temperature (°C) at a depth of 0.1 m was recorded adjacent to each collar simultaneously with soil CO_2 efflux measurements using a thermocouple probe (Li-8100-201) connected to the Li-8100. The volumetric soil water content at 0.06 m depth was also measured with a handheld theta probe (ML2, Delta-T Device Ltd, Cambridge, UK) at three replicates around the collars within 5 cm of each collar immediately following each soil CO_2 efflux measurement. For each of the stands mean CO_2 efflux rates were calculated from the 20 chamber measurements obtained during individual sampling events.

2.3 Chemical and biological properties of the soil

After the soil respiration measurements were completed for a given sampling period, the soils to the depth from 0-10 cm and 10-25 cm below each PVC collar were collected to determine organic carbon (OC) and total nitrogen (TN), fine root density and microbial lipid concentration (PLFA analysis). Immediately after collection, the soil samples were sieved with 2 mm mesh size and all visible fine roots were picked out. Root samples were then washed with distilled water and oven-dried at 65 °C for 48 h for biomass determination. After homogenization of each soil sample, aliquots (*c.* 2 g) were taken for total organic C and total organic N analysis. After air-drying, soil samples were finely grinded with a steel ball mill (Mixer Mill, Retsch MM 200) and dried overnight at 105 °C. Soil organic carbon and nitrogen contents were analyzed using a Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Germany).

Likewise, directly after homogenization of the soil, subsamples of about 10 g were collected separately underneath each collar for phospholipids fatty acid (PLFA) and neutral lipid fatty acid (NLFA) analysis. After removing all visible plant materials, the soil samples were then packed in small glass vials and kept frozen until analyzed. Lipid fatty acid extraction was carried out using frozen soil samples (1.5 g) extracted overnight with a chloroform-methanol citrate buffer mixture (1:2:08) by the modified method described by Bligh and Dyer (1959). The fatty acid 19:0 (nonadecanoic acid methyl ester) was added to the samples as an internal standard. Lipids were separated into neutral, glyco-, and phospholipids using solid phase extraction with silicic acid column (Bond Elut LRC-Si, Varian Agilent Technologies, Santa Clara, CA). Thereafter, the neutral and phospholipids were subjected to a mild-alkali methanolysis, and the resulting fatty acid methyl esters were separated by gas chromatography using an Agilent 7890A GC-MS (Varian Agilent Technologies, Santa Clara, CA). The fatty acids were quantified by comparison of the peak areas with those of the standard peak. Standard nomenclature was used to refer to the PLFAs according to the designation described in Zelles (1999). Phospholipids fatty acids i15:0, a15:0, i16:0, 18:1ω7c and cy19:0 were used to represent bacterial biomass (Zelles, 1999), while the PLFAs 18:2ω6 and 18:1ω9 were considered to have fungal origin (Frostegård and Bååth, 1996; Zelles, 1997), and the NLFA 16:1ω5 was used as marker for arbuscular mycorrhizal fungi (Olsson, 1999). At our study site, *C. lusitanica* is infected by arbuscular mycorrhiza (Wubet, unpublished). The sum of the mentioned PLFAs in addition to PLFAs being not assigned as biomarkers (16:1ω9, 16:1ω7c,

16:1ω5, 16:0, 10Me16:0a and 10Me16:0b) were used to represent microbial biomass (Federle, 1986; Frostegård *et al.*, 1993) expressed as nmol PLFA g^{-1} dry soil.

2.4. Statistical analysis

Individual collars were used as the statistical units for analyzing the spatial variation. Statistical differences in the temporal variability of the soil temperature and soil moisture between thinned and control stands were analyzed using student *t*-test, at 95% confidence interval. A t-test or, if normality was not fulfilled after transformation, a Mann-Whitney Rank Sum test were used to analyze the difference between thinned and control plot in concentrations of fine roots (i.e., fine root biomass), OC and TN, total PLFAs and 16:1ω5 NLFA. For each collar from thinned and control stands the relationships between soil CO_2 efflux rate and soil chemical and biological parameters (fine root biomass, OC and TN, PLFAs) was examined with linear regression. The statistical analysis (α 0.05 level) was performed using R 2-11.0 (R Development Core Team, 2009) or SigmaPlot version 11 (Systat Software Inc., San Jose, CA, USA).

The effects of thinning, season, soil moisture and soil temperature (and their interaction) on soil CO_2 efflux variability were tested by linear mixed effect (LME) model. The model was set up with respiration rate at a collar as dependent variables with treatment (thinned and control) and season (wet and dry) as fixed effects. We nested the random effects of collar numbers within the experimental plot. Because of repeated measurements on the same sampling unit, correlation between the residuals within the same collar was expected. To account for this possible autocorrelation, a first-order continuous autoregressive structure (CAR(1)) error process was incorporated. Plotting the residuals of soil CO_2 efflux as a function of soil moisture showed a clear curvature and hence the quadratic effect of soil moisture is also included in the LME model. We used maximum likelihood to calculate parameter estimates and associated standard errors for each fixed effect, with the package nlme using program R (Version 2.10.1, www.r-project.org; Ihaka and Gentleman, 1996; Pinheiro and Bates, 2000). We examined residual plots to ensure that variance was homoscedastic and after fitting we have found out that the residual variation of soil CO_2 efflux increased with increased fitted values. Thus we used a log transformation of the soil CO_2 efflux data to improve homogeneity of variance (Pinheiro and Bates, 2000). We also used nonlinear regression analysis with the objective to describe the dependence of soil respiration on soil moisture or with soil temperature. More details about statistical approaches can be found in Yohannes *et al.* (2011).

3. Results

3.1. Seasonal variability in soil CO_2 efflux under thinned and unthinned stands

For the period of May 2009 to May 2011 Fig. 2.1 shows the temporal patterns of air temperature and soil temperature at 0.10 m depth (Fig. 2.1a), precipitation and soil moisture at 0.06 m depth (Fig. 2.1b), as well as the soil CO_2 efflux rate (Fig. 2.1c) for the thinned and control stands.

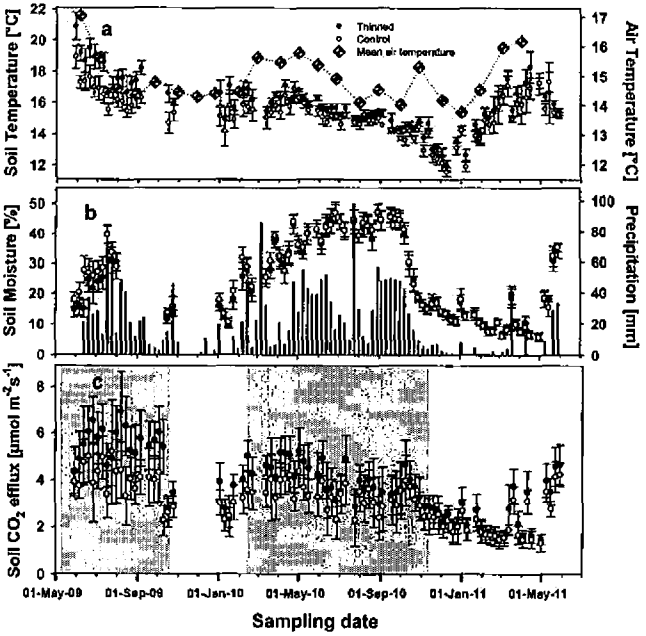

Fig. 2.1: Seasonal patterns of air and soil temperatures (a), precipitation (bar graph) and soil moisture (b), and soil CO_2 efflux rate (c). Each data point for soil respiration, soil temperature and soil moisture is a mean of twenty measurements. *Error bars* indicate standard deviation. For the air temperature the monthly average temperatures measured at the Kuke weather station are presented. Data gaps are due to instrument failure. Periods with light grey background indicate wet periods as calculated by the approaches of Gibbs and Maher (1967).

Average soil temperatures during the measurement period were 15.9±1.7 °C and 15.2±1.5 °C at the thinned and control stand, respectively. In general, soil temperatures within the thinned stand were significantly higher as compared with the unthinned stand (p=0,005), with higher coefficient of variation CV between the stands than within the stand (Fig. 2.2). In contrast,

there were no significant differences in volumetric soil water content between thinned and control stands ($p>0.05$). The matrix of within-stand coefficient of variation versus the between-stand coefficient of variation lined up almost equally along the 1:1 line (Fig. 2.2), indicating that the thinning treatment unlike soil temperature did not change soil moisture regime.

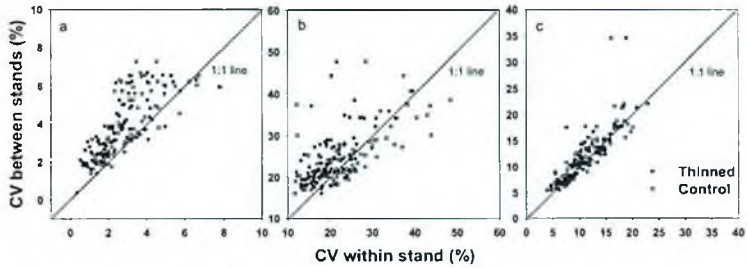

Fig. 2.2: Coefficients of variation between stands versus CV within-stands in the thinned and control plantation stands: soil temperature (a), Soil CO_2 efflux (b), and soil moisture (C). Please note the difference on the scale of both-axis between the figures.

Across the measurement campaign, mean soil CO_2 efflux rate varied from 1.5 to 6.9 μmol m^{-2} s^{-1} and from 1.3 to 5.2 μmol m^{-2} s^{-1} at the thinned and control stand, respectively with seasonal minimum in January 2010 and April 2011 and maximum in June 2009 and June 2010 (Fig. 2.1c). Soil respiration rates over the period of observation were significantly ($p<0.001$) higher within the thinned than within the control stand, and differences in the flux density appeared to be more pronounced during wet periods. The coefficient of variation of the seasonal soil CO_2 efflux rate ranged from 9% to 40% (thinned stand) and 12% to 48% (control stand), being not statistically different ($t = 1.479$ and $p = 0.088$). However, the coefficient of variation of the soil CO_2 efflux rate was larger between stands than within stand (Fig. 2.2b), indicating the potential influences of stand specific biotic and/or abiotic factors on soil CO_2 efflux variability. In general, the seasonality of soil CO_2 efflux pattern reflects the changes in soil moisture and different significantly between wet and dry periods (Table 2.3).

Table 2.3: Summary statistics of linear mixed effect model describing the relationship of soil respiration to thinning treatment, covariates soil temperature and volumetric soil moisture content, and interaction term.

Factor	Coefficient	Standard error	df	F	*p*
Intercept	-1.0582	0.1008	1	2376.6	<0.0001
Thinning	0.1259	0.0456	1	13.8	0.0098
ST	0.0651	0.0057	1	1162.9	<0.0001
SM	0.0896	0.0056	1	2651.6	<0.0001
Season	0.0662	0.0135	1	920.3	<0.0001
SM^2	-0.0015	0.0001	1	2125.6	<0.0001
ST:SM	0.0004	0.0003	1	1.6	0.2017

df, degree of freedom; *SM*, soil moisture; *ST*, soil temperature.

A non linear regression shows a significant (p<0.05) effect of soil moisture on soil CO_2 efflux rates that explained about 50% and 48% of the variability under thinned and control stand respectively (Fig. 2.3).

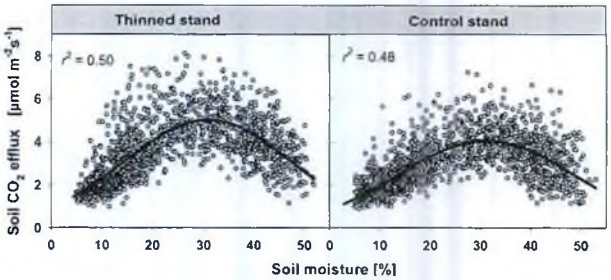

Fig. 2.3: Relationship between soil CO_2 efflux rate (*SR*) and volumetric soil water content (*SM*) at 0.06 m soil depth. The parameters of the Gaussian function *SR* = a*exp(-0.5*((*SM*-x_0)/b)^2) are: a = 5.01, b = 16.80, x_0 = 30.97 (thinned stand); a = 4.08, b = 17.47, x_0 = 31.18 (control stand).

Soil CO_2 efflux rates as a function of soil moisture was fitted by Gaussian curve, which suggested the existence of thresholds in dependency of soil CO_2 efflux rate on soil moisture (Fig. 2.3). Soil respiration rates coincidently increased with increasing volumetric soil water content up to 31%, but the opposite was the truth after soil water content exceeded the abovementioned threshold.

Compared with soil moisture, soil temperature had a minor but significant impact on seasonal variability of soil CO_2 efflux. Soil temperature explained 17% and 10% of the temporal variability of the soil CO_2 efflux rate in unthinned and thinned stands, respectively (Fig. 2.4).

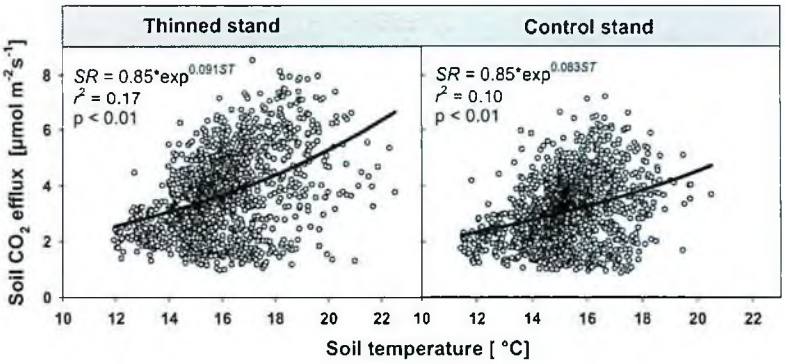

Fig. 2.4: Relationship between soil CO_2 efflux rate (*SR*) and soil temperature (*ST*) at 0.1 m soil depth.

3.2. Soil CO_2 efflux as related to biological parameters and stand management

The variance components in the LME model analysis indicate that the nested effect of collars around individual tree attributed to 19% of the total variance of the random effect. This shows the likely effect of inherent characteristics (biotic and abiotic) of the soil underneath the collar to the overall soil CO_2 efflux variability. Organic carbon concentration in soil collected from each collars in 0–10 and 10–25 cm soil layers differed between the thinned and control stands, with mean values of 114 ± 27 and 40 ± 13 g C kg^{-1} soil for the former stand and 93 ± 20 and 21 ± 6 g C kg^{-1} soil for the latter (n = 20, $p<0.05$ in both soil layer). Similarly the mean TN contents from 0-10 and 10-25 cm soil depth were 10 ± 2 and 4 ± 2 g N kg^{-1} soil for the thinned and 9 ± 2 and 2 ± 0.5 g N kg^{-1} soil for the control stand, being significantly different ($p<0.05$) at the 10-25 cm soil depth.

