

# **PHYSIOLOGICAL ECOLOGY OF THE FRANKINCENSE TREE**

Tefera Mengistu Woldie

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# PHYSIOLOGICAL ECOLOGY OF THE FRANKINCENSE TREE

Tefera Mengistu Woldie

## **thesis**

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To my father Mengistu Woldie-mariam who has left me during my early age  
To my brother Tsegaw Mengistu who decided to be a farmer while sending me to school  
To children of the world to whom education has become only a dream so far





The degradation of frankincense tree dominated woodlands has been attributed to climatic conditions and human activities. We lack however information on how such factors influence the resource balance and productivity of trees. The aim of this study was to evaluate the impact of resin tapping on the whole tree carbon gain, storage and allocation pattern of frankincense trees (*Boswellia papyrifera* (Del.) Hochst) in the dry woodlands of northern Ethiopia. I hypothesized that the intensive resin tapping of frankincense trees reduces tree vitality, particularly under relatively dry conditions. I established experimental plots in the highland woodlands of Abergelle and the lowland woodlands of Metema, and applied tapping treatments to similar sized adult trees (DBH 20 +/- 3cm). For these trees I also collected data on leaf gas exchange, crown traits, carbon storage, carbon allocation, growth and frankincense production during a period of two years (2008-2009).

Trees follow similar leaf gas exchange patterns in contrasting environments, but differ in annual crown carbon gain between highland and lowland sites. Highland trees of *Boswellia* had a higher photosynthetic capacity, were exposed to higher light conditions, but had a shorter leaf lifespan than lowland trees. Integrating these effects, I showed that the annual crown carbon gain is higher in the highland trees than in lowland trees. Lowland trees are mainly constrained by clouded conditions and resultant low light levels during the wet season, limiting their carbon gain. Moreover, carbon gain was also restricted by atmospheric drought, and much less by soil water deficit during the growing season. The production of frankincense was not affected by the annual tree carbon gain implying that trees with smaller total leaf area may suffer sooner from carbon starvation by tapping.

Tapping reduced storage carbohydrate concentrations in wood, bark and root tissues indicating that continuous tapping depletes the carbon reserves. A large part of the carbohydrate concentration in the plant tissues was starch. *Boswellia* trees have more total nonstructural carbohydrates (TNC) concentrations and pool sizes in wood than in root and bark tissues. Because tapped trees face depleting carbon storage pools during the dry tapping season and cannot fully replenish these pools during the wet season, tapped trees may face higher risks of carbon starvation compared to untapped trees in the long term.

Estimated total annual carbon sinks to the different plant components were 38-68% of the annual carbon gain in both study sites. However, *Boswellia* trees also establish mycorrhizal associations which may consume an additional 20% of gross primary production. On a whole-tree basis, the percentage of autotrophic respiration may exceed all other costs. The foliage construction costs and incense production are the second and third largest carbon sinks, respectively. Contrary to our expectation, the sum of all dry season carbon costs was higher than the total amount of consumed TNC during the dry season. The high carbon costs during the dry season imply that trees do not fully depend on TNC to pay for the carbon costs during the dry season. With the exception of carbon allocation to foliage production and maintenance, a higher gross primary production does not enhance an overall increase in carbohydrate investments in the other sinks. Therefore, the carbon allocation pattern is constrained not exclusively by the absolute amount of carbon gained but also by other factors.

The results clearly indicate that continuous tapping depletes the amount of stored carbon, the leaf area production and the reproductive effort. These negative effects were however site specific and could possibly be apparent sooner for smaller trees than for larger ones. Thus, guidelines for resin tapping of *Boswellia* trees should consider tapping intensity, tapping frequency, environmental conditions and tree size and should focus on maintaining vital trees and populations for the future.

*Keywords:* *Boswellia papyrifera*, carbon balance, drylands, Ethiopia, frankincense, tapping

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# *Chapter 1*

## *Introduction*

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### **Land Degradation in Tropical Dry Woodlands**

Drylands cover about 41% of the earth's land surface and are inhabited by more than 2.5 billion people (Mortimore 2009). About 72% of the global dryland areas occur within developing countries and face increasing pressure for goods, services and values to people (Campbell 2000). African drylands are among the most exploited systems (Campbell 2000), and are being degraded or transformed to agricultural lands at increasing spatial scale (Bongers and Tennigkeit 2010). The dry woodlands, co-dominated by trees and grasses, are sometimes considered hotspots for biodiversity, and support valuable and renewable resources of economic importance (Timmermann and Hoffmann, 1985). Despite their economic potential, the communities that inhabit dry woodlands of Africa are poor, often overexploit the remaining resources, or transform the woodlands to persistent agricultural croplands. The final consequence of such trends is that some of the resources provided by these woodlands are on decline and will influence the peoples' livelihood. Given such increasing pressure on the remaining woodland resources, more basic information is required to provide a sustainable alternative for the woodland management.

In addition to the land use change, climate plays another important role (Olson et al. 2004, Bongers and Tennigkeit 2010) in dryland systems. Rainfall is erratic and temperatures are generally high making drought a recurrent phenomenon. The distinctly seasonal rainfall patterns provide water during the short wet season alternating with periods of drought (Eamus 1999). This implies that plants face a seasonal water deficit (Murphy and Lugo 1986, Walter 1971, Bullock et al. 1995). Under these circumstances, plants either tolerate drought or avoid drought by, for example, dropping leaves and thus limit transpiration during the dry season, to survive in these environments. For certain African dry woodlands however, rainfall intensity and frequency vary considerably also within the wet season itself (Renner 1926, Johnson 1962), suggesting that even deciduous trees may encounter drought stress, but during the wet season. Such dry spell conditions have significant effects on the annual carbon gain and allocation patterns of plants challenging their survival in the dryland systems.

### **Plant Survival in Tropical Dry Woodlands**

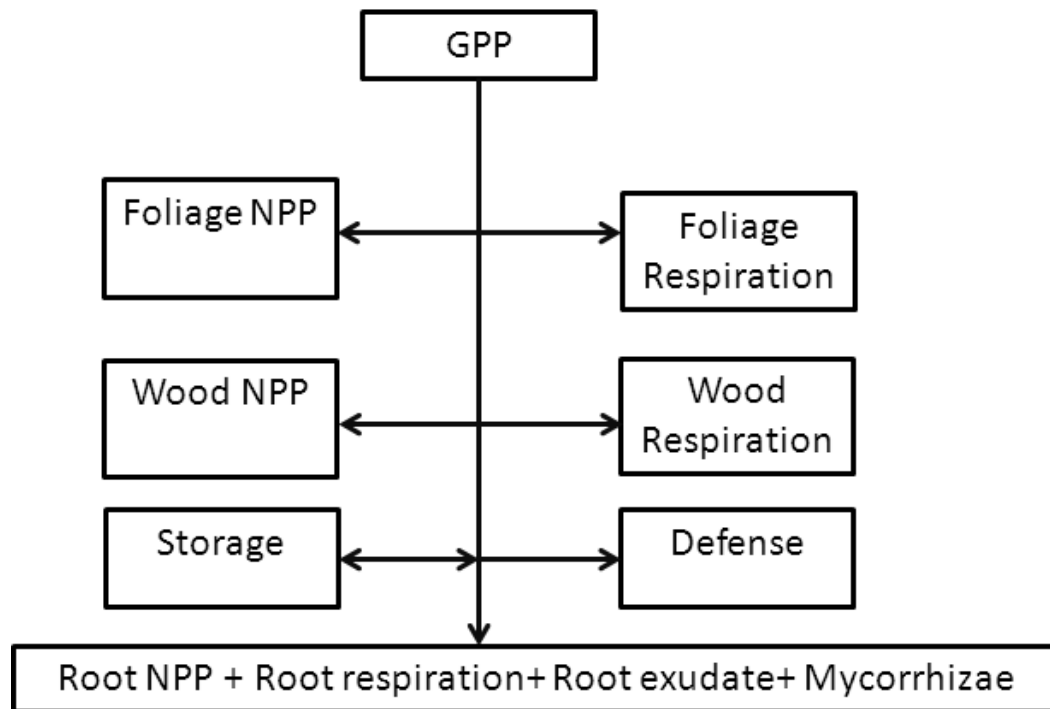
The fact that tropical dry woodlands face long dry and shorter wet seasons implies that plants should employ functional and/or phenological adjustment strategies (Zweifel et al. 2007, Tuzet et al. 2003, McDowell et al. 2008, Cowan and Farquhar 1977) either to tolerate or avoid water stress. Trees respond to such seasonality by dropping leaves, thus limiting physiological activity during the drought stress period, or by very specific physiological adaptations to deal with drought. Examples of such adaptation are producing long roots to access deep persistent water resources, or physiological control of excessive water loss. However, an extended dry season is still expected to reduce the productivity of the trees, and of whole dry woodland vegetations.

Developing specific traits and physiological adaptations are important to ensure survival in such ecosystems. For example, deciduous and evergreen species have adopted different physiological strategies in carbon gain. Deciduous species with short leaf lifespan invest large amounts of nitrogen in leaves to support better assimilation rate (Poorter et al. 2006, Eamus et al. 1999), such that each day of the wet season when soil water is freely available – their short-lived leaves fix large amounts of carbon.

Trees also modify their carbon investment in response to climatic conditions. Most species can modify their allocation to root and shoot by proportionately allocating their carbon to favor growth towards the most limiting resources. Having a more extended root system in moisture stressed environments helps plants to scavenge moisture patches and underground water sources. This type of root architecture is an adaptive trait, as it enables the plant to capture resources efficiently. However, the controls to these morphological adjustments are still poorly understood (Yang and Midmore 2005). Dryland plants are also adapted to suit the rigors of high temperature. These plants have smaller leaves, non-porous covering on their leaves (wax) and leaf hairs to reduce moisture loss. Other plants cope with the extremes in temperature and rainfall by becoming dormant during the drought period, and escaping difficult times. For example, annual plants finish their lifecycle (from germination to flower and seed) within one growing season.

### Whole-Tree Carbon Balance

Allocation of carbohydrates to plant organs is indispensable for growth and survival. However, in moisture-pulsed dry environments, trees cannot maintain a constant carbon balance owing to the dynamics in carbon acquisition and limitation. Under extreme drought conditions, carbon gain is constrained by water stress and plants may starve by carbon depletion. If carbohydrates are indeed the limiting resources within the plant, this may result in trade-offs among different carbon sinks or demands (Bazzaz et al. 1987). For example, water stress may trigger higher investments in roots relative to shoots, light stress may result in higher investments in the shoot relative to the roots, and damage to plant organs may stimulate investments in defense metabolites. Such conflicting sink demands are captured by a general scheme of the carbon budget scheme in plants (e.g. Litton et al. 2007). This thesis work started from such a scheme (Figure 1), where I distinguish between a single carbon source, the gross primary productivity (GPP) by leaf photosynthesis and sinks that refer to maintenance respiration of living tissues, growth of different tissues (NPP), fluxes to a carbon storage pool, and defense against damage..



**Figure 1.** Schematic presentation of how gross primary productivity is partitioned into above ground (biomass growth = NPP; respiration, storage and defense) and below ground sinks.