The concentrations of microbial biomarker (nmol total PLFA g^{-1} dry soil) used as a proxy for microbial biomass assessment were 22% larger ($p<0.05$) in the soil from 0-10 cm depth sampled from the thinned stand than in those under the control (Fig. 2.5).

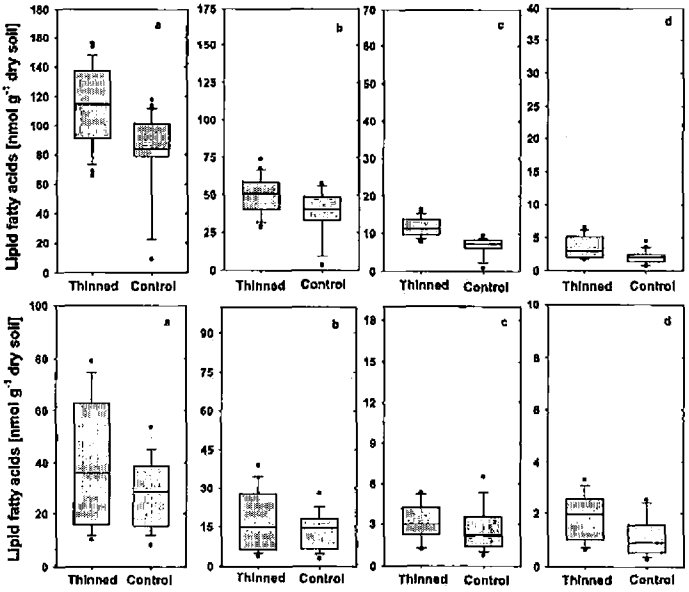

Fig. 2.5: Total microbial (a), bacterial (b) and fungal markers (c) PLFA concentrations (nmol PLFA g^{-1} dry soil) and total AMF marker (d) concentration (nmol NLFA g^{-1} dry soil) collected from thinned and control stand from 0-10 cm (top graph) and 10-25 cm soil depth (bottom graph), n = 20. Box plots indicate the distribution by percentiles. The lower and higher part of the box indicates the 25th and 75th percentiles, respectively. The value of the error bars are the 10th and 90th percentile, and the 0th percentile (median) is given by horizontal line within the box. Please note the difference on the scale of Y-axis.

The soils from the thinned stand also were characterized by larger concentrations of PLFAs being attributed to bacteria (nmol bacterial PLFA g^{-1} dry soil) and fungi (nmol fungal PLFA g^{-1} dry soil), being 19% ($p<0.05$) and 39% ($p = 0.025$), respectively, more than in the soils from the control stand. At both soil depths, soils under the thinned stand contained also larger a concentration of the NLFA16:1ω5 arbuscular mycorrhizal fungi biomarker ($p<0.05$) (Fig. 2.5). The mean (n=20) fine root biomass was 145±25 (0-10 cm soil depth) and 105±10 (10-25 cm soil depth) at the control stand, being 11%, $p= 0.023$) and 12% ($p<0.001$) respectively smaller as for the thinned stand (not shown).

There was no significant relationship between the final soil CO_2 efflux measurement with OC, fine root biomass, and PLFAs / NLFA analyzed in the soil samples taken directly underneath each collars. However, the microbial fatty acids content in 0-10 cm soil depth were positively

related with fine root biomass (Pearson's $r = 0.74$ at $p<0.05$ and $r = 0.69$ at $p<0.05$), OC ($r = 0.52$ at $p<0.05$ and $r = 0.18$ at $p<0.05$), and with NT ($r = 0.59$ at $p<0.05$ and $r = 0.10$ at $p<0.05$); values in parenthesis are for thinned and control stands, respectively.

4. Discussion

4.1. Seasonal variability of soil CO_2 efflux in relation to soil moisture and soil temperature

Not surprisingly, the soil respiration in both stands showed a similar seasonal pattern, with the higher rates observed in wet periods and the lower rates during dry periods. This result is consistent with our previous study conducted in adjacent natural forest (Yohannes *et al.*, 2011). Our finding is also in agreement with results reported for other tropical forests with seasonality in precipitations (e.g. Epron *et al.*, 2004; Nsabimana *et al.*, 2009; Werner *et al.*, 2007). However, a positive effect of soil moisture on soil respiration could only be identified when it was below 31%; soil water contents above this threshold led to a decrease in soil respiration (Fig. 2.3). It should be also noticed that, the threshold for the evergreen *Cupressus lusitanica* stand reported here, is equal to the threshold of evergreen tree species (*Podocarpus falcatus* and *Prunus africana)* examined in the natural forest and exceeded that for deciduous *Croton macrostachys* (Yohannes *et al.*, 2011). The decline in soil CO_2 efflux rate at extremely wet conditions could possibly result from physiological stress of heterotrophic microorganisms due to oxygen deficiency that decreases decomposition rates of soil organic matter (Linn and Doran, 1984). Alternatively, periods with the most precipitation are associated with conditions of low photosynthetic active radiation. This may decrease carbon assimilation rates and, consequently, the rates of associated autotrophic soil respiration (Moyano *et al.*, 2008).

Although the effect of soil temperature on the seasonal pattern of soil respiration has been widely confirmed by many researchers (e.g., Lloyd and Taylor, 1994, Davidson *et al.*, 1998), our result indicated that soil respiration was only slightly positively related to soil temperature. This suggests that soil temperature was not the key determinant of soil respiration at the study site. The apparent weak contribution of soil temperature unlike to soil moisture is partly due to the relatively small temporal temperature fluctuations in this ecosystem, being not sufficient enough to drive seasonal variations in soil respiration (Nsabimana *et al.*, 2009; Yohannes *et al.*, 2011). Further, at times of high soil temperature soil CO_2 efflux was restrained by low soil moisture (Fig. 2.4).

4.2. Soil CO_2 efflux in relation to CN and biotic factors

Regression analysis of the soil CO_2 efflux rates either with fine root biomass, soil OC, TN, or the different microbial parameter sampled underneath the respective collars showed no correlation. The lack of any relationship is surprising as soil CO_2 efflux from forest soil derived both from respiration of plant roots and soil microorganisms, positive relationship between soil respiration rate and fine root biomass and/or microbial parameters have been frequently reported (e.g., Pregitzer *et al.*, 2000; Lee and Jose, 2003; Oscar, 2007). In young stands of trembling aspen and paper birch, King *et al.* (2001) found that increased fine root biomass was accompanied by an increase of soil CO_2 efflux by 39%. Fine roots influence soil physical and chemical environment via exudation of carbon-rich substances (Kuzyakov and Cheng, 2001). This argument highlights the decisive role of fine roots to change soil OC concentration which is one of the main determinants of soil CO_2 efflux variability (Wiseman and Seiler, 2004). In our case, we speculate that the lack of any relationship can be due to the overriding effect of abiotic parameters in the control of the soil CO_2 efflux rate at this particular date. At that time the averaged volumetric soil moisture underneath the individual collars was 33%, and with that above the threshold level of c. 31% as identified in Fig. 2.3. The negative impact of the high soil moisture on soil respiration rates may have leveled off effects of different concentration of the organic substrate or the microbial biomass (Fierer and Schimel, 2002).

4.3. Thinning effects on soil CO_2 efflux

The LME model analysis revealed that, averaged soil CO_2 efflux rates at the thinned stand was 13% larger than at the control. Thinning alters the abiotic stand parameters such as increasing the soil temperature or improving the light conditions due to loss of competitors. Soil temperatures increased with thinning intensity (e.g Thibodeau *et al.*, 2000; Ma *et al.*, 2010; Yang *et al.*, 2011), and is caused by higher insolation to the soil surface under a more open canopy. As soil temperature affects the soil CO_2 efflux rates at the investigated stands to a certain extent (Fig. 2.4), the increase of the temperature might have contributed to the larger soil respiration of the thinned stand. In contrast, soil moisture, the major abiotic driving factor at the stands under study, did not get altered by the management impact.

A major reason of any thinning operation is the improvement of access to the light, nutrient, and water supply for increasing stand productivity and obtaining better timber from the potential crop trees (Tang *et al.*, 2005). After three years of thinning treatment, the basal area increment (at diameter breast height, 1.3 m from the ground) of the experimental trees in the

thinned stand was on average 20% larger than trees under study in the control stand (Nenninger *et al.*, unpublished). As the soils are highly fertile and water supply is also not limited (in addition to the precipitation, there is lateral water input to the stand due to its downslope position), the major reason for this increased growth rates was rather the improved conditions for incoming photosynthetic active radiation. Larger biomass production requires more nutrient acquisition by the trees, thus leading to larger fine root biomass and larger colonization by arbuscular mycorrhiza, as it can be concluded from the larger concentrations of 16:1ω5 NLFA in the thinned stand soils. Hence, both components of the autotrophic continuum may have enhanced soil respiration at the thinned stand due to higher energy demand, thus increasing the autotrophic respiration (Ma *et al.*, 2004).

However, fine roots, after their death, are also a source of heterotrophic respiration. Larger concentrations of biomarkers indicative for total microbial biomass (all PLFAs), bacteria (i15:0, a15:0, i16:0, 18:1ω7c and cy19:0 PLFAs) and fungi (18:2ω6 and 18:1ω9 PLFAs) suggesting fuelling of heterotrophic activity by higher substrate supply, i.e. by rhizodeposition, at the thinned stand. Such positive relations between fine roots and microbial biomass parameters have been frequently reported (e.g. by Wardle, 1992; Lee and Jose, 2003). Also Hwang and Son (2006) found larger fine root input to soil after thinning and argued that this was enough organic matter input to offset potential losses of soil OC by soil respiration. This might also explain our observation that despite the higher soil CO_2 efflux rates, OC and TN concentrations tended to be even larger at the thinned stand.

In general, our results concur with the findings of other studies reporting that thinning treatments led to elevated soil CO_2 efflux rates in plantation forests (Messina *et al.*, 1997; Ohashi *et al.*, 1999; Kowalski *et al.*, 2003; Ma *et al.*, 2004; Concilio *et al.*, 2005). However, there are also reports showing the opposite effect of thinning on the soil CO_2 efflux densities (e.g., Tang *et al.*, 2005; Sullivan *et al.*, 2008). Varying results on the effect of thinning treatment on soil CO_2 efflux variability could possibly arise from differences in thinning intensity, the timing of the post-thinning monitoring period, tree species and age of the study trees and sampling design (e.g., Nilsen and Strand, 2008; Sullivan *et al.*, 2008; Jónsson and Sigurdsson, 2009). In our study collars were placed randomly around trees as it has been shown that measurement of fluxes in a closer distance to the nearest tree give more reliable estimates of greenhouse gas emissions from a given stand than measuring further from the tree stem (Butterbach-Bahl *et al.*, 2002). Oshashi *et al.* (1999) designed their sampling points

in a similar way and also found larger soil respiration rates of the thinned stand as compared to the control.

In the present study, only selective thinning was applied in which only competitors' trees were removed that found next to trees assigned as potential crop trees. Concilio *et al.* (2005) also carried out selective thinning and measured a 43% and 14% higher soil respiration rate in the thinned areas as in the control of a mixed-conifer forest and a hardwood forest, respectively. According to Son *et al.* (2004) soil respiration was higher at a lightly thinned than at heavily thinned stand. A heavy management impact probably reduces the carbon translocation from plant to soil more, and with that root respiration and the supply of readily available OC source for heterotrophic respiration, as potentially the decomposition of the old organic matter is accelerated by more favorable abiotic conditions. Therefore, differences in thinning intensity might be also one of the reasons for the inconsistence reports among similar studies.

Further, the period when the soil respiration measurements have been done after the thinning might have an influence on the result. In a nine-year old Japanese cedar forest Ohashi *et al.* (1999) reported that 3-4 years after thinning soil respiration was higher in a thinned stand than those of intact stand, but there was no difference 5 years after the thinning. In our result, the differences in average soil CO_2 efflux rate between thinned and control plot was larger in the first measurement year than the second. The largest soil CO_2 efflux during the early periods since thinning can be attributed to an increased heterotrophic respiration resulted from an accompanied change in the microclimate that favors decomposition of dead tree roots and slashed materials from the thinned trees. At our site, it is also likely that decomposition of dead roots from thinned trees influenced total soil respiration, nevertheless decomposition rate in the tropic is very fast (Trumbore *et al.*, 2006) and less likely to prolonged more than a year. Therefore it is reasonable to assume that, change in soil CO_2 efflux between thinned and unthinned stand particularly after two years of management intervention could emanates from other factors than the priming effect of the dead roots.

5. Conclusions

Thinning of the *C. lusitanica* stand was carried out in order to increase productivity of the trees and raise the economic value of the timber. In this study we investigated an important part of the C cycle, the soil CO_2 efflux, as influenced by thinning and in connection to the

weather variables. The soil respiration rate was on average 13% larger in the thinned stand as it was estimated by the LME model. Soil moisture could have been identified as the key abiotic driver. Due to the pronounced seasonality in precipitation, soil moisture overrides the effect of soil temperature on soil CO_2 efflux rate that was found to be minor. While soil moisture did not differ between the thinned and control stands, temperature in soils (10 cm depth) of the former was significantly higher due to higher insolation. This higher soil temperature likely contributed to a certain extent to the higher soil CO_2 efflux at the thinned stand. However, it is likely that the increased in soil respiration is the result of the trees' response on the silvicultural treatment. Higher fine root concentration of the thinned stand may suggest both, a higher autotrophic respiration and a higher heterotrophic respiration, as is also indicated by higher concentrations of biomarkers representing the microbial biomass. Despite the higher C losses as soil CO_2 efflux, the ON and TN concentrations in soil rather tended to increase. Hence, fostering tree growth by thinning obviously increased the organic C input to soil, counter balancing the C losses.

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Study 3

Tropical forest degradation accelerates carbon cycling in the plant-soil-atmosphere system

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Abstract

Anthropogenic disturbances of montane tropical forests lead to a shift from late-successional to pioneer trees, differing in their ecophysiological traits such as rates of photosynthesis and transpiration. In an Ethiopian Afromontane forest we compared the coniferous gymnosperm late-successional *Podocarpus falcatus* with the deciduous angiosperm pioneer *Croton macrostachys* to test whether this also affects carbon allocation from tree canopy to belowground and to soil CO_2 efflux. For that we ^{13}C pulse-labeled the tree canopies and followed the temporal resolution of recently assimilated carbon in the plant-soil-atmosphere system for one year. Tracer uptake efficiency was *c.* 60% for both tree species, but ^{13}C excess in leaves of *C. macrostachys* declined more rapidly with a larger size of a fast pool (64% *vs.* 50% of the assimilated carbon) having a shorter mean residence time (14h *vs.* 55h) as for *P. falcatus*. Phloem sap velocity was about 4 times faster for the former, where the label also appeared earlier in the arbuscular mycorrhiza (AM). Finally, in the soil CO_2 efflux the label showed up after 24h under *C. macrostachys* and after 72h under *P. falcatus*, and within the one year chasing period 32% of the assimilated carbon appeared in soil CO_2 efflux under the pioneer angiosperm as compared to 15% in case of the late-successional gymnosperm. Presumably, this larger carbon allocation belowground is due to the large energy investment of the pioneer into the AM to acquire nutrients, whereas in case of *P. falcatus* more of the recently assimilated carbon stays in the plant-soil system as structural carbon. With continuing forest degradation the shift in abundance towards the angiosperm pioneer is proceeding. Likely, such a change towards the pioneer will affect the forest carbon balance, besides leading to a decrease of tree biomass carbon accumulation; more recently assimilated carbon will be lost as soil CO_2 efflux due to the higher energetic investment of the plant belowground.

Keywords: Africa, arbuscular mycorrhiza, carbon allocation, *Croton macrostachys*, forest degradation, *Podocarpus falcatus*, soil CO_2 efflux, stable carbon isotopes, tree canopy labeling,

1. Introduction

To quantify CO_2 exchange between African terrestrial ecosystems and the atmosphere is extremely important in the context of the United Nations Framework Convention on Climate Change (UNFCCC) activities, including the Clean Development Mechanisms (CDM) of the Kyoto protocol and the Reducing Emissions from avoided Deforestation and forest Degradation (REDD). Recently, tropical ecosystems, and particularly African intact forests, have been recognized to be a reasonable carbon sinks in a global carbon cycle (Stephens *et al.*, 2007; Ciais *et al.*, 2009). However, despite several evaluations have been presented on the size of the African carbon stocks (Bombelli, 2009; Ciais *et al.*, 2009; Lewis *et al.*, 2009), the quantification of the carbon fluxes still remain poorly understood (Lewis *et al.*, 2009). The lack of data on African carbon budget in representative African forest ecosystems together with scant information of forest dynamics under anthropogenic pressure makes the regional estimates and biogeochemical models of the carbon cycle highly uncertain (Bombelli, 2009; Ciais *et al.*, 2009). The situation is further complicated as most of the information available refers to intact forests. However, besides deforestation increasing anthropogenic disturbance of natural forests such as intensive removal of trees for timber, firewood collection, and particularly forest grazing puts increasing pressure to forest regeneration with consequences for biodiversity and the stand structure (Tesfaye *et al.*, 2002).

Yet to date there has only been a limited data available on carbon exchange in the large Afromontane forest area in eastern and south eastern Africa. Among them the Ethiopian highlands contribute to more than 50% of the tropical Afromontane vegetation of Africa (Tamrat, 1993). Ethiopia is exemplary for the ongoing processes of deforestation with annual rates of about 1.1 % (FAO, 2009) and forest degradation. The Afromontane forests in central and southern Ethiopia have been described as *Podocarpus* mixed forests and characterized by a mixture of evergreen and deciduous tree species with predominance of *Podocarpus falcatus* (Thunb.) Mirb. and *Croton macrostachys* Del. (Euphorbiaceae) in upper canopy (Friies, 1992). These two coexisting tree species represent different functional types (Whitmore, 1989). While evergreen gymnosperm *P. falcatus* is a climax shade tolerant, the facultative deciduous angiosperm *C. macrostachys* is a pioneer, light demanding tree. *P. falcatus* in Eastern Africa is among the tree species that are locally threatened by illegal cutting and encroachment. Such activities create gaps that favor the abundance of the pioneer tree species *C. macrostachys* and cause problems in the natural regeneration of *P. falcatus* (Teketay, 1997; Tesfaye *et al.*, 2002). In consequence along with the drastic decline of *P. falcatus* (Legesse

Negash, 1995), *C. macrostachys* appears to become the first abundant indigenous tree species of Ethiopian highlands (Taye Bekele *et al.*, 1999).

Such a shift in relative abundance of different functional type of trees may alter the stand-level carbon balance. 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 (e.g., Running and Coughlan, 1988; Pretzsch *et al.*, 2008). The rapid growth of angiosperms as compared to gymnosperms is the result of the evolutionary gained ecophysiological features, including higher photosynthetic rates, fast uptake of available resources, rapid accumulation and cycling of nutrients associated with low investment in wood (Gamalei, 1991; Kozlowski, 1992; Aerts, 1995; Lusk *et al.*, 2003). One key parameter determining terrestrial ecosystem balance, in addition to the carbon accumulation in the biomass, is the temporal and spatial pattern of the assimilated carbon allocation within the plant-soil-atmosphere system (Trumbore, 2006). According to Chambers *et al.* (2001), the ratio of carbon allocated in fast-cycling tissues and compounds in the plant-soil system and the carbon incorporated in structural woody compounds or slow-cycling soil organic matter determines the long-term carbon sequestration. Consequently, the shift in forest vegetation composition and structure may influence CO_2 exchange rates at ecosystem scale (Hanson *et al.*, 2000; Gifford, 2003; Schulze, 2006).

During the last few years considerable progress has been made of ^{13}C and ^{14}C labeling experiments to unravel the quantitative distribution of tree belowground carbon allocation, which represents a sizeable portion of forest gross primary production (Giardina *et al.*, 2005; Litton *et al.*, 2007), and its impact to soil respiration for boreal (Carbone *et al.*, 2007; Högberg *et al.*, 2008; Pumpanen *et al.*, 2009) and temperate (Horwarth *et al.*, 1994; Plain *et al.*, 2009; Ruehr *et al.*, 2009) forests. Root-associated respiration in forested ecosystems contributes about 30–50% of the CO_2 released by whole plant (Poorter *et al.*, 1990) and more than half of annual soil respiration (Hanson *et al.* 2000; Högberg and Read, 2006). There is growing evidence that soil respiration is closely linked to photosynthesis, and recently gained carbon supply may influence the rate of soil CO_2 efflux on short-time (from hours to days) scales as root respiration consumes recently fixed photosynthates (Högberg *et al.*, 2001; Davidson *et al.*, 2006; Högberg and Read, 2006; Trumbore, 2006; Bahn *et al.*, 2009). The proportion of assimilated carbon lost by autotrophic respiration and root exudation tends to be higher for tropical forests than for other forest ecosystems (ranging from 0.65 to 0.85), likely due to a high proportion of acquired carbon investment to root-mycorrhizal respiration (Lloyd and

Farquhar, 1996; Lloyd *et al.*, 2001). Much of the allocated carbon to the soil is made available to mycorrhizal fungi and other soil biota closely associated with roots (Högberg and Read, 2006), and hence the contribution of recently assimilated carbon to soil CO_2 efflux is large (Högberg *et al.*, 2008). Despite of this fundamental progress in the plant-soil interactions of carbon dynamics, it is primarily restricted to boreal and temperate forests, while our knowledge with this respect is scarce for trees of tropical forest ecosystems.

The degraded Afromontane Munessa-Shashemene forest in southern Ethiopia provided an excellent opportunity to study *in situ* temporal and spatial pattern of carbon exchange in the tree-soil-atmosphere system with respect to the ecophysiological traits of coexisting tree species. We exposed two individual trees to $^{13}CO_2$ to follow the translocation of recently assimilated carbon to belowground and its subsequent release in soil $^{13}CO_2$ efflux during a course of one year. We have chosen *P. falcatus* and *C. macrostachys* as two main coexisting tree species with contrasting ecophysiological traits for this study. The specific goals of this experiment were to comparatively evaluate (i) the timing of the recently fixed carbon allocation belowground and its respiratory release by the autotrophic continuum (roots with associated rhizospheric microbiota) and (ii) to assess the fraction of newly assimilated carbon in belowground respiration with respect to tree species ecophysiological traits. We hypothesize that, similar to temperate and boreal forests, there is also a close temporal coupling between canopy photosynthesis and soil respiration in tropical forest trees, when the respiration of the autotrophic continuum is driven by recent photoassimilates. We further suggest that the alteration of the forest vegetation composition caused by forest degradation may have a large impact on carbon cycling within tree-soil-atmosphere. We hypothesize that in case of the angiosperm *C. macrostachys* the velocity of recently assimilated carbon translocation from the tree canopy to soil CO_2 efflux will be faster and the contribution of recent photosynthates to soil CO_2 efflux will be greater as for the gymnosperm *P. falcatus*.

2. Material and Methods

2.1. Site description

The study was conducted in Munessa-Shashemene forest situated in the Ethiopian highlands, on the eastern escarpment of the southern Main Ethiopian Rift Valley, about 250 km south of Addis Ababa (7°26' N, and 38°52' E). The climate is sub-humid with mean annual temperatures of about 15°C; and annual rainfall of about 1500 mm (Fritzsche *et al.*, 2007). The rainfall pattern is, in general, bimodal but the rainfall occurrence and duration erratically

varies between years (Yohannes *et al.*, 2011). The soils are rich in clay and iron oxides, being classified as Mollic Nitisol according to the WRB system (FAO, 1998). The study plot was established in a *Podocarpus-Croton* patch of the natural forest (2300 m a.s.l.). The forest is strongly degraded by grazing and illegal logging activities. The pioneer *C. macrostachys* is favored by disturbances and concentrates in plots with canopy gaps. The dominant canopy species are *Croton macrostachys* Hochst. ex Del (143 trees ha^{-1}) and *Podocarpus falcatus* (73 trees ha^{-1}), tree species with relatively less abundance include *Prunus africana, Syzygium guineense* (Wild.) DC., *Celtis africana* Burm. f. and *Pouteria adolfi-friederici* (Engl.) (Tesfaye *et al.*, 2010).

2.2. Experimental setup

In July 2008, two pairs (control and experimental) of *C. macrostachys* and *P. falcatus* trees of the third height class (Tesfaye *et al.*, 2010) were selected at the distance of *c.* 100 m. The trees in pairs were similar in height but differed in stem diameter and foliage dry mass (Table 3.1).

Table 3.1: Main eco-physiological traits and characteristics of experimental trees.

Sample	*Croton macrostachys*	*Podocarpus falcatus*
Functional group	Gymnospserm	Angiosperm
Leaf habit	Facultative shedding, deciduous	Evergreen, coniferous
Life strategy	Pioneer	Late successional
Growth rate	Fast	Slow
Mycorrhiza type	Arbuscular mycorrhiza	Arbuscular mycorrhiza
Shade tolerance	Light demanding	Shade tolerant
Height, m	5.1*; 5.6†	6.2*, 5.6†
§DBH, cm	4.5*, 4.1†	11.5*, 9.4†
§Foliage biomass, g	780*	4910

* labeled tree; †control tree; §dry weight, §DBH (diameter at breast height, 1.3m from the ground).

Five permanent PVC collars (20 cm diameter) were randomly installed in 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 a soil CO_2 efflux. The ground area underneath the canopies where the collars have been placed covered a surface of 12.7 m^2 under *C. macrostachys* and 16.7 m^2 under *P. falcatus*. We assumed these areas to contain the main part of the roots of each considered tree (Abate, 2004). The roots were trenched around the corresponding areas at 50 cm depth.

2.3. Pulse labeling

Short-term $^{13}CO_2$ pulse labeling of the target trees was carried out in two consequent cloudless days (on November, 12 and November, 14, 2008) on the offset of the main rainy

season. For carrying out the ^{13}C pulse-labeling experiment, rectangular wooden frames made of eucalyptus poles were constructed around the individual trees. Greenhouse plastic cover (UVA Clear, Ginegar Plastics Products Ltd, Israel: 120 micron, 88% light transmission in PAR) was pulled over the frame and tightly sealed before the labeling event within 30 min. To prevent transport of $^{13}CO_2$ from the chamber space into soil pores, the chamber was also sealed with the plastic film from the bottom side at 1.60 m distance from the forest floor. The corresponding chambers volumes were of 58.4 m^3 for *C. macrostachys* and of 71.1 m^3 for *P. falcatus*. Five electric fans (12V, 0.21A) installed for circulated the air in the chambers while labeling. Inside chamber CO_2 concentration, air temperature, and relative humidity were monitored continuously using a LI-8100 infrared gas analyzer (LI-COR Inc., Lincoln, NE, USA). Fifteen minutes after the chambers were sealed, and the decline of the CO_2 concentration inside the chambers clarified carbon dioxide uptake, ^{13}C-labeled CO_2 was generated by injecting diluted sulfuric acid into a flask containing isotopically enriched $Na_2{}^{13}CO_3$ solution (99 atom% ^{13}C; Campro Scientific, Berlin, Germany), and a quantity of 12.5 mM of $^{13}CO_2$ per 1 m^3 was added to each chamber. The labeling duration was 85 min and 105 min for *C. macrostachys* and *P. falcatus*, respectively. Thereafter the plastic cover was removed and then the chamber was opened (defined as sampling time 0).

There were no significant differences in environmental conditions in both labeling experiments. During the labeling period the air temperature inside the *C. macrostachys* and *P. falcatus* chambers increased to a maximum of 29.4°C and 27.3°C, respectively, being *c.* 5°C higher than the ambient temperature. Relative air humidity increased from 58% to 79% within the *C. macrostachys* chamber and from 56% to 82% within the *P. falcatus* chamber, as compared with the ambient relative air humidity, which increased from 58 to 67% and 56 to 69% during the labeling period of the two consecutive days. Photosynthetically active radiation (PAR) within the chambers averaged 1050 and 990 μmol m^{-2} s^{-1} for *C. macrostachys* and *P. falcatus*, respectively. While the ambient PAR ranged between 495 and 1070 μmol m^{-2} s^{-1}.