As far as I know, there is no study that quantified this carbon balance concept at tree level. Prior studies focused on global scale (Edwards et al. 1990, Mantlana 2008, Heimann 2009), aboveground to belowground comparisons (Cairns et al. 1997, Gower et al. 2001) or stand level studies (Litton et al. 2007). Evidently, our knowledge on plant carbon balance of larger trees lags behind smaller trees or other life forms (Veneklaas and Poorter 1998). In addition, some of the most dominant vegetation types in tropical environments like the dry tropical woodland systems still remain without being well studied (Heimann 2009). In this study, I aimed at understanding how the carbon is acquired and allocated to different sinks at the whole plant level for the economically important frankincense producing tree *Boswellia papyrifera* (Del.) Hochst, inhabiting dry woodlands in northern Ethiopia. I used the carbon balance approach to elucidate the impact of climate parameters and human induced factors (e.g. tapping) on the carbon gain, allocation pattern and productivity of adult trees of this species. The study focuses on the carbon balance of frankincense trees from leaf to whole tree scale, and from diurnal to annual patterns. This approach allows us to understand the impact of tapping frankincense on tree functional traits, and to speculate about the possible consequences of climate and thus, climate change. This study is one of the first that quantifies carbon gain and carbon allocation patterns at whole-tree level. Moreover, it is one of the few detailed studies on physiological responses in functional traits of a dry woodland species. Such patterns are better known for, for example, rain forests (Poorter et al. 2006, Markesteijn et al. 2007, Poorter 2009, Niinemets 1997) or tropical savannahs (Eamus et al. 1999, Goldstein et al. 2008, Bucci et al. 2008), but are particularly scanty for African dry woodlands (but see Biao et al. 2010, Yoshifugi et al. 2006, Kushwaha et al. 2010).

### ***Boswellia Papyrifera* and Its Frankincense Production**

The species *Boswellia papyrifera* (Del.) Hochst of the Burseraceae family has a centre of geographic distribution in the Horn of Africa (Lovett and Friis, 1996). The tree is distinctly deciduous during the dry season. It produces resin after bark injury, which provides protection against herbivores (Chantuma et al. 2009), prevents desiccation and further decay (Phillips and Croteau 1999, Langenheim 2003). Since long, this resin, also known as “Olibanum”, is known for its own distinctive fragrance and medicinal properties (Rahman et al. 2005). The practice of

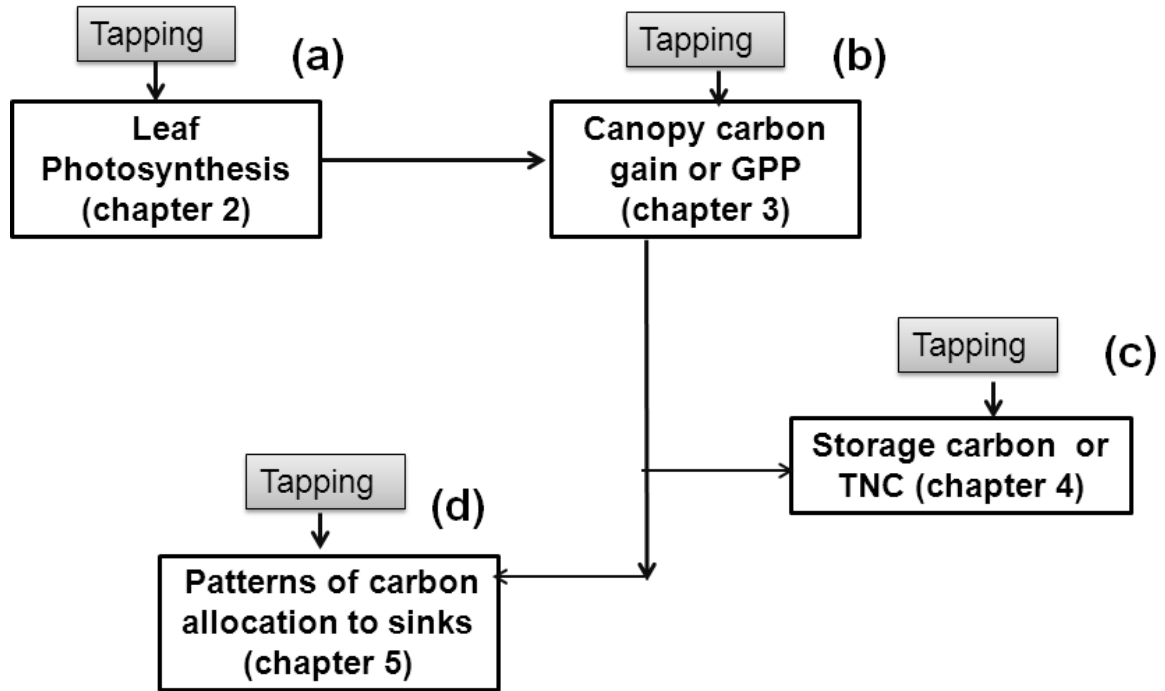
tapping is commenced by a cut of the cambium, and is renewed at each tapping day by removing additional bark from the upper edge. During tapping, the cut surface gradually moves upward and each year a fresh cut is started at a different site. Traditionally, people wound the bark with a small hand axe such that the frankincense exudes and can be collected and used as incense in religious ceremonies or as export item.

The incision on the bark triggers sink stimulation similar to rubber production (Chantuma et al. 2009) and drains the resin (frankincense) to exude. The resin acts as additional carbon drain and the costs of resin production are assumed to limit plant productivity due to assimilate competition among coupling demands. Since harvesting occurs during the dry season, tapping largely depletes storage carbohydrates and therefore trees may restrict growth, reproduction and ultimately result in carbon starvation. Therefore, understanding impact of frankincense tapping for *Boswellia* tree carbon gain and allocation pattern will help us to design the frequency and intensity of tapping.

Currently, the international market for frankincense is rapidly increasing, probably challenging the potential of the remaining *Boswellia* woodlands to provide sufficient frankincense to satisfy this growing market. In woodlands of northern Ethiopia, *Boswellia* trees are tapped at large scale and this will certainly continue given that the remaining tree populations are rapidly colonized by people and exploited to satisfy a growing global market demand. Moreover, trees are also challenged by the recurrent drought episodes in these regions. Altogether, existing procedures are damaging the trees (Rijkers et al. 2006, Kindeya 2003, Lemenih et al. 2004, Ogbazghi et al. 2006). Therefore, resin extraction requires research focus especially on the effect of continuous tapping on the tree carbon balance. So far, there are few studies on the ecology and reproductive effort of *Boswellia* (Rijkers et al. 2006, Ogbazghi et al. 2006, Abiyu et al. 2010, Eshete et al. 2011) while a lot is still unknown on the physiology of frankincense production in relation to the plant carbon balance.

I started by relating plant carbohydrate sources and sinks including frankincense tapping with the carbon budget scheme (Figure 1). In the model, understanding seasonal and annual patterns in carbon balance requires quantifying the carbohydrate source (Figure 2 a, b) and sinks (Figure 2 c, d) while tapping is considered as an additional sink. Based on the main hypothesis that tapping *Boswellia* trees drains carbon, I hypothesized that intensive frankincense production

impacts carbon gain, limits the carbon available for other sinks, and thus increases the risks for carbon starvation of the trees.



**Figure 2.** Study and organization of the chapters based on the basic carbon flow scheme.

In this dissertation, I report the impact of frankincense tapping on leaf physiology (Figure 2 a), annual carbon gain (Figure 2 b), storage carbohydrate dynamics (Figure 2 c) and carbon allocation patterns (Figure 2 d).

My main aim is to understand the impact of tapping on leaf gas exchange property (chapter 2), tree annual carbon gain (chapter 3) and carbon allocation pattern (chapters 4 and 5) of *Boswellia papyrifera* (Del.) Hochst in northern Ethiopia.

I relate this to four research questions:

- (1) How do external climate factors and physiological mechanisms explain the variation in leaf gas exchange characteristics of *Boswellia papyrifera*? (Chapter two)
- (2) How do climatic factors link to crown functional traits to affect annual carbon gain and resin yield? (Chapter three)
- (3) How does frankincense tapping influence the concentration and seasonality of non-structural carbohydrate storage? (Chapter four)

- (4) How do the multiple carbon sink demands respond to tapping and to seasonal variation?  
(Chapter five)

### **Thesis Outline**

This study evaluates the impact of frankincense tapping on the carbon gain and allocation pattern of *Boswellia* trees. It consists of six chapters which includes the general introduction (chapter one), four research data papers (chapter two to five), and a synthesis (chapter six). To evaluate *Boswellia* physiology in contrasting sites, I established five experimental plots in the highland and lowland of northern Ethiopia in 2007 (Fig. 1). Tapping treatments were applied to similar size adult trees (DBH 20  $\pm$  3cm) and data on leaf gas exchange, crown traits, storage carbon and growth traits was collected for two years (2008-2009). This data was used for all the chapters.

In chapter 2, I analyze diurnal patterns in leaf transpiration and leaf carbon gain (GPP, but at the leaf level only). Using a mechanistic path-model, I tested how climate and physiological responses contributed to both leaf transpiration and leaf carbon gain during the wet season.

In chapter 3, I scale morphological and physiological functional plant traits to annual carbon gain estimates at the whole tree level (GPP, Fig 1.). More particularly, I investigate the effect of tapping on the annual carbon gain, via its possible effects on the crown area and leaf photosynthesis.

In chapter 4, I quantified the dynamics in the major carbon source during the dry season, the carbon storage (TNC) pools (Figure 1), and I evaluated the possible effects of tapping on these pools. I therefore collected bark, root and stem samples at different periods during the years, and quantified the glucose, sucrose, fructose and starch concentrations and total carbon contents. Moreover, I scaled the sample values to the whole tree, taking into account the biomass of the different storage tissues. This chapter thus evaluates the impact of frankincense tapping on total non-structural carbohydrate (TNC) storage of the tree.

In chapter 5, I relate the carbon sources during wet season (leaf/crown carbon gain) and dry season (TNC) to the different sinks, both during dry and wet season. I thus quantified for the whole year (Chapter five, Figure 1) and for the dry and wet season separately (Chapter five,

Figure 2). This chapter explains the carbohydrate allocation patterns of *Boswellia* trees with seasonally changing phenological and physiological events.

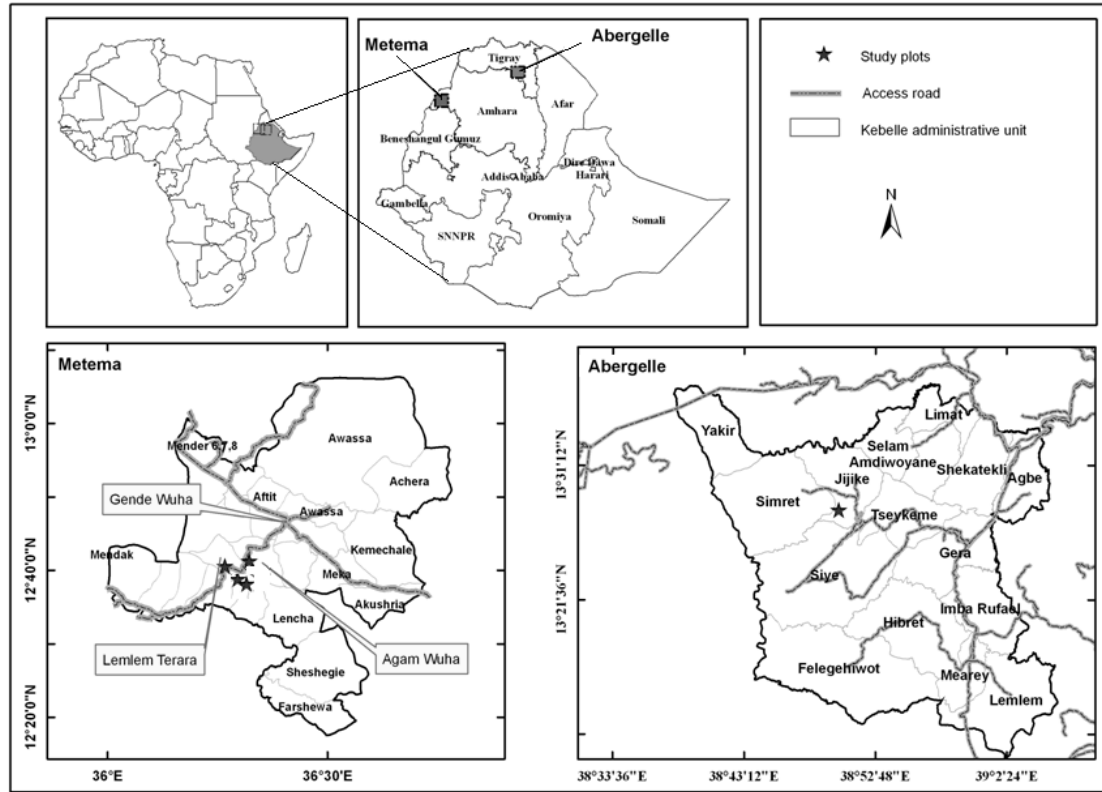
In chapter 6, I summarize the main outcomes of the preceding chapters and discuss them in the light of carbon allocation theory. This chapter also formulates conclusions with respect to sustainable harvesting of frankincense, which may have far reaching significance for the management of *Boswellia* woodlands.

### **Description of Study Areas**

This study is conducted in two *Boswellia* populations at low and high altitudes sites in the northern Ethiopia (Figure 3). Permanent plots were established and adult trees of *B. papyrifera* (Del.) Hochst were selected for detailed investigation in both sites.

The Abergelle site is at relatively high altitude (1400-1650 meters). This site is dry and is characterized by erratic rainfall and a short wet season (chapter 2, Fig. 1). The site is dominated by hills and shallow soils that limit plants to form deep roots. The vegetation is characterized by *Combretum-Terminalia* and *Acacia-Commiphora* woodland (NBSAP 2005), dry forest dominated by *Boswellia papyrifera*, *Acacia etbaica*, *Terminalia brownii* and *Lannea fruticosa*.

The Metema site is at relatively low altitude (810-990 meters). The site is less dry and has relatively better rainfall distribution and the topography is flatter than Abergelle. The soils are deeper and predominantly have vertic properties (Birhane et al. 2010). The vegetation is categorized as *Combretum-Terminalia* woodland (NBSAP, 2005) where *Acacia spp.*, *Balanites aegyptiaca*, *Boswellia papyrifera*, *Combretum spp.*, *Stereospermum kunthianum* and *Terminalia brownii* are the dominant species.



**Figure 3.** Location of the study sites. The two dark squares in the upper middle panel show the location of the study sites in Ethiopia. Red-marked stars in the lower two panels indicate the location of the plots in the lowland, Metema (left) and highland, Abergelle (right).

## Chapter 2

### *Leaf gas exchange in the frankincense tree (*Boswellia papyrifera*) of African dry woodlands*

Tefera Mengistu, Frank J. Sterck, Masresha Fetene, Wubalem Tadesse and Frans Bongers  
(Submitted for publication)

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*Abstract*

A conceptual model was tested for explaining environmental and physiological effects on leaf gas exchange in the deciduous dry tropical woodland tree *Boswellia papyrifera* (Del.) Hochst. For this species we aimed at (i) understanding diurnal patterns in leaf gas exchange (ii) exploring cause-effect relationships among external environment, internal physiology and leaf gas exchange and (iii) exploring site differences in leaf gas exchange in response to environmental variables. Diurnal courses in gas exchange, underlying physiological traits and environmental variables were measured for 90 trees in consecutive days at two contrasting areas, one at high and the other at low altitude.

Assimilation was highest in the morning and slightly decreased during the day. In contrast, transpiration increased from early morning to midday, mainly in response to an increasing VPD. The leaf water potential varied relatively little and did not influence gas exchange during the measurement period.

Our results suggest that the same cause-effect relationships function at contrasting areas. However, leaves at the higher altitude had higher photosynthetic capacities reflecting acclimation to higher light levels. Trees at both areas nevertheless achieved similar leaf assimilation rates since assimilation was down-regulated by stomatal closure due to the higher vapor pressure deficits at the higher altitude, while it became more light limited at the lower altitude. Gas exchange was thus limited by a high VPD or low light levels during the wet season, despite the ability of the species to acclimate to different conditions.

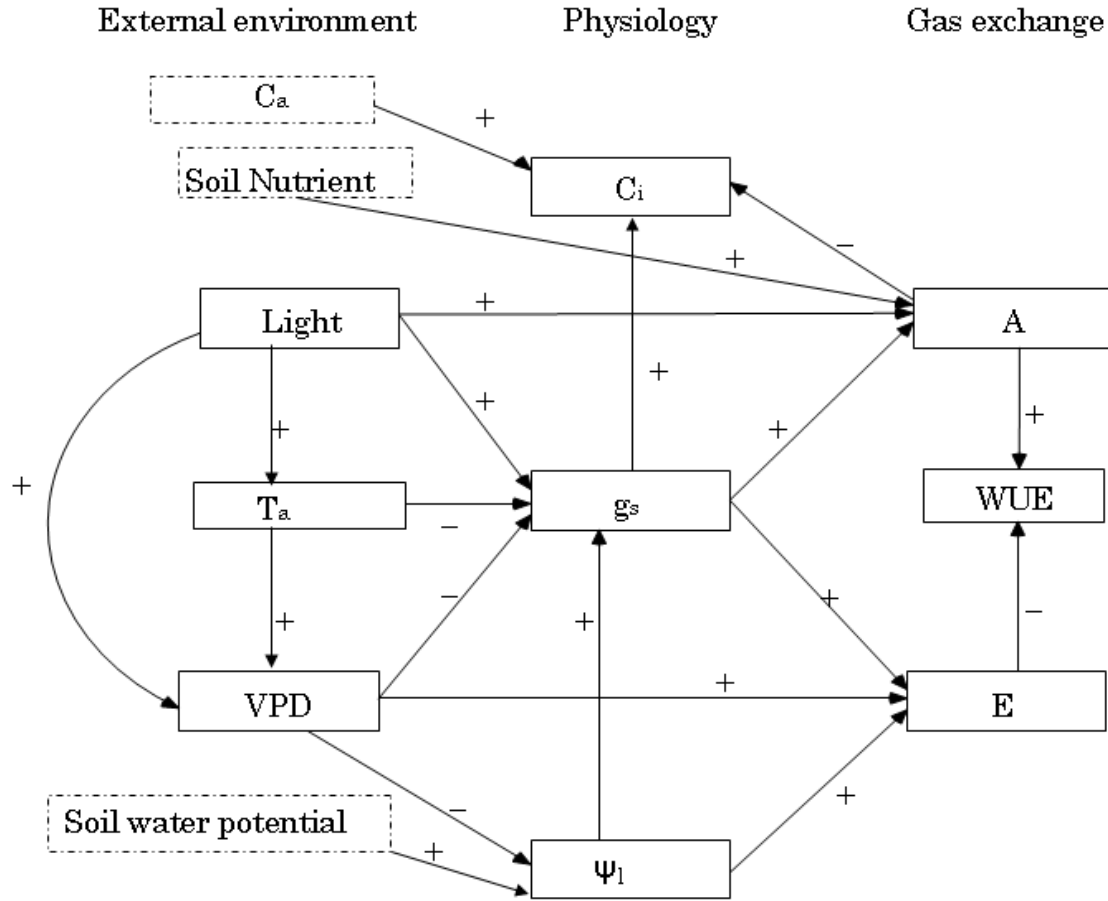
*Keywords:* *Boswellia*, diurnal variation, path analysis, photosynthesis, tropical dry woodlands.



## **Introduction**

Trees of tropical dry woodlands face strong seasonality in rainfall and thus in water stress, and typically encounter relatively long dry (8-9 months) and short wet (3-4 months) seasons (Murphy and Lugo 1986, Walter 1971, Bullock et al. 1995). In such water stressed environments, trees face trade-offs between carbon uptake and water loss (Cowan and Farquhar 1977, Collatz et al. 1991). When trees close their stomata to avoid water loss with increasing drought in air and soil (Zweifel et al. 2007), this comes at the cost of low carbon gain (Tuzet et al. 2003, McDowell et al. 2008). It has been suggested that trees optimize the carbon-water acquisition by minimizing water loss relative to the amount of CO<sub>2</sub> uptake (Cowan and Farquhar 1977, Sandquist and Susan 2007), which is often expressed by water use efficiency, or carbon gain to water loss ratio. Alternatively, deciduous trees avoid the most water stressed conditions by dropping their leaves during the dry period and supporting leaves only during the wet season (Mulkey et al. 1996, Singh and Kushwaha 2005). For certain African dry woodlands however, it is well known that rainfall intensity and frequency vary considerably within and across wet seasons (Renner 1926, Johnson 1962, Vincens et al. 2007), suggesting that deciduous trees might also encounter drought stressed conditions during the wet season.

Based on theoretical and empirical studies in other dry woodland systems, such as Mediterranean systems (e.g. Tuzet et al. 2003, Zweifel et al. 2007, McDowell et al. 2008) and Neotropical systems (e.g. Goldstein et al. 2008, Bucci et al. 2008), we start from a conceptual cause-effect model that captures how environmental conditions and physiological traits affect leaf photosynthesis, transpiration and water use efficiency (Figure 1).



**Figure 1.** A conceptual model for the causal relationship of external climatic variables, internal physiological traits and the leaf gas exchange traits (symbols as in Table 2). Cause-effect paths are indicated by arrows and the direction of response is indicated by a minus or plus signs. Note that soil and leaf water potential range from zero (“water saturation”) to negative values (more water stress). The factors taken into account in this study are indicated by solid boxes, and those beyond the scope of this study are indicated by dashed boxes.