2.4. Sample collection

Leaves, phloem sap, soil CO_2 efflux, and soil samples from 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 with 4 hours interval, while soil CO_2 efflux was collected 12 hours after the labeling.

Leaves of all age classes were randomly sampled from four directions from top, middle and bottom part of the labeled and control trees crown. All foliage samples were dried for 48 h at 65°C and ground before subsequent analyses. Phloem soluble sugars were extracted according to the phloem exudation method described by Gessler *et al.* (2004). Briefly, pieces of phloem tissues (about 1 cm x 1 cm) were removed with a scalpel from stems of labeled and control trees at 1.3 and 0.5 m height. The wood tissues 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.

Within four hours of collection of the soil samples, they were sieved <2 mm, and the adhering soil method (Phillips and Fahey, 2006) was employed to gain the rhizosphere soil for analysis of the neutral lipid fatty acid (NLFA) 16:1ω5, a marker for arbuscular mycorrhiza (AM) (Olsson, 1999). The adhering soil as well as the bulk soil was kept frozen until NLFA extraction.

A LI-8100 infrared gas analyzer supplied with 8100-103 Survey Chamber (LI-COR Inc., Lincoln, NE) was used for soil CO_2 efflux measurements and air sampling. A soil chamber was tightly fitted on the PVC collars (n=5), and the air samples for estimating ^{13}C signature evolved from soil were collected from the LI-COR stream. For this, a T-fitting with septum was installed between the analyzer unit and the survey chamber. At each sampling time, gas samples were taken from all 5 PVC collars at the reference and labeled plots, respectively. For every collar 15 ml of air was sampled with a 20-ml syringe equipped with 6-cm long needle and injected into a previously evacuated 12 ml glass vials (Exetainer, Labco Ltd, High Wycombe, UK) to produce an overpressure preventing any contamination with ambient air during storage. In addition, at each sampling time, five air samples were taken at the labeled and control plots at approximately 100 ppm steps of increasing CO_2 concentration inside the chamber to establish Keeling plots (Keeling, 1958).

2.5. Stable carbon isotope analysis and calculations

The stable carbon isotope composition was analyzed for leaves, water soluble phloem sugars, NLFA extracted from adhering and bulk soil, and soil-derived CO_2. For stable carbon isotope ratios in leaves, *c.* 1 mg of finely ground material was weighed into tin capsules and analyzed either on a Thermo Finnigan MAT DELTA^{plus} Advantage isotope ratio mass spectrometer (Thermo Electron Corporation, Waltham, USA) coupled to an Euro EA 1110 C/N analyzer (EuroVector SpA, Milan, Italy) or on an Elementar IsoPrime 100 IRMS (IsoPrime Ltd.,

Cheadle Hulme, UK) coupled to an Elementar vario MICRO cube EA C/N analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

For compound-specific isotope 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, in the first step, sample compounds were separated by the HPLC system. In the second step, the separated compounds were oxidized to CO_2 in the Finnigan LC IsoLink Interface, excess O_2 was removed by oxidizing elemental copper, and the O_2-free gas stream was transferred to the mass spectrometer for stable isotope analysis. Standards were referenced with EA/IRMS (Euro EA 1110 CN analyzer coupled to a Finnigan MAT DELTA^{Plus} 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 Wild *et al.* (2010). Based on the individual concentrations and isotope composition of the sugars glucose, fructose and sucrose a weighted average of the isotope composition of the hexoses in the phloem sap was calculated.

Lipid extraction followed the method of Frostegård *et al.* (1993). About 1g and 1.5g of adhering and bulk soil, respectively, were extracted using a chloroform-methanol citrate buffer (1:2:0,8, v/v/v), followed by solid phase extraction with silica SPE columns (Varian Bond Elut LRC-Si, Agilent Technologies, Santa Clara, CA) to obtain the neutral lipids by chloroform extraction. Thereafter, the neutral lipds were subjected to mild alkaline methanolysis to obtain the NLFA methyl esters. The derivatized NLFA were separated by gas chromatography (Agilent 7890A, Agilent Technologies, Santa Clara, CA), oxidized to CO_2 by an Isoprime GC V Interface, and measured for stable carbon isotopes on an Elementar IsoPrime 100 IRMS. Integration for each peak was checked and corrected manually, and the $\delta^{13}C$ enrichment of the NLFA was corrected for the carbon added in the methanolysis step of the fatty acid analysis procedure.

The stable carbon isotopic ratios in gas samples were analyzed by continuous-flow isotope-ratio mass spectrometry on a Thermo Finnigan Delta V Advantage Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled to a Finnigan GasBench. Isotope values are expressed in δ notation (‰), relative to the Vienna Pee Dee Belemnite (VPDB) standard.

The isotopic signature of the air respired from soil was estimated by calculation of the intercept *a* of the Keeling plot relationship at each sampling time.

$$\delta^{13}C \text{ in soil } CO_2 \text{ efflux} = \delta^{13}C \text{ in sample } CO_2 + a\ (1/[CO_2] \text{ sample}) \quad (1)$$

Keeling plots with $R^2 < 0.9$ were discarded. To correct the isotopic composition of soil CO_2 for the discrimination during diffusion in the soil, 4.4‰ was subtracted from the calculated $\delta^{13}C$ values (Cerling *et al.*, 1991).

For the foliage biomass and the soil CO_2 efflux, the $\delta^{13}C$ values (‰) were converted to ^{13}C sample excess as follows. First, the $\delta^{13}C$ notations were converted to the absolute isotope ratio ($^{13}C/^{12}C$) of the sample (R_{sample}):

$$R_{sample} = [(\delta^{13}C/1000+1)]\ R_{standard} \quad (2)$$

where $R_{standard}$ = 0.0111802, the standard value for isotope ratio of VPDB. The fractional abundance (A) of ^{13}C relative to $^{12}C+^{13}C$ was then related to R by equation:

$$A = R_{sample}/(R_{sample}+1) \quad (3)$$

The excess of ^{13}C in the leaves of the labeled trees (*excess* $^{13}C_{label}$, g) was calculated by multiplication of the difference in the fractional abundances in leaves of the labeled trees (A_{label}) and the control tree ($A_{control}$) with the leaf biomass carbon of the labeled trees (C_{label}, g):

$$excess\ ^{13}C_{label} = (A_{label} - A_{control})\ C_{label} \quad (4)$$

To calculate the excess ^{13}C in CO_2 efflux under the labeled trees as compared to that under the control trees at each sampling point, the fractional abundance A (equation 3) and the soil CO_2 efflux rate (C_{sample}, g C m^{-2} h^{-1}) were used to calculate the amount of ^{13}C evolved from soil ($^{13}C_{sample}$, g ^{13}C m^{-2} h^{-1}) under the labeled and control trees:

$$^{13}C_{sample} = A\ C_{sample} \quad (5)$$

The excess of ^{13}C in soil CO_2 efflux under the labeled trees at a given time ($excess\ ^{13}C_{label}$, g m^{-2} h^{-1}) was calculated by subtracting the mass of ^{13}C in soil CO_2 efflux under the control trees ($^{13}C_{control}$, g m^{-2} h^{-1}) from that under the labeled trees ($^{13}C_{label}$, g m^{-2} h^{-1}):

$$excess\ ^{13}C_{label} = {}^{13}C_{label} - {}^{13}C_{control} \quad (6)$$

The cumulative excess of ^{13}C in soil CO_2 efflux between two sampling points ($\sum_{t-1}^{t+1} excess\ ^{13}C$, g ^{13}C m^{-2} time $period^{-1}$) was calculated according to:

$$\sum_{t}^{t+1} excess\ ^{13}C = ((excess\ ^{13}label_t + excess\ ^{13}label_{(t+1)})/2)(t+1-t) \quad (7)$$

where excess $^{13}label_t$ and excess $^{13}label_{t+1}$ is the excess of ^{13}C in soil CO_2 efflux (g ^{13}C m^{-2} h^{-1}) at two consecutive sampling points, and t and $t+1$ define the time interval (h) between the two sampling points. The cumulative excess ^{13}C in soil CO_2 efflux during the 365 days chasing period ($\sum excess\ ^{13}C$) 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 in the one-years time course efflux using SigmaPlot 11 (Systat Software Inc., San Jose, CA, USA). Mean residence time *(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 $^{12}CO_2$ (Mordacq *et al.*, 1986; Tohjima *et al.*, 2009). Therefore, the ^{13}C assimilation was estimated by two independent indirect ways. Approach I 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 the label (Svejcar *et al.*, 1990). Thus the measured CO_2 assimilation 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 (Ehleringer, 1991), 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 (Bonham, 1989; Abate, 2004). Fifteen *Croton macrostachys* and 15 *Podocarpus falcatus* trees of third height class were selected based on comparable tree height and diameter at breast height. From each tree, 15 randomly selected branches were cut and 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 single exponential function $y = a\ exp^{bx}$, with R^2 of 0.70 and 0.87 for *C. macrostachys* and *P. falcatus*, respectively, with $P<$ 0.0001. The foliage biomass of each branch was estimated and summed up to obtain the tree foliage biomass. From this the foliage biomass carbon was calculated by multiplication with the leaf carbon concentration of 437 mg g^{-1} for *C. macrostachys* and 462 mg g^{-1} for *P. falcatus*. Then the overall uptake of the ^{13}C label by the tree leaves was determined by equation 4.

3. Results

3.1. ^{13}C assimilation and recovery in foliage

Carbon dioxide concentration declined inside the chambers during the labeling, indicating a photosynthetic uptake by both trees (Fig. 3.1).

The rate of carbon uptake by angiosperm *C. macrostachys* was greater (12.7±2.2 μmol m^{-2} chamber basal area s^{-1}) than that by gymnosperm *P. falcatus* (8.5±3.1 μmol m^{-2} chamber basal area s^{-1}). The overall ^{13}C assimilation as estimated by the decline in the CO_2 concentration in the chamber headspace with above mentioned corrections (approach 1) were *c.* 0.4 and 0.5 mol, or 5.2 and 6.5 g by *C. macrostachys* and *P. falcatus*, respectively.

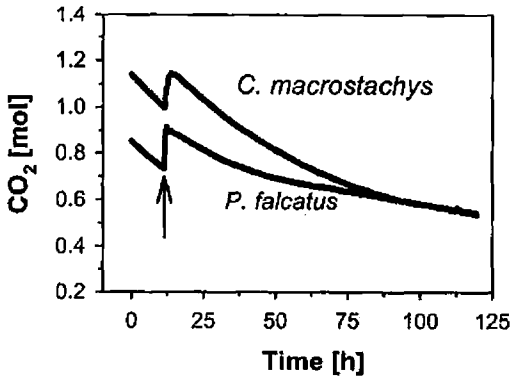

Fig. 3.1: Change in the apparent amount of CO_2 (mol) in chambers of *Croton macrostachys* and *Podocarpus falcatus* during the $^{13}CO_2$-labeling period. The decline in the amount of CO_2 reflects the predomination of photosynthesis over leaf and stem respiration. The arrow shows the release of 12.3 mmol $^{13}CO_2$ m^{-3} chamber volume.

Before labeling there were no significant differences between the labeled and the reference plots in ^{13}C natural abundance of foliage the foliage (Fig. 3.2a, b). Immediately after the chambers were opened (i.e., sampling time 0), the $\delta^{13}C$ of the foliage of *C. macrostachys* and *P. falcatus* labeled trees was significantly elevated as compared to control ($P< 0.001$), with a $\delta^{13}C$ value of 1557±871‰ for *C. macrostachys* and of 248±50.5‰ for *P. falcatus*. Nevertheless, the greater net assimilation rate of *C. macrostachys* was offset by the larger foliar biomass of the *P. falcatus* resulting in only minor differences in the total amount of ^{13}C in the foliage of the two species.

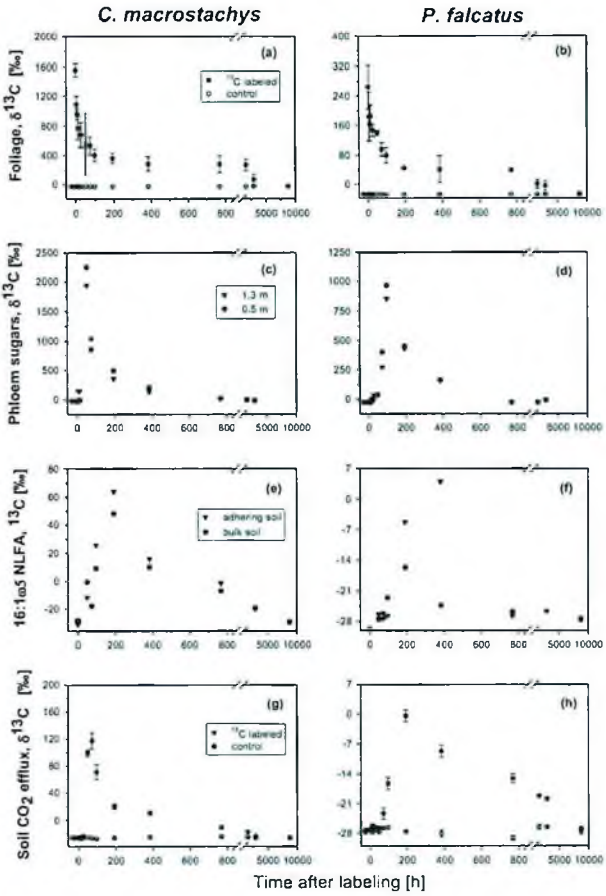

Fig. 3.1: Time course of the ^{13}C label in plant-soil compartments of *Croton macrostachys* and *Podocarpus falcatus* during the one year chasing period. Shown are (a) leaves of *C. macrostachys*, (b) leaves of *P. falcatus*, (c) weighed sum of soluble sugars in tree phloem sap at 1.3 m and 0.5 m above ground of *C. macrostachys*, (d) weighed sum of soluble hexoses in tree phloem sap at 1.3 m and 0.5 m above ground of *P. falcatus*, (e) 16:1ω5 NLFA in adhering and bulk soil under *C. macrostachys*, (f) 16:1ω5 NLFA in adhering and bulk soil under *P. falcatus*, (g) soil CO_2 efflux under *C. macrostachys*, (h) soil CO_2 efflux under *P. falcatus*. For the sake of clarity of the figure, we omitted to show the $\delta^{13}C$ values for the control of soluble sugars in tree phloem sap and the 16:1ω5 NLFA in adhering and bulk soil. The former was -25.53±0.85‰ for *C. macrostachys* and -25.36 ± 0.33 ‰ for *P. falcatus*, and the latter was -27.7±1.4‰ for *C. macrostachys* and -29.3±1.1‰ for *P. falcatus*. For leaves and soil CO_2 efflux data are means ± standard deviation (n=5). No replicates were taken for phloem sap extraction and soil cores to avoid destructive impact to the plant-soil system.

Excess of ^{13}C in the foliage of, i.e. the ^{13}C assimilation calculated with approach 2, was 5.9±0.3 for *C. macrostachys* and 6.9±1.3 g for *P. falcatus*. This result fits well to the ^{13}C assimilation as estimated by the $^{13}CO_2$ uptake rate and suggests a pulse labeling efficiency of 61.3% for *C. macrostachys* and 59.4% for *P. falcatus*. In both species, the label in leaves became ^{13}C-depleted with time, and within the one year period of observation approached the isotopic signature of the leaves in the reference trees (Fig. 3.2a, b). The tree species differed in their ^{13}C depletion rate, being faster for *C. macrostachys* than for *P. falcatus* (Fig. 3.3).

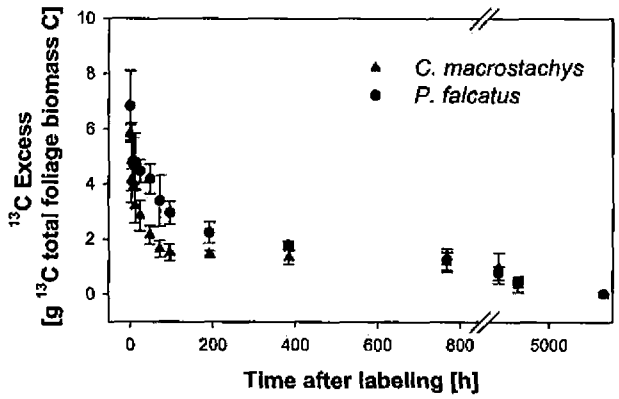

Fig. 3.2: Time course of excess of ^{13}C in total foliage biomass of *Croton macrostachys* and *Podocarpus falcatus* during the one year chasing period. Data are means ± standard deviation (n=5). Parameters are shown in Table 3.2.

Table 3.2: Relative size of fast and slow pool in foliage and soil CO_2 gas samples, 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}C.

Carbon pool	*C. macrostachys*			r^2	*P. falcatus*			r^2
	Size	MRT	Half		Size	MRT	Half	
	%	h	h		%	h	h	
Leaves								
Fast pool	65	14	10	0.97	45	55	38	0.94
Slow pool	30	2000	1386		38	1111	770	
Soil CO_2 gas								
Fast pool	17	417	289	0.99	15	2000	1386	0.97
Slow pool	23	10000	6930		-	-	-	

3.2. ^{13}C translocation in belowground

There was a significant difference between the trees in the velocity of the recent photoassimilates translocation down to the root system via phloem. Soluble sugars in phloem

sap of *C. macrostachys* were enriched in ^{13}C at 1.3 m stem height already 4 to 8 hours after labeling, and the tracer peaked in the following 40 hours at 1.3 and 0.5 m stem heights (Fig. 3.2c). For *P. falcatus* the first evidence of the elevated ^{13}C in phloem sap sugars was 4 hours later, and $\delta^{13}C$ reached a peak at 1.3 m and 0.5 m height between 72 and 96 hours after the labeling event (Fig. 3.2d). The soluble phloem sugars of *C. macrostachys* were represented by mono- and disaccharides with predominance of sucrose (up to 70%). In contrast, the phloem sap of *P. falcatus* contained in addition the indirect product of photosynthesis D-1-*O*-methyl-*muco*-inositol (OMMI), with a proportion of about 42% of the transported sugars and polyols. Within the chasing period in both trees the mono- and disaccharides in the phloem sap became exponentially ^{13}C depleted (r^2>0.93, P<0.0001), reaching the natural abundance of the control of *c.*-26‰ before the end of the chasing period. In contrast, the OMMI in the *P. falcatus* phloem sap remained isotopically enriched with about 10‰ above the control even after one year (data not shown).

The adhering soil (0-10 cm depth) underneath *C. macrostachys* showed a significantly larger concentration of AM NLFA 16:1ω5 compared to *P. falcatus* (18.1±4.59 *versus* 10.39±4.81 nmol g of dry soil^{-1}). The same was true for the bulk soils (11.1±1.8 *vs.* 6.1±2.7 nmol g of dry soil^{-1}). The AM fungal NLFA biomarker became ^{13}C enriched synchronal to the transport of the label within the phloem (Fig. 2e, f). Under *C. macrostachys* the AM ^{13}C NLFA in adhering soil was elevated as compared to natural abundance already 48 hours after the labeling, and peaked 4 to 8 days after the labeling. The temporal pattern of the tracer in the AM NLFA in bulk soils was similar in shape, but showed smaller ^{13}C enrichment. For *P. falcatus* highest ^{13}C enrichment of the AM fungal NLFA in adhering and bulk soil occurred later (8 to 16 days after labeling) and less pronounced.

3.3. ^{13}C recovery in soil CO_2 efflux

The soil CO_2 efflux rate did not differ significantly between the tree species at the beginning of the chasing period (i.e., 4.8±0.3 μmol m^{-2} s^{-1} under *C. macrostachys* compared with 4.6±0.5 μmol m^{-2} s^{-1} under *P. falcatus*). With decreasing soil moisture during the first four months after labeling, the CO_2 efflux rates decreased concurrently to 3.1±0.3 μmol m^{-2} s^{-1} and 2.8±0.6 μmol m^{-2} s^{-1} for the former and for the latter (data not shown).

The timing when the tracer appeared in the soil CO_2 efflux under the labeled trees reflected the temporal course of the label in the phloem sap and the AM NLFA. Beneath *C. macrostachys* soil CO_2 from all 5 collars coincidently showed a remarkable enrichment in ^{13}C

(99.3±4.6‰) already 48 hours after the labeling, tending to a maximum within the next 24 hours (Fig.2.2g). In soil CO_2 efflux evolved from the 5 collars under *P. falcatus* the first evidence of the ^{13}C label occurred around 72 hours after the labeling, and the maximum enrichment (3.9±1.4‰) was recorded at day 8 of the chasing period (Fig.2. 2h). The time lag between ^{13}C photosynthetic uptake and release in soil CO_2 efflux was calculated by fitting a quadratic function to the relationships between $\delta^{13}C$ and the time after labeling (Dannoura *et al.*, 2011). The lag times between photosynthesis and soil CO_2 evolution were 29 and 51 hours for *C. macrostachys* and *P. falcatus*, respectively. In both trees, the $\delta^{13}C$ values in soil CO_2 efflux exponentially depleted with time (r = 0.95 for *C. macrostachys* and 0.93 for *P. falcatus*), being faster *for C. macrostachys*, but approaching natural abundance level at the end of the chasing period under both tree species (Fig. 2.2g, h). Despite the comparable values of the total ^{13}C assimilated by the trees during the labeling, the label recovered in the cumulative soil CO_2 efflux after the one year period under *P. falcatus* (14.9±2.4%) was half of that than under *C. macrostachys* (32.2±3.3%) (Fig. 3.4).

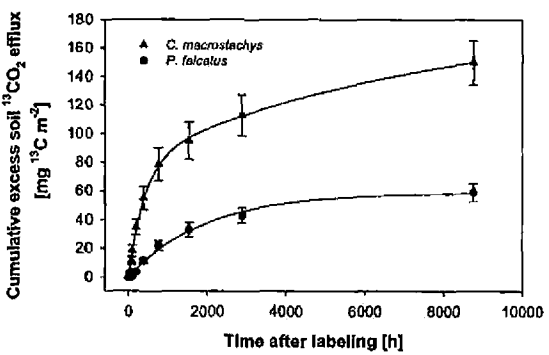

Fig. 3.3: Time course of cumulative excess of ^{13}C in soil CO_2 efflux under *Croton macrostachys* and *Podocarpus falcatus* during the one year chasing period. Data are means ± standard deviation (n=5). The curves are fitted with a double exponential function for *C. macrostachys* and a single exponential function for *P. falcatus*. Parameters are shown in Table 3.2.

In case of *C. macrostachys* the efflux of the label with soil CO_2 followed a double-exponential function, showing that 17% of the overall assimilated carbon was released as fast pool within 17 days (Table 3.2). In contrast, the kinetics of the label recovery in cumulative soil CO_2

efflux under *P. falcatus* did not allow distinguishing between two sources of the soil respiration.

4. Discussion

4.1. Labeling

For the whole tree ^{13}C labeling in the remote field area, we used a crown labeling chamber approach suggested by Simard *et al.* (1997), who isolated the soil surface from the chamber headspace. The crown labeling chamber (i) prevented diffusion of $^{13}CO_2$ to soil pores and later back to the atmosphere and (ii) avoided labeling of understory vegetation and thus increases the accuracy of $^{13}CO_2$ efflux measurements under the target trees (Plain *et al.*, 2009). We used two independent approaches to calculate the total amount of assimilated ^{13}C by the trees. The first one was based on the average rate of the $^{13}CO_2$ uptake estimated as a function of the carbon dioxide concentration decline over the labeling time, while the second approach estimated the ^{13}C excess in leaves immediately after the labeling. Despite some assumptions, like chamber temperature and pressure application for the ideal gas law along with uncertainties due to correction of the absorption spectra of the infrared gas analyzer in the first case, and the rough foliage biomass evaluation in the second one, the two values gave reasonable estimates of the labeling efficiency (Brodribb *et al.*, 2007). Hence, we feel confident that the overall uptake of the ^{13}C label was adequately expressed.

In agreement with the functional trait, the photoassimilation rate of the angiosperm pioneer *C. macrostachys* was higher as compared to gymnosperm late-successional *P. falcatus*. Also Lüttge *et al.* (2003) and Fetene and Beck (2004) showed that *C. macrostachys* had much higher photosynthesis and transpiration rates than *P. falcatus*. Larcher (1969) and Bazzaz (1979) reported larger photosynthesis and growth rates being achieved by pioneer tree species than by late-successional trees. It is also further widely accepted that species with a longer leaf lifespan (i.e., conifers) have a lower photosynthetic capacity per unit mass as well as per unit area (Wright *et al.*, 2004).

4.2. Kinetics of label recovery and carbon allocation

The pattern of the recently fixed ^{13}C allocation belowground and with soil CO_2 efflux back to atmosphere differed significantly between *C. macrostachys* and *P. falcatus* in time and magnitude. The kinetics of the label recovery in the foliage of both trees suggests that there were two main pools of the recently assimilated carbon (Table 3.2). These pools differed in size and MRT, but for both trees the first fast pool of recent carbohydrates exceeded the

second one, especially for *C. macrostachys*, where this pool was twice the size of the second one. While the labeled products of photosynthesis from the fast pool in *C. macrostachys* leaves were transported in about 14 hours; for *P. falcatus* this process took more than 2 days. This is even longer than the MRT of 32 hours for the recently fixed carbon in the fast pool in needles of young Scots pine trees (Högberg *et al.*, 2008). Nevertheless, part of recently assimilated carbon remained in leaves most likely as reserve compounds, such as starch, or being incorporated in structural components. Leaves with a longer lifespan, as in case of *P. falcatus*, tend to accumulate secondary compounds (i.e., cellulose and phenolics), while those with a shorter lifespan, as the *C. macrostachys* leaves, contain larger amounts of proteins (Poorter and Villar, 1997).

The time lag between the ^{13}C uptake and the appearance of ^{13}C enriched sugars in phloem sap was about twice longer for *P. falcatus* than for *C. macrostachys*. The velocity of the phloem transport in the two study trees were approximately calculated as 0.4 m h^{-1} (*C. macrostachys*) and 0.1 m h^{-1} (*P. falcatus*). Due to coarse time resolution of phloem sap sampling (4 hours during the first day and then at daily course for the next 4 days), these numbers are subjected to a source of error. Nevertheless, this agrees with estimates that most recently assimilated carbon reaches the phloem within hours to days (e.g., Keitel *et al.*, 2003; 2006; Plain *et al.*, 2009; Kuptz *et al.*, 2011) and supports the marked differences in carbon phloem transport velocity between deciduous and evergreen species (Pumpanen *et al.*, 2009; Kuptz *et al.*, 2011). The different time resolution of belowground transport between gymnosperm *P. falcatus* and angiosperm *C. macrostachys* could be attributable 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 (Gamalei, 1991; Kozlowski, 1992; Brodribb *et al.*, 2007). The differences in the non-structural carbohydrate composition between the trees species also reflects the evolutional traits of the phloem with uniformity of transport forms of assimilates represented mainly by sucrose in angiosperms (Gamalei, 1991) such as *C. macrostachys*. In contrast, OMMI being present in the phloem sap of *P. falcatus* and other cyclic polyols are ubiquitous in gymnosperms. As they are no direct products of the primary metabolism, they behave more conservatively, becoming more slowly enriched as sugars but keeping the label over the whole period of observation.

Mirroring the temporal resolution of the ^{13}C in the phloem sap, the 16:1ω5 NLFA under *C. macrostachys* showed an earlier and much more pronounced ^{13}C enrichment followed by a

steeper decline than that under *P. falcatus*, implying a faster turnover of carbon through the fungal symbiont in case of the former. We observed a similar temporal pattern of 16:1ω5 NLFA biomarker enrichment in the adhering and bulk soils under both trees, presumably indicating the time for translocation of the recently assimilated carbon within the root-AM continuum.

In line with the different velocities of the ^{13}C flux in the phloem sap and through the mycorrhiza, the time lag between the ^{13}C assimilation by the foliage and the tracer release in soil CO_2 efflux was almost 2 times longer for gymnosperm *P. falcatus* than for angiosperm *C. macrostachys*, with estimated carbon transfer rates from the tree canopy to the roots of about 0.12 m h^{-1} and 0.18 m h^{-1}, respectively. For the angiosperm this result is in accordance to the 8-m tall European beech trees, for those Plain *et al.* (2009) reported a time lag of 2 to 3 days. Also for the gymnosperm values are comparable with reports in the literature. Andrews *et al.* (1999) identified a time lag of 7 days for a *Pinus taeda* forest that was labeled in a FACE experiment. As the time lag between the ^{13}C assimilation and the tracer release in soil CO_2 efflux is defined by the phloem transport velocity, it reflects the evolutionary differences between angiosperms and gymnosperms.