Accordingly, light is expected to have a positive direct effect on photosynthesis and also, via its positive effect on stomatal conductance, on both photosynthesis and transpiration (Farquhar and Sharkey 1982, Tuzet et al. 2003). Water stress is expressed in terms of soil water potential and vapor pressure deficit, where the latter is driven by diurnal variation in temperature and relative humidity. Both types of water stress are expected to reduce the leaf water potential and stomatal conductance (Farquhar and Sharkey 1982, Zweifel et al. 2007) and, in turn, result in

lower transpiration and photosynthesis. Transpiration demand may influence the stomatal conductance when it affects the epidermal turgor (Mott et al. 1999). Soil nutrient stress might reduce photosynthesis owing to insufficient nutrient availability for replacing photosynthetic proteins, but not influence transpiration directly. Apart from such external environments and physiological cause-effect relationships, there are various possible interactions amongst physiological traits. For example, stomatal closure and high photosynthesis rates will result in lower internal CO<sub>2</sub> concentrations, and stomatal conductance is expected to reduce in response to lower leaf water potentials (Meinzer et al. 2001, Tuzet et al. 2003). We thus have a conceptual cause-effect model for the diurnal trends in environmental factors, physiological traits and gas exchange responses.

Most of our current knowledge of tropical systems comes from rain forests, where the discussion is dominated by pioneer-shade tolerance concepts (Poorter et al. 2006, Markesteijn et al. 2007) or canopy-understory concepts (Poorter 2009, Niinemets 1997). Other studies are more on tropical savannas (e.g. Sarmiento et al. 1985, Myers et al. 1997, Eamus and Cole 1997, Eamus et al. 1999, Goldstein et al. 2008, Bucci et al. 2008). There is relative scarcity of knowledge on leaf gas exchange patterns of dry woodlands, and of African dry woodlands in particular. In this study, we present in-situ results on leaf gas exchange rates in response to external environmental conditions and in association with possible underlying physiological mechanisms for a deciduous tree species of poorly studied but extensive African dry woodlands. For deciduous trees in such dry woodlands, diurnal leaf gas exchange patterns might follow similar qualitative patterns as trees of temperate forests or wet tropical rain forests (Weber and Gates 1990, Ishida et al. 1996, Souza et al. 2008): a typical hump-shaped pattern in transpiration and photosynthesis, with a possible midday depression or a gradual decline after morning peaks in gas exchange in temperate (Bassow and Bazzaz 1998) and dry tropical savanna (Eamus et al. 1999).

In this study, we analyzed in-situ leaf-level gas exchange rates for naturally grown frankincense trees, *Boswellia papyrifera* (Del.) Hochst, in two contrasting areas: one at relatively low altitude (Metema) representing extensive *Boswellia* woodlands in the lowlands, and the other at higher altitude (Abergelle) representing more isolated populations. We tested our conceptual model for trees in both areas, representing contrasting conditions for this species. More specifically: (i) we tested whether diurnal patterns in leaf gas exchange are similar to those

observed for more frequently studied tree species in wet tropical and temperate forests: a hump-shaped pattern for gas exchange with a possible midday depression (Mulkey et al. 1996, Pathre et al. 1998, but see Bassow and Bazzaz 1998, Eamus et al. 1999). (ii), we used path analysis to test whether the hypothesized relationships in our conceptual model apply to both areas, and thus whether the external factors and physiological mechanisms explained the variation in in-situ leaf gas exchange, and (iii) we considered how possible differences in gas exchanges between the two contrasting areas could be explained by the variation in the external factors. To our knowledge, this is one of the first attempts to quantify and understand such physiological response patterns for naturally grown, resin producing trees of dry tropical woodlands.

## Materials and Methods

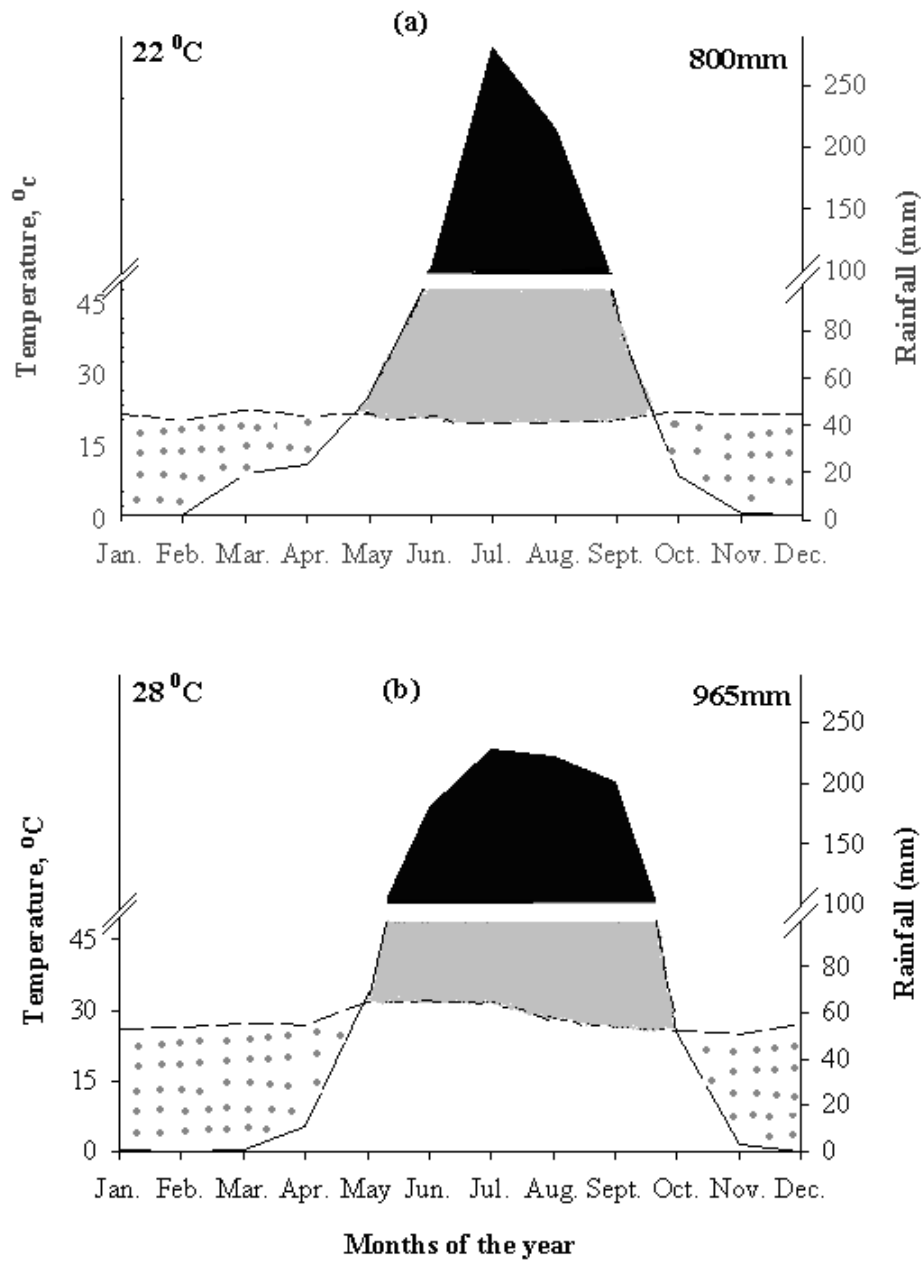
### *Species and study areas*

The species *Boswellia papyrifera* (of the family Burseraceae) occurs in dry woodlands of the Sudano-Sahelian region of Nigeria, Chad, Sudan, Central African Republic, Uganda, Ethiopia, Somalia and Eritrea (Lovett and Friis 1996, Ogbazghi et al. 2006). In Ethiopia, the species is indigenous and occurs in the northern, western and central parts of the country (Tengnas and Azene 2007, Tadesse et al. 2007). The species is intensively exploited for the frankincense than before, putting an extra pressure on the declining populations (Rijkers et al. 2006, Ogbazghi et al. 2006, Abiyu et al. 2010).

*B. papyrifera* is a deciduous tree up to 13m tall, with a stem diameter up to 35 cm (Ogbazghi et al. 2006, Abiyu et al. 2010) and with an approximately circular crown. The tree is monoecious and has compound leaves that contain 9-20 pinnate, veined, leaflets supported by petioles. It grows mainly in tropical dry woodlands with the centre of geographic distribution in the Horn of Africa (Lovett and Friis 1996). The species is mainly found on rocky steep slopes and hilly areas with the roots not going deep but extending sideways on shallow surface soils (Ogbazghi et al. 2006). Flowering and fruiting occurs during the dry leafless season. Upon wounding, the tree produces a water-soluble resin (frankincense) with distinctive fragrance.

Adult trees of *B. papyrifera* were selected for this comparative field study at Metema, an area at relatively low altitude (810-990 meters) and Abergelle at relatively high altitude (1400-1650 meters). The Abergelle site is drier and is characterized by more erratic rainfall and a shorter wet season than Metema (Figure 2). This site is dominated by hills and shallow soils that

limit plants to form deep roots. The vegetation is characterized by *Combretum-Terminalia* and *Acacia-Commiphora* woodland (NBSAP 2005), dry forest dominated by *Boswellia papyrifera*, *Acacia etbaica*, *Terminalia brownii* and *Lannea fruticosa*. The less dry Metema site has relatively higher rainfall distribution (Figure 2) and the topography is flatter than Abergelle. The soils are deeper predominantly with vertic property (Birhane et al. 2010). The vegetation is categorized as *Combretum-Terminalia* woodland (NBSAP, 2005) where *Acacia spp.*, *Balanites aegyptiaca*, *Boswellia papyrifera*, *Combretum spp.*, *Stereospermum kunthianum* and *Terminalia brownii* are the dominant species.



**Figure 2.** Climate diagrams of the two study sites: Abergelle (a) and Metema (b). Dotted lines refer to average monthly temperature and solid lines to rainfall. For both climate variables monthly averages for the years 1973 –1979 and 1995- 2009 are presented. Data was not available for 1980-1994.

### *Measurements for diurnal patterns*

We measured leaf gas exchange on 90 naturally grown *B. papyrifera* trees. Trees were selected from a similar diameter at breast height (DBH) class of  $20\pm 3$  cm to avoid possible size effects. At Abergelle, diurnal gas exchange patterns were measured for 30 trees on a randomly selected leaf from the canopy over a series of three consecutive days (20-22 July, 2009). For the 60 selected trees at Metema, the same measurements were done during 10 consecutive days (05-14 July, 2009). We synchronized the gas exchange measurements on each tree with the measurements of external environmental conditions and leaf water potential.

For each tree, leaf gas exchange was recorded in the morning (8-11 h), around midday (12-14h), and in the afternoon (15-17h). We measured the net photosynthetic rate, transpiration rate, sub-stomatal CO<sub>2</sub> concentration, leaf temperature and stomatal conductance using a portable photosynthesis system LcPro (ADC, Hoddesdon, UK.). The LcPro is an open-system Infra Red Gas Analyzer (IRGA), allowing ambient fresh air to pass through the plant leaf chamber. The LcPro was calibrated at the ADC company before the measurements started. The LcPro also automatically recorded the photosynthetically active radiation incident on the leaf. Using a thermo-hygrometer, we synchronized all these measurements with records of relative atmospheric humidity and temperature. The saturated vapor pressure, actual air vapor pressures and pressure difference between leaves and atmosphere, hence referred to as VPD, were calculated using the Tetens equations (Campbell and Norman 1998). Overall, gas exchange was expected to vary based on the macro-climatic conditions, since self shading due to vertical layering of leaves was minimal. Leaf water potentials were measured for two leaflets at each of the three measurement times per selected tree, using a pressure chamber (Scholander et al. 1965). The harvested leaves were wrapped in a close-fitting polyethylene bag at excision and placed in the pressure chamber within few minutes to avoid spuriously low water potentials due to dehydration (Turner and Long 1980). Before sunrise, we also measured predawn leaf water potentials for each tree (between 4:30-6:00hrs).

### *Light response curves*

Apart from this diurnal time series schedule, light response parameters were measured for small samples of leaves at Abergelle and Metema. For this purpose, a detachable mixed Red/Blue LED light source chamber (2 cm x 3 cm) was fixed on top of the LcPro leaf chamber. Leaves

were enclosed inside the leaf chamber without any light for 30 minutes to enable dark respiration estimates. Subsequently, light levels were increased stepwise resulting in a light series over a realistic light intensity range: 0, 50, 100, 200, 400, up to 2000  $\mu\text{molm}^{-2}\text{s}^{-1}$  using steps of 200  $\mu\text{mol}$  light intervals. At each light level, we waited until stable photosynthetic rates were achieved. Light response curves were constructed per leaf using the non-rectangular hyperbola (Thornley and Johnson 1990).

$$A = \frac{\phi I + A_{\max} - \sqrt{\{(\phi I + A_{\max})^2 - (4\theta\phi I A_{\max})\}}}{2\theta} - R_d$$

In this model, the parameters are the irradiance ( $I$ ), the light saturated photosynthetic rate ( $A_{\max}$ ), dark respiration rate ( $R_d$ ), the quantum yield ( $\phi$ ), light compensation point ( $I$ ) and the curvature factor ( $\theta$ ).

### *Statistical data analysis*

In the GLM analysis, we tested for the effects of site and time on gas exchange, taking site and time as fixed factors and tree as a random factor. Prior to the analysis, we tested for normality and homogeneity of the variance, using the Kolmogorov-Smirnov and Levene tests, and transformed if required. The same data were used in the Pearson's correlation analysis on environmental factors, physiological traits, and gas exchange rates.

We also tested for differences in leaf gas exchange, environmental conditions, leaf water potentials and light response curves between the two areas, i.e. Abergelle and Metema. For this purpose, we pooled all data and used a one-way ANOVA with the Welch statistic procedure in order to obtain robust F-statistics for the mean comparisons while correcting for the unbalanced design (N= 90 at Abergelle and N=178 at Metema). Because we have two areas only, we can only reflect on possible causes of observed gas exchange differences between these areas.

To test our initial conceptual model (Figure 1), we used path analysis. The initial conceptual model was tested for Chi-square ( $\chi^2$ ) values and significance levels ( $P$ ). If the path model was not significant, the model was trimmed by deleting non-significant paths and expanded by adding the possible direct paths between external environmental, internal physiological and gas exchange traits. We stuck to possible mechanistic cause-effect relationships during this process. In this way, more significant path models were obtained for both areas, characterized by a lower  $\chi^2$  and higher  $P$  (i.e.,  $P > 0.1$ ; the higher the  $P$ , the better the



model fits the data). We thus can come up with the most significant path model per site. We also tested whether these models applied to both sites using the structural weight model and assuming the same paths and equal path strength for both sites, using AMOS-software. As data input for the path model, we used the measurements at different time periods during the day as replicates i.e N= 90 at Abergelle, N=178 at Metema (two records were excluded from Metema data set due to machine reading signal failure).

**Table 1.** The plant physiological and environmental variables used, with their symbols and units

Symbols	Variables Name	Units
$A$	Net Photosynthetic rate	$\mu\text{molm}^{-2}\text{s}^{-1}$
$A_{\text{max}}$	Maximum light saturated photosynthetic rate	$\mu\text{molm}^{-2}\text{s}^{-1}$
$C_i$	Sub-stomatal $\text{CO}_2$ concentration	ppmv
$C_a$	Atmospheric $\text{CO}_2$ pressures	ppmv
$g_s$	Stomatal conductance for water	$\text{molm}^{-2}\text{s}^{-1}$
$Q_l$	Photosynthetically active radiation incident on the leaf	$\mu\text{molm}^{-2}\text{s}^{-1}$
$R_d$	Dark Respiration rate	$\mu\text{molm}^{-2}\text{s}^{-1}$
$R_h$	Relative humidity	%
$T_a$	Air Temperature	$^{\circ}\text{C}$
$T_l$	Leaf Surface Temperature	$^{\circ}\text{C}$
$E$	Transpiration rate	$\text{mmolm}^{-2}\text{s}^{-1}$
WUE	Water use efficiency	$\mu\text{mol}/\text{mmol}$
VPD	Vapor pressure deficit	kPa
$\Theta$	Curvature factor	-
$\Psi_l$	Leaf water potential	bars
$\Gamma$	Light Compensation Point	$\mu\text{molm}^{-2}\text{s}^{-1}$
$\Phi$	Quantum Yield	$\text{molCO}_2\text{mol}^{-1}\text{light}$

## Results

### *Environmental conditions*

The leaves of trees at the high altitude site (Abergelle) were exposed to higher light levels, higher vapor pressure deficits and slightly lower atmospheric  $\text{CO}_2$  pressures than at the low altitude (Metema) site (Table 2). In line with the atmospheric  $\text{CO}_2$  pressures, the internal leaf  $\text{CO}_2$  pressures were also higher at the low altitude site. Temperatures during the measurement

period were surprisingly similar between both areas. Despite the variation in average light levels, the average assimilation rate was similar across areas. Transpiration rate was higher at the high altitude site, corresponding with the higher transpiration demand as indicated by the higher vapor pressure deficit. This high transpiration demand might also contribute to the lower water use efficiency at higher altitude.

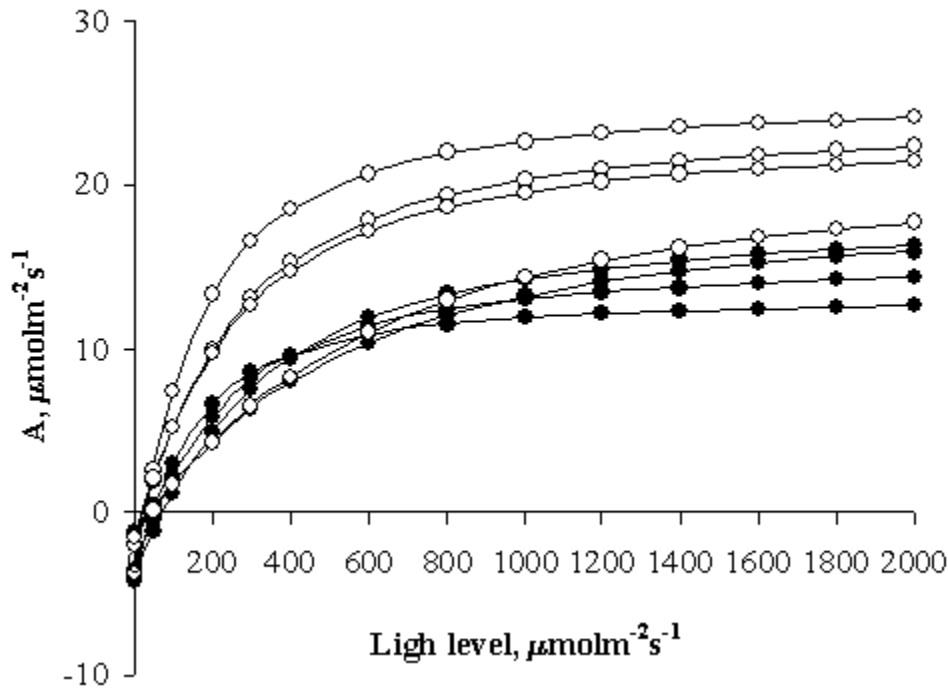
**Table 2.** Differences in *Boswellia* leaf physiological traits and environmental variables between Abergelle and Metema sites.

Traits	Abergelle		Metema	
	Units	Mean±s.e.	Mean±s.e.	F-value
<b>Ecophysiological:</b>				
Photosynthetic rate ( <i>A</i> )	$\mu\text{molm}^{-2}\text{s}^{-1}$	6.16±0.37	5.72±0.30	1.16NS
Transpiration rate ( <i>E</i> )	$\text{mmolm}^{-2}\text{s}^{-1}$	3.12±0.10	1.80±0.08	99.23***
Stomatal conductance ( <i>g<sub>s</sub></i> )	$\text{molm}^{-2}\text{s}^{-1}$	0.22±0.01	0.23±0.01	0.03NS
Substomata CO <sub>2</sub> concentration ( <i>C<sub>i</sub></i> )	ppmv	264±0.5	297±0.5	28.8***
Leaf Nitrogen	%	2.83±0.26	2.42±0.18	2.1NS
Leaf water potential ( $\Psi_l$ )	bars	-1.40±0.11	-1.93±0.09	14.16***
Water use efficiency (WUE)	$\mu\text{mol}/\text{mmol}$	2.16±0.24	3.42±0.19	8.6**
Maximum photosynthesis ( <i>A<sub>max</sub></i> )	$\mu\text{molm}^{-2}\text{s}^{-1}$	22.14±1.291	14.89±0.989	0.008***
Quantum yield ( $\Phi$ )	$\text{molCO}_2\text{mol}^{-1}\text{light}$	0.086±0.020	0.06±0.013	0.43 NS
Dark respiration rate ( <i>R<sub>d</sub></i> )	$\mu\text{molm}^{-2}\text{s}^{-1}$	2.17±0.550	3.23±0.686	0.27 NS
Curvature ( $\theta$ )	Dimensionless	0.47±0.008	0.48±0.008	0.44 NS
Compensation point ( <i>I</i> )	$\mu\text{molm}^{-2}\text{s}^{-1}$	55±18.3	73±5.55	0.39 NS
<b>Environmental:</b>				
PAR incident on the leaf ( <i>Q<sub>l</sub></i> )	$\mu\text{molm}^{-2}\text{s}^{-1}$	804.42±40	243.05±32	112.39***
Atm. CO <sub>2</sub> concentration ( <i>C<sub>a</sub></i> )	ppmv	375±0.4	396±0.3	25.8***
Temperature ( <i>T<sub>a</sub></i> )	°C	27.71±0.3	27.57±0.2	0.03NS
Relative humidity ( <i>R<sub>h</sub></i> )	%	49.31±1.2	62.81±1.0	68.51***
Vapor pressure deficit (VPD)	kPa	3.26±0.1	2.11±0.08	74.42***

Mean and the standard error (s.e.) of each variables is presented; the sample size n = 90 for Abergelle and n = 178 for Metema. Differences are tested with ANOVA and their significance is shown for each of the variables. All tests were significant at  $P < 0.05$ . (\*\*\*)  $P < 0.001$ ; \*\*,  $0.01 < P < 0.001$  and NS not significant).