Despite the total soil CO_2 efflux rate under the trees during the chasing period was comparable (Yohannes *et al.*, 2011), the total amount of the label recovered in the soil CO_2 efflux over the period of observations was half for gymnosperm late-successional *P. falcatus* as compared with pioneer angiosperm *C. macrostachys*, suggesting a larger share of recently assimilated carbon on total soil CO_2 efflux for the latter. This refers particularly for the first *c.* 2 weeks of the chasing period, and the temporal resolution of the cumulative label recovered in soil CO_2 efflux under the study trees suggested that two different carbon sources with different MRT contributed to soil respiration under *C. macrostachys*. The first pool with a MRT of 17 days is closely connected to the current photosynthesis and possibly reflects the direct use of recent assimilates by root respiration, transfer to fungal symbionts with subsequent mycorrhizal respiration, and exudation into the rhizosphere becoming available for rhizospheric bacteria (Högberg and Read, 2006; Kuzyakov and Gavrichkova, 2010). With 17% of the overall assimilated ^{13}C, this first pool represented about the same share of the overall assimilated label, as was found in the soil CO_2 efflux under *P. falcatus* during the course of the whole year of observation. This clearly shows the faster carbon allocation in case of the deciduous pioneer. Recently assimilated carbon by *P. falcatus* was translocated to the belowground at a slower rate. The carbohydrates were partly stored in leaves and phloem

and then allocated to roots during the whole period of observation. This more conservative flow of photoassimilates to the autotrophic continuum may contribute to the lack of clear distinction of different pools in soil CO_2 efflux.

Both trees species under investigations are associated with AM, with larger colonization for C. *macrostachys* as compared with *P. falcatus* (Wubet *et al.*, 2009). This is also obvious from the larger 16:1ω5 NLFA concentrations in soil under the pioneer. The late secondary successional species have a lower dependence on AM fungi, as compared to pioneer trees, where the AM influence root system development and mediated increased carbon fixation by the canopy (Zangaro *et al.*, 2003). Mycorrhizal colonization further increases the proportion of plant carbon allocated below ground (Douds *et al.*, 1988), and has been associated with higher rates of root respiration (Valentine and Kleinert, 2007) and soil CO_2 release (Langley *et al.*, 2005). According to Kozlowski (1992), AM symbionts may use about one quarter of the carbon assimilated by the tree. Higher rates of respiration by the autotrophic community in AM-colonized plants can be related to enhanced 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 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 (Hughes *et al.*, 2008). In fact, the deciduous pioneer *C. macrostachys* is characterized much larger nutrient concentrations and turnover as the coniferous late-successional *P. falcatus* (Gindaba *et al.*, 2004), thus enhancing the necessity of carbon consuming acquisition of soil nutrients.

The second pool describes the soil $^{13}CO_2$ efflux released by heterotrophic decomposition of structural biomass (Ryan and Law, 2005). Since the leaf litter was collected over the chasing period and thus 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 rhizobiota. No data are available about the root turnover of the trees under investigations, but assuming, that root lifetimes in broadleaf tropical forests (annual precipitation >1000 mm) range from 0.4 to 3.2 years (Gill and Jackson, 2000), and the root life span is longer for slow growing than for fast growing tree species (Eissensat and Yanai, 2002), we suggest a higher longevity of roots from slow growing *P. falcatus* as compared with the fast growing *C. macrostachys*. 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 general, the temporal pattern of the recently fixed carbon allocation belowground is mainly defined by certain consequent steps: (i) phloem loading and the time needed to transport the carbohydrates from assimilating organs to sink (particularly the roots), (ii) utilization of the carbon as a source for autotrophic continuum (roots and associated microorganisms including mycorrhiza) and, finally, (iii) the physical diffusion through the soil (Kuzyakov and Gavrichkova, 2010). While due to the similarity in physical soil properties (Yohannes *et al.*, 2011), the latter should be the same for both investigated trees, the other processes differed with respect to the dynamics of carbon exchange depending the trees on physiological traits. A picture emerges that the angiosperm pioneer *C. macrostachys* pumped recently assimilated carbon much faster and at higher quantities to the soil than the gymnosperm late-successional *P. falcatus*. We acknowledge that our findings are limited to two individual trees at annual timescale. However, it has been known that such functional attributes as photosynthetic capacity, maximum leaf conductance and $\delta^{13}C$ signature seem to be clearly different between pioneer and non-pioneer tree species with minor or no overlap between the two groups (Meinzer, 2003). In addition, the adaptive evolution the life forms from evergreen gymnosperm to angiosperm involved an intensification of such ecophysiological constrains as phloem loading capacity, increasing the linear velocity of the phloem transport, sinks strength and quantitative coordination of the chemical content of the phloem exudates (Gamalei, 1991). All these adaptive traits resulted in an accelerated growth activity of angiosperms pioneers, associated with higher rates of water and nutrient uptake coincidently with increased substrate availability enhances energy demand by AM-colonized roots in the belowground autotrophic continuum (Hughes *et al.*, 2008).

5. Conclusions

Our study shows that canopy chamber ^{13}C pulse-labeling of c. 6 m tall trees is also possible under non-optimal conditions with limited facilities and use of local equipment for chamber construction. Restrictions with transport of $^{13}CO_2$ in pressured bottles can be solved by the use of $Na_2{}^{13}CO_2$ and releasing $^{13}CO_2$ by dissolving the salt in H_2SO_4. A more frequent sampling at the early phase of the chasing period would be advantageous to track more detailed the kinetics of the fast pool of the recent assimilates. However, the use of a diode laser spectrometer for continuous measuring of $^{13}CO_2$ is difficult in remote areas due to its high energy demand, and the invasive phloem sap and soil sampling must be kept at a minimum to prevent impact of the experiment on the studied processes.

As was shown before for trees of boreal and temperate forests, also for the two investigated trees in the Ethiopian Afromontane forest there is 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. With that our study supports recent findings showing a close temporal coupling between tree canopy photosynthesis and belowground activity. Further, the different functional traits of the two tree species are also reflected in their carbon allocation. The transport of recently assimilated carbon from the tree canopy to soil CO_2 efflux in case of the angiosperm pioneer *C. macrostachys* was faster as for the gymnosperm late-successional *P. falcatus*. Within the one-year course of the ^{13}C chasing, about twice as much carbon was released back to the atmosphere as CO_2 in case of the pioneer than in the late-successional tree. Presumably, this is due to the large energy investment of the pioneer into the AM mycorrhiza to acquire nutrients. In consequence, more carbon is pumped belowground that is turned over faster in soil. Conversely, in case of *P. falcatus* more of the recently assimilated carbon stays in the plant-soil system (presumably as structural carbon components in above and below-ground biomass and more slowly turning over soil organic matter).

As the continuing forest degradation in the Afromontane forest under study leads to the formation of more gaps, the shift in abundance towards the angiosperm pioneer is proceeding. Knowing that generalizing gas exchange studies carried out on individual trees to canopies and whole forests is problematic (Ehleringer and Field, 1993), we suggest that such a change in the angiosperm and gymnosperm abundance will affect the forest carbon balance. Probably this shift in tree species abundance may not affect the soil organic carbon storage on short time scale, but it likely leads to a decrease of tree biomass carbon accumulation (see also Schulze *et al.*, 2006) and to an acceleration of the carbon exchange in the tree-soil-atmosphere system.

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List of Publications

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DEKLARATION

ERKLÄRUNG ZUR DISSERTATION

gemäß §6(1) der Promotionsordung der Naturwissenschaftlichen Fakultät der Gottfried Wilhelm Leibniz Universität Hannover für die Promotion zum Dr. rer. nat.

Hierdurch erkläre ich, dass ich meine Dissertation mit dem Titel

"IMPACT OF TREE SPECIES AND FOREST MANAGEMENT ON CARBON CYCLING IN THE PLANT- SOIL-ATMOSPHERE SYSTEM"

selbständig verfasst und die benutzten Hilfsmittel und Quellen sowie gegebenenfalls die zu Hilfeleistungen herangezogenen Institutionen vollständig angegeben habe.

Die Dissertation wurde nicht schon als Masterabeit, Diplomarbeit oder andere Prüfungsarbeit verwendet.

CURRICULUM VITAE

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APPENDIX

Table A.1: Basic characteristic of the soils from 0-10 cm and 10 -25 cm soil depths underneath the collars installed around the experimental trees

Tree/ Soil depth	Sand	Silt	Clay	C	N	pH	CEC	BS
	—— g kg^{-1} ——			– g kg^{-1} –			$mmol_{(+)}$ kg^{-1}	%
***C. macrostachys* plots**								
0-10 cm	211±6	379±20	410±26	133±37^{a}	11.3±2.7^{a}	6.7±0.1^{a}	732±24^{a}	100
10-25 cm	219±2	301±24	480±25	50±8^{b}	4.9±0.8^{b}	6.5±0.4^{a}	498±63^{b}	100
***P. falcatus* plots**								
0-10 cm	180±3	409±20	411±17	116±26^{a}	10.8±2.1^{a}	6.5±0.2^{a}	565±55^{c}	100
10-25 cm	214±4	304±20	482±24	49±9^{bc}	5.2±0.9^{b}	6.2±0.2^{a}	376±42^{d}	100
***P. africana* plots**								
0-10 cm	195±5	405±37	400±32	107±19^{a}	11.0±1.6^{a}	6.6±0.1^{a}	640±45^{e}	100
10-25 cm	222±8	319±27	459±36	43±8^{bc}	5.0±0.9^{b}	6.5±0.2^{a}	497±49^{b}	100
***C. lusitanica* (Managed Stand)**								
0-10 cm	210±15	395±62	395±67	114±27^{a}	10.2±2.3^{a}	6.0±0.5^{a}	638±92^{e}	100
10-25 cm	189±8	371±65	440±70	39±15^{c}	4.3±1.6^{b}	5.7±0.6^{a}	449±69^{f}	100
***C. lusitanica* (Unmanaged Stand)**								
0-10 cm	203±20	376±20	421±20	90±23^{d}	8.8±2.2^{c}	6.3±0.5^{a}	568±24^{e}	100
10-25 cm	190±26	329±7	481±27	21±6^{e}	2.0±0.5^{d}	5.7±0.7^{a}	447±74^{f}	100

Values are mean and standard deviation (for C, N and pH n = 15 (natural forest stand); and n = 20 plantation forest stand), and n = 3 for other parameters). Different letters in each column indicate significant differences ($P < 0.05$) with one-way ANOVA, post hoc Tukey's test.

Table A.2: Proportion of PLFA profiles, expressed as mol% in soil from *C.macrostachys*, *P. falcatus*, Cupressus managed and Cupressus unmanaged stand from 0-10 cm and 10-25 cm soil depth.

PLFA	Natural forest stand				Plantation forest stand			
	C. macrostachys plots		*P. falcatus* plots		Managed Cupressus		Unmanaged Cupressus	
	0-10 cm	10-25 cm	0-10 cm	10-25 cm	0-10 cm	10-25 cm	0-10 cm	10-25 cm
i15	10.72(0.78)	10.81(1.03)	10.89(0.84)	11.24(1.69)	10.22(0.90)	10.15(1.30)	9.32(1.06)	8.12(0.72)
a15	4.92(0.28)	4.97(0.52)	6.64(0.37)	6.96(0.80)	5.01(1.14)	5.09(1.16)	4.63(0.47)	4.36(0.53)
i16	4.82(0.1)	5.31(0.18)	5.32(0.01)	6.48(0.45)	4.97(0.35)	5.61(0.37)	5.05(0.37)	4.73(0.30)
16:1ω9	1.38(0.18)	1.44(0.14)	1.34(0.06)	4.54(2.81)	1.41(0.13)	1.53(0.09)	1.12(0.39)	1.02(0.44)
16:1ω7c	7.10(0.47)	6.69(0.63)	8.01(0.18)	4.14(3.49)	7.47(0.49)	6.47(1.49)	7.66(0.99)	6.69(0.72)
16:1ω5	4.79(0.38)	4.53(0.34)	5.04(0.12)	4.56(0.65)	4.70(0.61)	4.31(0.84)	4.85(0.54)	4.43(0.67)
16:00	17.65(2.16)	16.85(1.06)	15.89(1.82)	14.95(1.10)	16.95(1.87)	17.23(1.11)	18.45(1.76)	19.00(2.54)
10:Me16a	3.98(0.34)	3.99(0.11)	3.46(0.27)	2.76(0.32)	3.89(0.37)	3.42(0.76)	3.95(0.21	3.74(0.07)
10:Me16b	11.91(0.95)	13.51(0.33)	10.46(.50)	11.03(1.46)	12.81(1.19)	14.09(1.82)	11.99(2.89)	12.34(1.36)
18:2ω6	1.56(0.19)	1.53(0.23)	1.84(0.22)	2.04(0.30)	1.82(0.61)	2.24(0.56)	2.34(0.64)	2.86(0.38)
18:1ω9	7.98(0.23)	7.80(0.22)	7.62(0.17)	7.52(0.62)	8.38(1.14)	8.12(1.31)	8.89(0.04)	9.95(0.52)
18:1ω7c	13.56(1.34)	12.41(0.69)	16.02(0.68)	15.34(1.62)	13.79(0.77)	12.65(0.67)	14.00(0.32)	14.30(0.66)
Cy19	9.64(0.50)	10.18(1.08)	7.47(0.35)	8.55(1.38)	8.58(0.26)	9.09(1.45)	7.77(1.99)	8.47(2.43)
Total PLFAs*	262.2(32.8)	196.7(12.5)	382.7(60.9)	221.6(56.7)	271.5(39.1)	136.1(9.3)	166.2(57.1)	76.5(6.4)

Values are Averaged mol% of individual fatty acids (SD in parenthesis, n = 3) * Total amounts of PLFAs in nmol g^{-1} dry weight of soil

Table A.3: Avergae soil CO_2 efflux rate and soil temperature at the natural forest stand throughout the measurement campaign (n = 15).