### Light response curves

The light response curves showed that leaves exhibited the typical asymptotic trend in photosynthesis with increasing light levels. Mean  $A_{\max}$  (Metema  $\sim 15$  and Abergelle  $\sim 22$   $\mu\text{molm}^{-2}\text{s}^{-1}$ ) was however significantly lower ( $P = 0.008$ ) and achieved at lower light levels for Metema ( $\sim 1200$   $\mu\text{molm}^{-2}\text{s}^{-1}$ ) than for Abergelle ( $\sim 1600$   $\mu\text{molm}^{-2}\text{s}^{-1}$ ). In line with this, leaf nitrogen content tended to be lower at Metema, but this trend was not significant (T-test,  $P = 0.09$ ,  $N = 21$  for Abergelle and  $N = 57$  for Metema). Dark respiration ( $R_d$ ), light compensation point ( $I$ ), quantum yield ( $\phi$ ) and curvature ( $\theta$ ) did not differ significantly between the two areas (Figure 3).

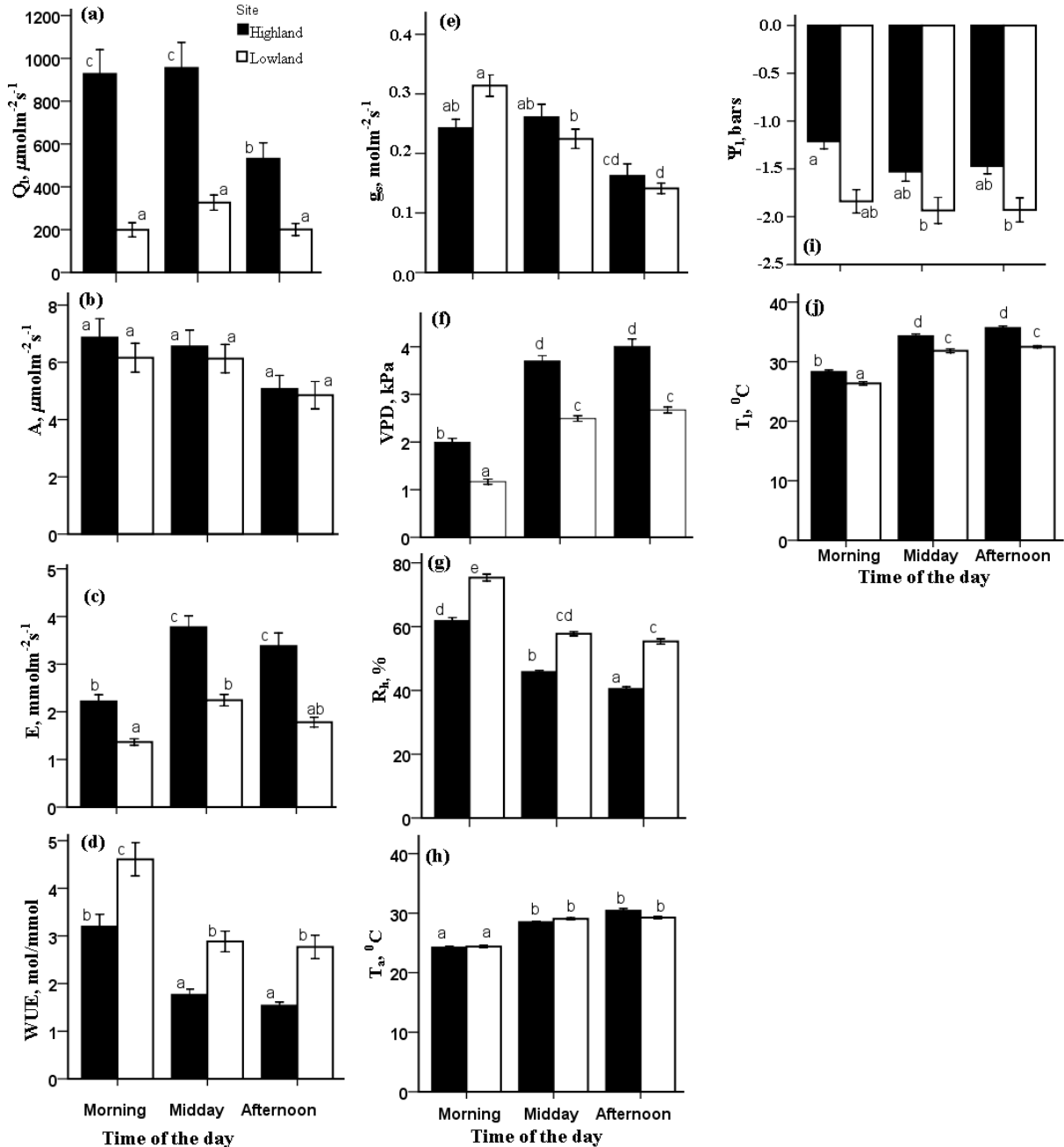


**Figure 3.** Photosynthetic light response curves for Metema (filled circles) and Abergelle (open circles).

### Diurnal patterns

While gas exchange measurements started only two hours after sun rise, assimilation and conductance started at relatively high values in the early hours of the day. The diurnal trend in transpiration suggested an increase until midday followed by stable pattern (Figure 4). The variation in assimilation and transpiration was however large at any time during the day.

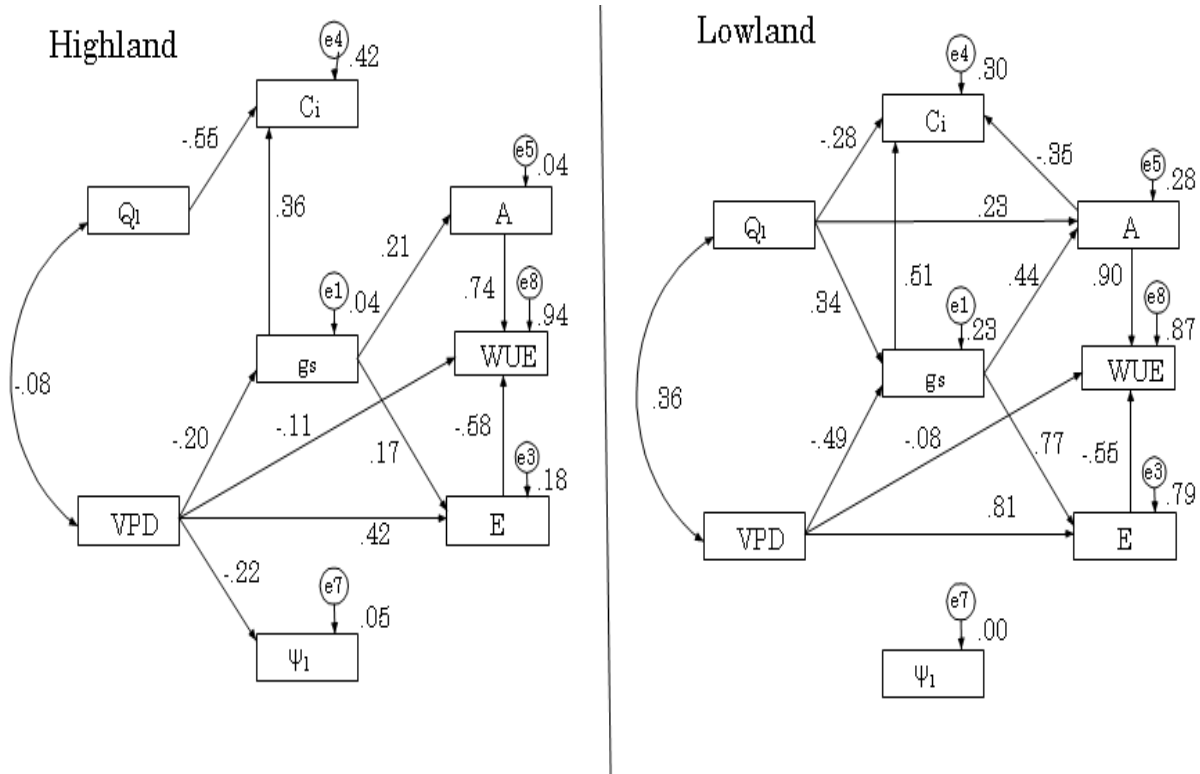
Similarly, light conditions varied considerably at any time of the day. Temperature showed a clear hump-shaped pattern during the day and was mirrored by the trend in the vapor pressure deficit (Figure 4).



**Figure 4.** Diurnal time course of light (a), leaf level photosynthetic rate (b), transpiration rate (c), water use efficiency (d) stomatal conductance (e), vapor pressure deficit (f), relative humidity (g), temperature of the external environment (h) Leaf water potential (i) and leaf temperature (j) in Abergelle (dark bars) and Metema (white bars) areas during mid-growing season.

*Gas exchange response patterns*

The path models of both areas partially confirmed our initial conceptual model, but the explained variation was rather low for assimilation and much higher for transpiration (Figure 5).



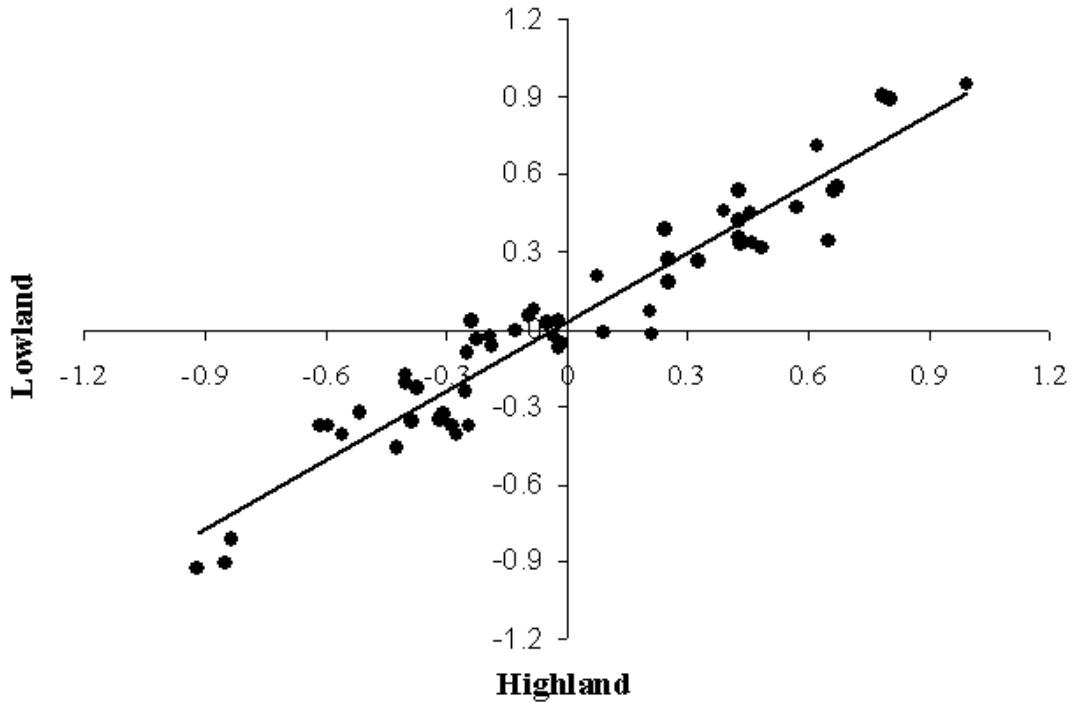
**Figure 5.** Path diagrams for describing dependence of gas exchange variables on external climatic and internal physiological traits in Abergelle (left) and Metema (right). Significant path values are indicated by numbers along arrows. The value at the right top of each variable box indicates the fraction of variation in that variable explained by the model.

We ended up with a separate path-model for each site, because there was no model significant for both sites pooled. Moreover, assuming the same path and path strengths for both sites never resulted in a significant model ( $\chi^2 > 100$ ,  $P < 0.001$ ). At the lower altitude, light had a positive direct effect on assimilation coupled with its indirect effect via stomatal conductance, which in turn, had positive effects on internal leaf  $CO_2$  pressure, assimilation and transpiration. The effect of light on stomatal conductance and subsequent gas exchange was not important for the high altitude Abergelle leaves. Here light levels might be close to saturation during most time of the day. Light had a direct negative effect on leaf internal  $CO_2$  concentration ( $C_i$ ) in both

areas. Probably this results from high photosynthetic rates utilizing internal CO<sub>2</sub> that creates steep gradients by depleting the internal CO<sub>2</sub> concentration (see also the effect of assimilation on C<sub>i</sub> at Metema). Vapor pressure deficit (VPD) had a negative effect on the leaf water potential at Abergelle, but not at Metema. VPD influenced gas exchange both indirectly via stomatal conductance and directly by increasing the transpiration demand. Higher VPD negatively affected stomatal conductance, which in turn resulted in lower gas exchange rates. Opposed to these indirect negative effects on gas exchange, VPD had a positive direct effect on transpiration since it increased the atmospheric transpiration demand. We thus observed that higher VPD at Abergelle induced a number of direct and indirect impacts on physiological responses, whereas such effects of VPD were only accompanied by light effects at Metema. Leaf water potential did not play a significant role in gas exchange at either site. Apparently, the observed range of leaf water potential was too small to create water limiting effects on gas exchange during mid-growing season.

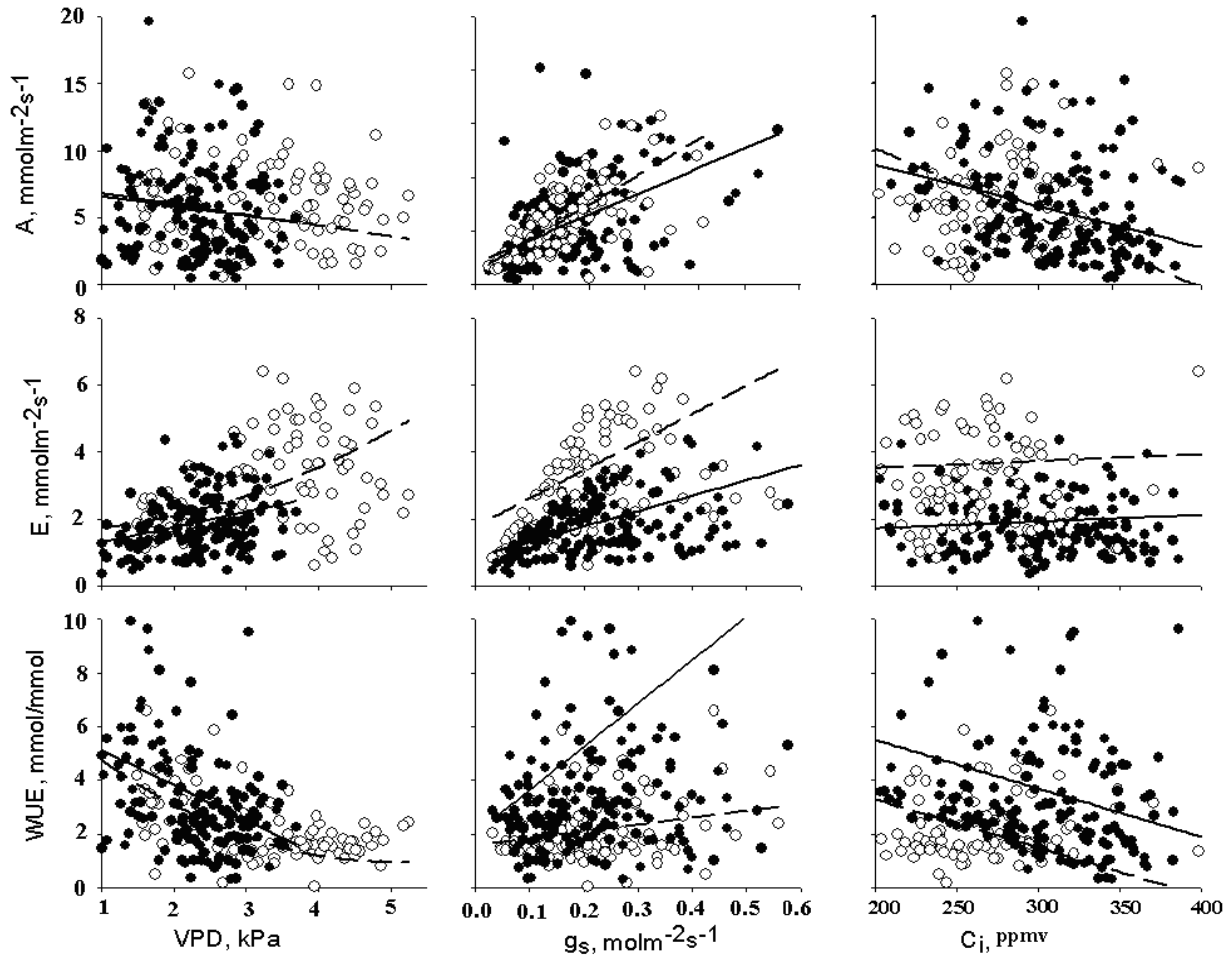
#### *Metema versus Abergelle*

Average net photosynthesis rate ( $A$ ) during the mid-growing season was 6.2 and 5.7  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , respectively for Abergelle and Metema and did not differ. Transpiration rate differed strongly between the two areas ( $P < 0.001$ ) (Table 2). Transpiration rate ( $E$ ) and leaf water potential ( $\Psi_l$ ) were also higher in Abergelle than Metema (Table 2). However, the water use efficiency (WUE) and sub-stomatal CO<sub>2</sub> concentration ( $C_i$ ) were higher in Metema than Abergelle (Table 2). Most correlations between traits are similar between sites. Hence, the correlation matrix (Appendix 1) resulted in similar patterns between the two areas irrespective of site variations (Figure 6).



**Figure 6.** Correlations between physiological and environmental trait are plotted for one site (Metema) against the other (Abergelle). Values are Pearson correlation coefficients.

Both from the correlation matrix and path models,  $g_s$  (internal physiological trait) and VPD (external climatic factor) are considered more important in affecting leaf gas exchange patterns at both areas (Figure 7), whereas the effect of light variation was statistically significant only at Metema.



**Figure 7.** Scatter diagram of physiological traits plotted against their direct causal variables from the path model in Abergelle (open circles) and Metema (filled circles); regression fitted lines are indicated by dotted lines (Abergelle) and continuous line (Metema).

## Discussion

In-situ gas exchange traits and environmental parameters were measured for *Boswellia* trees in dry woodlands of Ethiopia at two contrasting areas. Despite the limited number of days used for data collection, the daily range of environmental variability was wide enough to explore relationships. Extensive measurements of gas exchange in both areas allowed us to describe diurnal patterns in external environmental conditions, underlying physiological responses, and responses in gas exchange. Our results suggest that diurnal patterns in gas exchange differ from those observed for more frequently studied tree species in wet tropical and temperate forests (Weber and Gates 1990, Ishida et al. 1996, Souza et al. 2008). While we hardly observed any consistent hump-shaped pattern and no midday depression in assimilation as observed for other



trees (Weber and Gates 1990, Mulkey et al. 1996, Pathre et al. 1998), we found variable slightly decreasing assimilation after a morning peaks. Our results corroborate with some other studies in temperate (Bassow and Bazzaz 1998), Mediterranean (Gatti and Rossi 2010) and tropical savanna (Eamus et al. 1999) ecosystems. Results suggest that leaves maximized assimilation during the period of least atmospheric transpiration demand, mostly early in the morning and follow gradual closure of stomata as the transpiration demand increases (see also Zweifel et al. 2007, Bucci et al. 2008). High transpiration demand reflected by high vapor pressure deficit, largely determined an increased leaf transpiration rate during and after the midday.

We did not find one single path model that captured the cause-effect relationships underlying gas exchange for both sites. The sites nevertheless shared quite a number of cause-effects relationships. We showed how the light intensity and vapor pressure deficit influenced gas exchange, either directly or via underlying physiological traits. The two study areas shared similar physiological trait versus gas exchange trait correlations, suggesting that similar physiological mechanisms drive gas exchange in both systems (see similarities in Figures 5, 6 and 7). The path analysis nevertheless indicated significant differences between the high altitude and the low altitude area, representing the environmental extremes where the study species is encountered. A positive direct effect of light on assimilation was only observed at the low altitude site (Metema), where light levels varied across the non-saturated range. At the high altitude, light levels were much higher and mainly varied within the saturating range for assimilation. Both areas showed similar responses in gas exchange to variation in VPD: VPD down-regulated assimilation indirectly via limiting stomatal conductance and increased the transpiration. This is in agreement with studies in some tropical and temperate forests (Fetene and Beck 2004, Cunningham 2004, Passos et al. 2009).

Leaf water potential is considered a key functional trait because it is associated with the water stress status of trees and its influence on stomatal conductance (e.g. Meinzer et al. 2001, Tuzet et al. 2003, Zweifel et al. 2007, McDowell et al. 2008). However, we observed a narrow range of leaf water potential values (Figure 4) at the high altitude site Abergelle (-2.6 to -0.01bars) and the lower altitude site Metema (-5.9 to -0.3bars). During this measurement period, we did not see any influence of the leaf water potential on gas exchange in either of the study areas (Figure 5). This suggests that a deciduous species such as *Boswellia* is more challenged by atmospheric drought than by soil water deficits during the wet growing season. A relatively

shallow but extensive root system (Ogbazghi et al. 2006, Mengistu, unpublished data), or stem water storage, might buffer against the fluctuating and erratic rainfall conditions during the wet season. It may also explain why the species is able to start leaf flushing before the actual onset of the first rains after a period of eight dry months. Narrow ranges of leaf water potential of this deciduous tree species, with leafing only during a rainy season, are similar to the observations on Neotropical Cerrado trees (Bucci et al. 2008) and Mediterranean trees (Martínez-Vilalta et al. 2002) and contribute maintaining normal gas exchange during the wet season.