Date	*Croton macrostachys*				*Podocarpus falcatus*				*Prunus africana*			
	Soil CO_2 efflux, $\mu mol\ m^{-2}\ s^{-1}$		*Soil Temperature, °C*		*Soil CO_2 efflux, $\mu mol\ m^{-2}\ s^{-1}$*		*Soil Temperature, °C*		*Soil CO_2 efflux, $\mu mol\ m^{-2}\ s^{-1}$*		*Soil Temperature, °C*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
7/10/2008	5.91	0.77	17.36	0.37	5.64	1.45	15.98	0.20	4.75	0.77	16.29	0.33
7/26/2008	4.18	0.90	15.07	0.22	5.04	0.98	14.44	0.14	3.77	1.08	15.07	0.29
8/2/2008	5.18	1.01	15.73	0.14	5.54	1.03	15.09	0.15	4.59	0.99	15.43	0.12
8/9/2008	3.50	1.01	14.40	0.20	4.14	0.77	13.92	0.35	2.83	1.16	14.30	0.41
8/16/2008	4.90	0.96	15.44	0.62	5.56	1.23	15.28	0.10	4.61	1.15	15.85	0.22
8/23/2008	5.07	0.78	16.32	0.15	5.96	1.21	15.42	0.16	4.53	0.95	15.92	0.35
8/30/2008	5.03	0.85	16.65	0.24	5.32	1.11	15.48	0.17	4.38	0.93	15.75	0.27
9/6/2008	4.39	1.23	16.73	0.26	5.74	1.41	15.68	0.08	4.00	0.84	16.03	0.19
9/20/2008	5.03	1.17	16.62	0.24	6.02	1.35	15.72	0.18	4.32	1.15	15.98	0.23
9/27/2008	4.98	1.26	16.50	0.20	6.27	1.38	15.70	0.22	4.50	0.92	15.96	0.27
10/4/2008	5.21	0.78	16.90	0.48	5.06	0.80	15.59	0.23	4.59	0.72	16.10	0.24
10/11/2008	3.34	1.14	16.18	0.29	3.41	0.87	15.69	0.10	4.18	0.49	15.93	0.17
11/1/2008	3.87	1.23	16.74	0.55	5.37	0.84	16.44	0.31	4.45	0.78	16.34	0.19
11/8/2008	3.76	0.97	15.84	0.48	5.37	1.08	15.67	0.18	4.46	1.05	15.94	0.15
11/15/2008	4.70	0.68	15.18	0.67	4.30	0.74	14.60	0.53	3.94	0.68	15.24	0.53
11/22/2008	4.72	0.64	15.03	0.66	4.30	0.88	14.44	0.49	3.72	0.82	15.01	0.69
11/29/2008	4.89	0.96	15.83	0.54	4.22	0.67	15.28	0.48	4.04	0.52	15.86	0.74
12/6/2008	4.36	0.79	16.14	0.76	4.09	0.87	15.50	0.42	3.73	0.45	16.14	0.73
12/13/2008	3.92	0.64	15.19	0.87	3.58	0.77	14.74	0.53	3.05	0.61	14.97	0.81
12/20/2008	3.55	0.65	14.71	0.82	3.24	0.63	14.13	0.50	2.94	0.54	14.53	0.63
12/27/2008	3.41	0.62	15.39	0.90	3.22	0.52	14.83	0.54	2.72	0.43	15.08	0.86
1/3/2009	3.34	0.62	15.78	1.13	3.10	0.53	14.77	0.42	2.66	0.42	15.31	0.96
1/10/2009	3.23	0.57	15.83	1.07	2.89	0.61	14.74	0.50	2.30	0.39	14.81	0.67
1/17/2009	3.40	0.61	16.10	0.92	2.92	0.55	15.35	0.32	2.51	0.44	15.88	0.93
1/24/2009	4.72	0.81	16.72	0.91	5.64	0.69	15.87	0.31	4.53	0.41	16.69	0.99
1/31/2009	NA	NA	NA	NA	3.56	0.33	16.69	0.56	3.39	0.47	17.11	1.04
2/7/2009	3.31	0.71	18.57	1.46	3.34	0.67	17.09	0.29	3.01	0.48	17.64	0.75
2/14/2009	3.82	0.53	19.51	1.40	4.48	0.63	17.96	1.05	3.65	0.69	17.75	1.35
2/21/2009	2.81	0.72	19.09	1.56	3.45	0.72	17.37	0.72	2.48	0.44	17.34	1.21
2/28/2009	3.81	0.65	17.44	0.20	4.06	1.10	17.39	0.44	3.91	0.75	18.30	1.31
3/8/2009	2.45	0.55	19.96	1.17	2.47	0.45	16.91	0.44	2.15	0.48	18.29	1.49
3/14/2009	2.26	0.48	20.34	0.72	2.36	0.52	17.26	0.49	2.00	0.54	18.46	1.39
3/21/2009	2.65	0.62	20.93	0.60	3.01	0.43	18.13	0.55	2.90	0.49	18.81	1.68
3/28/2009	2.26	0.41	21.07	0.53	2.36	0.52	18.36	0.58	2.13	0.30	19.34	1.17
4/4/2009	3.06	0.47	18.05	0.29	3.60	0.81	16.93	0.20	2.89	0.53	17.30	0.56
4/11/2009	3.25	0.60	18.95	0.29	4.79	0.87	17.22	0.28	3.38	0.62	17.79	0.44
4/17/2009	4.22	0.74	18.82	0.38	6.17	1.54	17.30	0.33	5.01	0.60	17.82	0.30
4/25/2009	3.25	0.69	19.43	0.67	4.19	0.63	18.17	0.29	3.40	0.41	19.19	0.79
5/2/2009	2.58	0.42	20.13	1.32	2.78	0 53	18.30	0.48	2.49	0.32	19.26	0.75
5/9/2009	2.27	0.42	20.13	1.41	2.44	0.46	19.09	0.39	2.16	0.48	20.25	0.93
5/17/2009	3.02	0.93	16.48	0.52	5.05	0.66	15.58	0.50	4.02	0.85	16.24	0.26
5/24/2009	4.33	0.68	18.44	0.31	5.04	0.84	17.33	0.56	4.29	0.71	17.74	0.55
5/30/2009	3.90	0.78	20.55	2.01	3.99	0.66	18.84	1.87	3.55	0.52	19.04	1.05
6/6/2009	3.08	0.74	20.49	2.01	3.41	0.59	18.92	1.89	3.19	0.32	18.89	0.89
6/13/2009	3.43	0.62	19.98	1.88	3.98	0.95	18.92	1.51	3.50	0.46	19.42	1.08

Table A.3: Continued

6/20/2009	4.65	1.05	18.29	1.17	5.39	0.90	17.30	1.11	5.08	0.66	17.25	0.61
6/29/2009	4.80	0.92	17.47	1.07	5.48	1.18	16.44	0.56	5.34	0.44	16.73	0.35
7/4/2009	5.34	1.09	18.86	2.10	5.26	0.93	17.46	1.92	5.33	0.42	17.71	0.99
7/11/2009	4.79	1.14	16.58	1.24	5.35	1.15	15.76	0.70	5.01	0.70	15.90	0.47
7/18/2009	4.52	1.17	16.72	1.36	5.97	1.11	15.76	1.40	5.26	0.99	15.44	0.45
7/25/2009	4.96	1.22	16.37	0.80	6.05	1.27	15.68	0.62	5.38	0.68	15.75	0.23
8/1/2009	5.96	1.33	16.51	0.95	5.93	0.99	15.90	0.33	5.85	0.61	15.95	0.20
8/8/2009	3.63	1.51	16.21	0.78	4.20	1.00	15.99	0.77	5.95	1.01	16.41	0.34
8/15/2009	4.60	1.38	15.11	1.42	5.64	1.25	15.42	0.79	5.46	1.22	15.52	0.45
8/22/2009	5.11	1.42	15.69	1.53	5.18	1.07	16.21	0.22	5.05	1.19	15.91	0.25
8/29/2009	5.20	1.52	16.34	0.87	5.70	1.11	15.47	0.49	5.10	1.12	15.66	0.51
9/4/2009	4.37	1.38	16.36	0.46	5.24	1.38	15.55	0.35	5.13	0.71	15.90	0.33
12/18/2009	3.01	0.78	18.40	1.35	2.97	0.62	16.00	0.50	3.49	0.83	16.94	1.00
12/18/2009	2.76	0.86	16.67	0.96	2.81	0.66	15.76	0.44	3.00	0.63	16.35	0.82
12/26/2009	3.83	0.87	16.53	0.59	4.02	1.17	15.32	0.20	3.99	0.82	16.11	0.49
1/2/2010	2.62	0.60	16.98	1.19	2.64	0.50	14.72	0.48	2.67	0.60	14.49	0.95
1/9/2010	2.39	0.54	16.97	1.20	2.27	0.49	15.64	0.59	2.39	0.49	16.24	1.04
1/15/2010	2.42	0.64	18.98	1.44	2.24	0.48	15.97	0.40	2.27	0.48	17.56	0.91
2/6/2010	4.26	0.87	18.47	0.87	5.06	1.04	16.30	0.35	4.97	1.28	16.53	0.84
2/13/2010	4.26	1.35	17.88	0.73	5.76	1.37	16.60	0.17	5.23	1.01	17.22	0.62
2/20/2010	4.96	0.88	19.22	1.09	4.51	1.00	16.49	0.43	4.77	0.77	17.34	0.89
2/27/2010	4.25	1.57	18.57	0.54	5.65	1.31	16.63	0.30	4.79	1.29	16.70	0.54
3/27/2010	5.17	1.05	19.14	0.97	5.84	1.25	17.07	0.32	5.76	0.81	17.99	0.72
4/2/2010	4.93	2.07	18.75	0.44	6.89	1.69	16.82	1.01	5.59	1.25	16.97	0.51
4/17/2010	4.43	1.58	18.21	0.36	5.45	1.24	16.11	0.15	5.71	1.15	16.88	0.27
5/1/2010	5.10	1.78	18.65	0.67	7.23	1.22	17.17	0.23	6.74	1.44	17.78	0.56
5/8/2010	4.41	1.87	17.48	0.44	7.01	1.45	16.51	0.16	5.65	1.28	16.96	0.22
5/29/2010	5.14	1.75	17.42	0.34	7.12	1.38	16.57	0.18	5.97	1.25	17.13	0.28
6/5/2010	5.27	2.32	17.65	0.44	6.64	0.78	16.23	0.11	6.60	0.94	16.54	0.28
6/12/2010	4.66	1.89	16.91	0.50	5.95	1.08	16.16	0.27	5.36	1.18	16.61	0.35
6/19/2010	4.15	2.31	16.90	0.36	6.39	1.16	15.83	0.12	5.61	1.43	16.11	0.20
6/26/2010	5.15	2.06	16.91	0.21	6.34	1.06	15.62	0.14	5.77	1.35	15.98	0.20
7/10/2010	4.14	1.98	16.49	0.36	5.54	1.37	15.39	0.17	5.53	1.22	15.78	0.22
7/17/2010	3.94	2.02	15.59	0.32	5.44	1.38	14.94	0.12	5.70	1.27	15.50	0.23
7/24/2010	4.85	2.51	16.02	0.37	6.08	1.55	15.18	0.11	5.98	1.33	15.52	0.31

Table A.4: Average soil CO_2 efflux, soil temperature and volumetric soil moisture content at the plantation forest stand throughout the measurement campaign (n = 20).

	Managed Cupressus						Unmanaged Cupressus					
	Soil CO_2 efflux, $\mu mol\ m^{-2}\ s^{-1}$		*Soil Temperature, °C*		*Soil Moisture, %*		*Soil CO_2 efflux, $\mu mol\ m^{-2}\ s^{-1}$*		*Soil Temperature, °C*		*Soil Moisture, %*	
Date	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5/30/09	4.34	1.04	20.91	0.91	14.86	2.49	3.91	0.50	18.90	0.71	18.05	2.40
6/6/09	4.88	1.17	19.17	0.51	15.72	2.82	3.74	0.56	17.32	0.41	20.24	3.39
6/13/09	5.50	1.64	18.68	0.76	15.12	2.86	4.74	0.97	17.35	0.57	27.73	4.46
6/20/09	6.04	1.12	19.47	0.60	24.66	4.29	4.97	1.07	17.64	0.73	26.92	4.48
6/27/09	6.52	1.02	18.92	0.78	21.77	5.15	3.86	1.69	16.99	0.63	25.83	4.78
7/4/09	5.77	1.11	18.70	0.50	23.93	3.54	4.35	0.74	16.92	0.78	27.25	3.64
7/11/09	6.14	1.11	18.61	0.60	25.41	3.98	4.95	0.90	16.49	0.52	28.57	3.60
7/18/09	4.61	0.93	16.60	0.30	39.66	2.99	3.37	1.02	15.54	0.35	39.80	3.14
7/25/09	4.91	1.15	16.82	0.84	31.02	4.28	5.20	2.23	16.71	0.43	33.49	3.45
8/1/09	6.01	1.17	17.56	0.48	30.56	3.01	4.36	1.35	16.16	0.46	28.90	4.08
8/8/09	6.92	1.71	17.58	0.54	NA	NA	4.40	1.21	16.05	0.55	NA	NA
8/15/09	6.29	1.28	16.65	1.10	NA	NA	3.31	1.24	15.46	0.40	NA	NA
8/22/09	5.23	1.03	17.24	0.45	NA	NA	4.04	0.89	15.80	0.49	NA	NA
8/29/09	5.12	1.21	17.41	0.55	NA	NA	3.62	0.69	16.23	0.58	NA	NA
9/5/09	5.76	1.20	18.21	0.44	NA	NA	4.10	0.74	16.31	0.40	NA	NA
10/17/09	2.79	0.52	16.58	0.58	11.64	1.45	2.59	0.60	14.69	0.67	13.84	1.49
10/24/09	3.46	0.46	16.03	0.22	13.67	1.04	2.84	0.46	15.34	0.51	15.82	3.18
1/2/10	3.93	0.79	16.63	0.81	16.16	1.66	3.01	0.54	15.11	0.60	17.97	2.01
1/9/10	2.78	0.38	14.93	0.79	12.78	1.37	2.29	0.47	14.17	0.96	13.51	1.61
1/16/10	2.37	0.42	17.09	0.56	9.76	1.16	2.45	0.86	15.81	0.99	10.63	1.48
1/23/10	3.77	0.46	15.69	0.45	18.30	3.40	3.03	0.75	14.94	0.56	20.80	3.56
2/6/10	4.02	0.60	16.85	0.55	25.70	4.59	3.22	0.70	15.85	0.71	31.78	3.56
2/13/10	5.01	0.66	16.87	0.44	27.68	4.14	3.57	0.80	15.71	0.40	28.87	2.68
2/20/10	4.23	0.71	16.19	0.68	20.63	3.26	3.45	0.76	15.55	0.72	22.22	2.98
3/13/10	4.60	0.42	15.42	0.34	25.73	2.36	3.43	0.60	15.03	0.70	25.37	3.07
3/20/10	4.52	0.56	15.98	0.29	27.89	2.74	4.02	1.77	15.37	0.28	30.82	2.76
3/27/10	4.11	0.72	16.51	0.17	36.86	1.75	3.23	0.92	15.79	0.35	39.31	1.55
4/3/10	5.15	0.63	16.31	0.44	31.33	3.13	4.00	0.74	15.38	0.42	32.39	3.19
4/10/10	5.14	0.73	17.13	0.39	29.90	3.10	4.03	0.64	16.46	0.58	27.71	4.12
4/17/10	5.07	0.79	16.17	0.18	33.78	3.68	4.20	0.71	15.74	0.45	32.70	3.27
4/24/10	4.12	0.68	16.38	0.31	43.39	3.31	3.18	0.52	16.89	0.48	44.21	2.08
5/1/10	5.22	1.01	16.79	0.09	36.25	2.93	4.35	0.69	16.26	0.29	35.95	1.95
5/8/10	4.77	0.70	16.33	0.22	33.60	2.23	3.68	0.55	15.94	0.28	35.05	2.09
5/15/10	4.51	1.05	15.96	0.14	39.81	3.56	3.29	0.73	15.49	0.23	40.05	2.35
5/29/10	4.22	0.93	16.30	0.15	41.10	2.35	3.25	0.83	15.47	0.32	41.52	3.05
6/5/10	4.92	0.74	15.68	0.23	36.37	3.38	3.95	0.77	15.07	0.46	37.40	2.89
6/12/10	3.54	1.20	15.45	0.27	43.00	3.55	2.69	1.03	14.74	0.27	42.23	3.26
6/19/10	3.67	0.84	15.66	0.27	44.45	3.96	3.24	0.81	15.14	0.20	42.94	2.92
6/26/10	3.09	0.87	15.66	0.27	47.01	3.52	2.29	0.81	15.11	0.21	46.99	2.80
7/3/10	3.53	0.66	15.20	0.12	44.14	2.56	3.40	0.84	14.61	0.34	44.07	2.54
7/10/10	4.88	1.02	15.66	0.25	41.39	2.39	3.85	0.90	15.21	0.19	41.16	3.11
7/24/10	2.86	0.74	15.02	0.16	44.08	4.04	2.27	1.10	14.69	0.18	44.90	3.23
7/31/10	3.97	0.69	14.97	0.11	39.19	2.30	3.50	0.86	14.59	0.24	39.30	2.23
8/7/10	3.59	0.65	14.99	0.21	42.58	3.45	3.00	0.80	15.24	0.31	43.66	3.66
8/14/10	4.02	0.76	15.23	0.10	43.29	3.32	3.61	0.82	14.84	0.27	42.86	3.08
8/21/10	3.69	1.25	15.34	0.18	38.06	3.65	3.04	1.05	14.84	0.20	41.25	2.28
8/29/10	2.96	1.17	15.45	0.10	47.09	2.80	2.38	0.93	15.01	0.27	47.38	2.65

Table A.4: Continued

9/4/10	3.47	1.07	15.32	0.22	44.37	3.19	2.84	1.02	14.56	0.11	44.80	3.21
9/18/10	3.34	0.86	14.85	0.48	46.17	2.18	3.13	1.04	14.23	0.17	45.61	3.30
9/25/10	3.44	0.93	14.27	0.05	44.99	3.30	2.90	1.17	NA	NA	45.16	2.95
10/2/10	3.65	1.12	14.28	0.24	44.59	2.53	3.54	1.22	13.71	0.15	44.71	3.30
10/9/10	4.64	1.03	14.43	0.32	39.73	3.27	3.96	1.08	13.56	0.30	41.46	2.53
10/16/10	4.01	0.80	14.54	0.27	29.79	4.00	3.72	0.53	14.19	0.32	30.51	2.33
10/23/10	3.73	0.52	14.37	0.22	24.71	3.67	3.15	0.49	13.69	0.24	23.11	2.60
10/30/10	3.27	0.85	14.82	0.29	19.16	1.23	2.89	0.61	14.47	0.29	18.79	1.42
11/6/10	2.83	0.52	13.74	0.28	16.57	0.92	2.42	0.52	12.94	0.36	16.36	1.89
11/13/10	2.85	0.51	14.67	0.44	15.20	1.78	2.46	0.76	14.09	0.28	15.19	1.38
11/20/10	2.76	0.58	13.00	0.30	17.34	2.56	2.40	0.46	12.62	0.41	16.81	1.91
11/27/10	2.60	0.42	13.12	0.27	17.57	1.45	2.51	0.81	12.39	0.34	17.06	2.04
12/4/10	2.24	0.29	12.57	0.52	13.90	0.98	2.01	0.44	12.15	0.47	14.38	0.92
12/11/10	2.54	0.36	11.98	0.35	13.15	1.42	2.25	0.46	11.58	0.29	13.65	1.06
12/18/10	1.79	0.53	10.75	0.34	12.38	1.13	1.80	0.43	10.36	0.35	11.41	1.40
12/25/10	2.06	0.34	13.54	0.30	11.35	1.38	2.02	0.75	13.08	0.37	10.85	1.08
1/1/11	3.00	0.69	14.39	0.13	17.50	2.22	2.66	0.50	14.22	0.31	18.24	3.60
1/8/11	2.07	0.31	12.64	0.36	12.80	1.48	1.86	0.44	11.90	0.30	12.57	1.25
1/22/11	2.70	0.68	14.59	0.34	13.90	1.67	2.31	0.48	13.86	0.35	13.03	1.73
1/29/11	1.96	0.34	13.89	0.48	11.07	0.97	1.79	0.45	13.70	0.41	10.42	1.05
2/5/11	1.77	0.29	15.28	0.38	9.75	1.01	1.68	0.36	14.87	0.86	9.24	1.00
2/12/11	1.69	0.22	15.06	0.41	8.36	0.93	1.64	0.40	14.59	0.58	7.22	1.06
2/19/11	1.72	0.28	15.52	0.90	7.93	1.12	1.62	0.42	14.72	0.63	7.48	0.86
2/26/11	1.57	0.25	16.30	0.81	11.07	0.97	1.45	0.38	15.72	1.05	10.42	1.05
3/5/11	1.54	0.22	16.54	0.61	6.49	0.86	1.53	0.33	16.40	0.52	5.88	0.57
3/12/11	2.76	1.06	17.53	0.55	8.94	0.93	1.88	0.23	17.09	0.67	7.93	1.26
3/19/11	3.74	0.75	15.51	0.31	19.71	2.88	3.15	0.59	14.96	0.17	18.74	2.96
3/26/11	2.11	0.56	16.59	0.49	10.16	1.77	1.66	0.39	15.89	0.68	9.70	1.76
4/2/11	1.61	0.28	15.90	1.24	7.71	1.11	1.44	0.31	15.85	0.53	7.34	0.94
4/9/11	3.49	0.90	17.31	0.87	10.51	2.20	3.08	0.53	16.32	0.90	8.95	1.62
4/16/11	1.65	0.32	18.31	0.99	6.80	1.08	1.46	0.37	16.93	0.69	6.27	0.99
4/30/11	1.47	0.22	17.24	0.71	6.14	1.02	1.31	0.37	17.31	0.34	5.66	1.09
5/8/11	3.97	0.71	16.39	0.24	18.24	2.44	3.51	0.47	15.61	0.37	18.56	2.64
5/14/11	3.51	0.56	16.66	1.96	18.58	0.92	2.75	0.33	17.36	0.66	15.72	2.95
5/21/11	4.58	0.83	15.92	0.25	31.03	3.40	4.14	0.66	15.38	0.29	32.44	3.25
5/28/11	4.66	0.84	15.43	0.22	34.12	2.14	4.23	0.50	15.25	0.17	35.32	1.40

Table A.5: Time serious $\delta^{13}C$ values of AMF biomarker NLFA16:1ω5 from adhering and bulk soil samples collected from 0-10 cm and 10-25 cm soil depth across the sampling campaign.

Time	Adhering soil								Bulk soil							
	0-10 cm soil depth				10-25 cm soil depth				0-10 cm soil depth				10-25 cm soil depth			
	NLFA 16:1ω5		PLFA 16:1ω5		NLFA 16:1ω5		PLFA 16:1ω5		NLFA 16:1ω5		PLFA 16:1ω5		NLFA 16:1ω5		PLFA 16:1ω5	
	Cm	Pf	Cm	Pf	Cm	Pf	Cm	Pf	Cm	Pf	Cm	Pf	Cm	Pf	Cm	Pf
2 days	-11.30	-26.40	-19.94	-22.34	-22.86	-26.04	-20.74	NA	-0.38	-27.51	-18.83	-22.71	-13.41	-28.44	-20.34	NA
3 days	-17.29	-26.11	-21.78	-22.83	-18.78	-23.38	-3.32	-22.77	-17.72	-27.29	-20.90	-24.12	-6.64	-25.64	-19.73	-22.98
4 days	25.77	-26.68	-9.16	-23.15	-13.65	NA	-18.11	NA	9.11	-22.55	-16.13	-22.48	-16.92	NA	-21.24	NA
8 days	63.87	-5.26	-12.69	-22.58	-13.25	-14.33	-17.99	-21.93	48.11	-15.64	-17.34	NA	24.91	-14.47	-15.10	-21.74
16 days	15.91	3.99	-19.26	-21.50	47.61	-22.21	NA	NA	10.08	NA	-19.94	-23.13	4.00	-18.28	-18.01	-23.06
32 days	36.53	-26.71	-19.17	-22.22	-3.24	-21.58	-17.71	-21.70	-6.51	-24.33	-21.10	-23.00	-9.97	-20.44	-21.02	NA
64 days	-1.07	NA	-21.18	-22.54	-21.85	-18.29	-19.68	-22.78	-2.26	-25.75	-21.39	-23.31	-14.24	-11.69	-21.80	-21.37
120 days	-18.68	-25.68	-22.32	-22.90	-18.73	-23.43	-19.69	-22.18	-19.51	NA	-22.50	-21.41	-19.74	-33.08	-21.17	-22.05
1 year	-28.35	-27.25	-21.81	NA	-28.35	-25.77	-21.81	-23.48	-22.12	-27.64	-27.02	NA	-22.11	-27.11	NA	-22.45

NA represent samples not considered because of the major height were below 1 nano-ampere (nA); Cm, *Croton macrostachys* and Pm, *Podocarpus falcatus*

Table A.6: Time serious $\delta^{13}C$ values of foliage, phloem and soil CO_2 gas samples from *C. macrostachys* and *P. falcatus*.

Time	*Croton macrostachys*									
	Foliage samples				Phloem samples		Soil CO_2 gas samples			
	Labeled		Control		Height		Labeled		Control	
	Mean	SD	Mean	SD	0.5 m	1.3 m	Mean	SD	Mean	SD
-24	-28.7	0.3	-28.7	0.3	-25.1	-26.1	-26.7	0.5	-26.5	0.9
0	1558.6	87.1	-28.8	0.3	-25.5	-25.3	-27.2	0.3	-25.9	0.5
4	1092.5	109.5	-29.4	0.3	-24.0	-23.9	-26.1	1.2	-27.3	0.2
8	966.3	78.1	-29.2	0.8	-15.5	152.0	NA	NA	NA	NA
12	773.7	163.1	-29.1	0.6	-9.0	NA	-26.9	0.2	-27.5	0.2
24	679.1	171.7	-28.8	0.3	NA	NA	-24.0	0.3	-26.8	0.2
48	553.8	425.5	-28.6	0.4	2250.2	1941.9	99.2	4.6	-25.8	0.1
72	531.2	117.7	-29.0	0.6	853.9	1041.8	117.2	12.0	-27.1	0.2
96	404.9	79.5	-29.1	0.6	NA	NA	71.0	10.9	-28.1	0.5
192	363.4	69.3	-29.1	0.8	493.4	358.0	20.3	4.0	-26.7	1.2
384	288.7	98.3	-29.3	0.6	207.0	138.3	10.7	2.5	-25.5	0.6
768	285.9	112.7	-29.2	0.5	20.5	24.3	-10.7	0.3	-24.8	0.4
1536	275.1	75.1	-29.3	0.4	-6.1	6.1	-17.9	0.3	-25.7	0.8
2880	68.8	75.6	-27.7	0.9	-13.5	-17.8	-24.4	0.1	-26.9	0.1
8760	-27.4	1.6	-27.8	0.1	NA	NA	-26.4	0.2	-26.9	0.2
	Podocarpus falcatus									
-24	-27.8	0.5	-28.3	0.4	-25.8	-25.7	-27.2	0.2	-27.8	0.2
0	264.2	58.9	-28.4	0.3	-25.3	-25.5	-27.0	0.2	-27.2	0.2
4	184.2	66.1	-28.3	0.2	-26.3	-25.5	-27.0	0.2	-27.1	0.3
8	165.0	48.4	-28.3	0.1	-24.8	-25.7	NA	NA	NA	NA
12	186.0	30.7	-28.4	0.4	-25.1	2.1	-26.1	0.3	-27.8	0.4
24	147.4	17.4	-27.9	0.5	-1.3	38.8	-26.5	0.1	-27.2	0.2
48	140.1	6.9	-28.3	0.2	38.8	35.8	-26.7	0.3	-27.7	0.1
72	95.3	17.3	-28.0	0.3	402.1	272.4	-23.3	1.3	-26.8	0.2
96	79.8	20.6	-29.1	0.5	965.8	855.7	-16.3	1.4	-26.6	0.3
192	46.2	2.2	-28.9	0.0	454.6	430.6	-0.4	1.4	-27.6	0.2
384	41.9	36.9	-28.4	0.8	163.0	160.7	-8.7	1.5	-28.1	0.9
768	41.0	1.4	-28.4	0.1	-24.5	-25.9	-15.1	1.1	-29.2	0.5
1536	0.6	11.8	-28.4	0.5	-26.1	-25.5	-19.2	0.2	-26.5	0.6
2880	-3.7	13.3	-28.4	1.1	-8.2	-4.7	-19.9	0.2	-26.5	0.1
8760	-26.2	0.3	-27.5	0.6	NA	NA	-27.5	0.8	-26.9	0.2

Foliage and soil CO_2 gas sample, n = 5; Phloem sample, n = 1/height

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