The light-saturated photosynthesis ( $A_{\max}$ ), which is an important factor for whole plant carbon gain (Chazdon and Field 1987), reached a higher level at the high than at the low altitude area. Possibly, this light response reflects an acclimation response to the higher average light levels at higher altitude. However, the average assimilation rates under ambient condition did not significantly differ between the two areas. The path analysis suggests why: at the higher altitude site, assimilation was tuned-down by stomatal closure in response to a higher VPD. This confirms that leaves of tropical dry woodlands, subjected to adequate light levels but high transpiration demand, might not increase carbon uptake because gas exchange becomes indeed limited by VPD. Similar observations have been reported for dry woodland trees in Mexico (Lebrija-Trejos et al. 2010).

Overall, our results confirm that leaf gas exchange at the low altitude is both light and VPD limited, while at high altitude it is mainly VPD limited. Therefore, these between site differences are not only driven by site factors but also to species physiological differences in response to those factors (Figures 3). We found that gas exchange responses resulted from the interaction of various environmental factors and the species response to these factors. This is essential for describing how varying environmental conditions affect in-situ gas exchange.

## Conclusions

Diurnal patterns of photosynthesis in frankincense trees showed neither a clear bell-shaped pattern nor midday depression, but instead a weak decline following morning maxima. We show how strongly the atmospheric VPD, and thus transpiration demand, controlled stomatal conductance and in turn assimilation. Moreover, a higher VPD resulted in a higher transpiration and lower water use efficiency. Our results suggest that assimilation at the low altitude is both light and VPD limited, while at high altitude it is mainly VPD limited. At this latter site, the

higher VPD down-regulated stomatal conductance such that average assimilation rates at ambient conditions did not differ from the other site. We conclude that trees of the study species were capable of acclimating to a variety of environmental conditions. Such understanding of environmental and physiological mechanisms that influence leaf gas exchange responses to environmental conditions will help to design future management systems for these inhabitants of the vast but poorly studied dry woodland trees.

**Appendix 1.** Pearson's correlation matrix among physiological traits and environmental variables for Abergelle (lower left diagonal) and Metema (upper right diagonal) during mid-growing season.

<b>Abergelle</b>											
<b>/Metema</b>	$Q_i$	$T_i$	$C_i$	$A$	$E$	$g_s$	$R_h$	$T_a$	$\Psi_i$	WUE	VPD
$Q_i$		0.327**	-0.321**	0.315**	0.451**	0.207**	-0.242**	0.269**	-0.020NS	-0.009NS	0.150*
$T_i$	-0.082NS		-0.373**	0.032NS	0.554**	-0.331**	-0.812**	0.893**	-0.037NS	-0.374**	0.645**
$C_i$	-0.516**	-0.056NS		-0.209**	-0.053NS	0.263**	0.385**	-0.375**	0.049NS	-0.174*	-0.290**
$A$	0.007NS	-0.023NS	0.130NS		0.425**	0.472**	-0.015NS	-0.002NS	0.006NS	0.712**	-0.09NS
$E$	0.007NS	0.671**	-0.23NS	0.428**		0.459**	-0.455**	0.536**	-0.034NS	-0.229**	0.359**
$g_s$	0.079NS	-0.309**	0.331**	0.218*	0.063**		0.338**	-0.355**	0.016NS	0.187*	-0.303**
$R_h$	0.187*	-0.836**	0.034*	0.209*	-0.422**	0.210**		-0.923**	0.031*	0.345**	-0.654**
$T_a$	0.224*	0.804**	-0.031*	-0.128NS	0.429**	-0.177	-0.918**		-0.031NS	-0.405**	0.657**
$\Psi_i$	-0.016NS	-0.322**	0.119NS	0.112NS	-0.233*	0.093NS	0.203*	-0.169*		0.038ns	-0.108NS
WUE	-0.013NS	-0.595**	0.172*	0.622**	-0.371**	0.196*	0.649**	-0.557**	0.318**		-0.289**
VPD	-0.058NS	0.740**	-0.063NS	-0.070NS	0.338**	-0.149NS	-0.836**	0.757**	-0.210*	-0.454**	

Note: n = 90 for Abergelle and n=178 for Metema; \*\* Correlation is significant at 0.01 level and \* at 0.05 level; NS = not significant.

## Chapter 3

### *Annual carbon gain in *Boswellia papyrifera* (Del.) Hochst trees: effects of light, crown traits and frankincense tapping*

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(Submitted for publication)

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*Abstract*

Whole crown carbon gain depends on environmental variables and functional traits, and in turn sets a limit to growth sinks of trees. We estimated annual crown photosynthetic carbon gain of the frankincense tree, *Boswellia papyrifera*, in dry woodlands of Ethiopia and examined the effect of light, functional traits, and tapping on carbon gain and, in turn, considered the consequences for resin yield. During the rainy season, trees of the drier highland had a higher diurnal photosynthetic rate ( $0.35 \text{ molCO}_2 / \text{m}^2 / \text{d}$ ) than trees of the less dry lowland ( $0.23 \text{ molCO}_2 / \text{m}^2 / \text{d}$ ), since highland trees received more light and had higher photosynthetic capacities than lowland trees. Highland trees therefore achieved a higher annual carbon gain ( $1081 \text{ molCO}_2 / \text{yr}$ ) than lowland trees ( $776 \text{ molCO}_2 / \text{yr}$ ), despite their shorter leaf lifespan (69 days vs. 81 days). Intensive tapping reduced crown leaf area and the annual carbon gain in the lowland trees, but not in highland trees.

Although the lowland site is characterized by a longer wet season and with longer leaf lifespans, trees have lower annual carbon gain than the highland due to lower light intensity and lower photosynthetic capacities. These results highlight how the interplay between local conditions and functional traits determine regional variation in tree productivity. However, such differences in productivity and carbon gain did not influence frankincense yield across sites. We conclude that *Boswellia* trees of different populations are thus adapted to their local climate conditions.

*Key words:* *Boswellia*; crown assimilation; Ethiopia; frankincense; plant traits; tapping

## Introduction

Some trees in the families of Pinaceae (*e.g.* pines), Euphorbiaceae (*e.g.* rubber) or Burseraceae (*e.g.* Boswellia) produce gum and resin upon bark wounding or tapping. The frankincense producing Boswellia trees dominate large areas of dry woodlands in eastern and central Africa and elsewhere, where resin is often tapped by local communities for local or international markets (Ogbazghi et al. 2006, Tadesse et al. 2007, Mertens et al. 2009). Tapping creates a carbon sink that is at the cost of growth sinks, including vegetative growth and reproduction (Cannell & Dewar 1994, Rijkers *et al.* 2006, Chantuma et al. 2009). Moreover, dry woodland trees may suffer from irregular rainfall patterns, thus creating more limiting growth conditions during some years than others (Murphy & Lugo 1986, Bullock et al. 1995, Vanacker et al. 2005). Climate change may also affect rainfall patterns, and reduce the ability of trees to acquire and supply carbon to the different carbon sinks (Lacointe 2000, Hély et al. 2006, Bolte et al. 2010). It is a major challenge to understand how resin tapping will affect the ability of trees to acquire carbon and its subsequent impact on annual carbon gain.

For resin producing trees, the annual whole-crown carbon gain depends on a number of functional plant traits, environmental conditions and tapping intensity. Functional traits that affect crown carbon gain include leaf photosynthetic rates, total leaf area and average leaf lifespan (Kikuzawa & Lechowicz 2006, Selaya & Anten 2010). However, it is not clear how they scale-up to crown carbon gain in the field (Poorter & Bongers 2006) and *visé versa*. This information is especially limited for tropical dry forests and dry woodland trees (Yoshifugi et al. 2006, Kushwaha et al. 2010).

In the present study, we link light and crown functional traits to annual carbon gain and annual carbon gain to resin yield for frankincense producing trees of two populations. The first tree population occurred at lower altitude (810-900 m) with a longer and wetter dry season than the population at higher altitude (1400-1650 m). These two populations represent the climatic extremes of this species in Ethiopia. For both populations we determine: (1) the effect of tapping intensity on functional traits and crown carbon gain; (2) the possible effects of contrasting site conditions on crown carbon gain and (3) the impact of photosynthetic carbon gain on incense production. Because the resin is rich in carbon (Hamm et al. 2005, Mertens *et al.* 2009), tapping is expected to drain carbon reserves limiting the carbon availability for leaf formation. We

expect a higher tapping intensity to reduce crown leaf area and hence canopy carbon gain. We also expect that trees in the drier highland area, with a shorter rainy season, will be restricted in the crown carbon gain by the limited leaf lifespan, and thus be more affected by tapping compared to lowland trees. Higher leaf area and crown assimilation is expected to increase frankincense yield. We discuss leaf and canopy traits that enable *Boswellia* to survive in contrasting sites and compare their response to light scenarios.

## Methods

We studied crown assimilation and carbon gain of *Boswellia papyrifera* of the family Burseraceae in two contrasting woodlands in northern Ethiopia. Abergelle is at an altitude of 1400-1650 m (hence forward referred to as “highland” site), and Metema is at a lower altitude of 810-900 m (referred to as “lowland” site). The Abergelle site is drier and has erratic rainfall with the wet season shorter than Metema (Chapter 2, Figure 2). The less dry Metema site has a relatively better rainfall distribution (Chapter 2, Figure 2).

We selected trees with a DBH of  $20 \pm 3$  cm for the experiment. For each site, the experimental trees were randomly allocated to one of the three treatments, *i.e.* 0 (control), 6 and 12 incisions tapping. The tapping treatments were applied over two successive dry seasons (2007-2008 and 2008-2009). In the highland, we established one plot and selected 10 trees per tapping treatment for gas exchange out of which five were also used for estimating total leaf area. In the lowland, we established four plots with *a priori* assumption of local variation and five trees were selected per tapping treatment in each plot for both gas exchange and total leaf area.

To estimate total leaf area of a tree, we counted the total number of apices per tree, the number of leaves per apex (three apices per tree), the number of leaflets per leaf (three leaves per tree), and measured leaflet area (five randomly selected leaflets per tree) using ADC model AM 100 leaf area meter (ADC, Bioscientific, Hoddesdon, UK). For each tree, total leaf area was calculated as the product of the number of apices, the number of leaves per apex, the number of leaflets per leaf and the average leaflet area after full expansion.

To estimate the leaf lifespan and crown leaf area over the wet season (the dry season was completely leafless), we monitored weekly leaf size expansion and number on three apices, leaves and leaflets for each of the five trees per tapping treatment. To determine leaf lifespan, a



leaf was considered “born” when half of its size unfolded and total crown leaf area was “born” halfway between first leaf and full leaf area expansion. Similarly, the timing of crown leaf “death” was recorded when leaves changed color from green to yellow because this is assumed to be a critical stage beyond which leaves may not benefit from a positive daily carbon balance anymore (Reich et al. 2009). Effective crown leaf lifespan (only counting days during which leaves function at full expansion) was calculated as the time difference (in days) between crown leaf birth and death.

#### *Gas exchange measurements*

Gas exchange was measured on 01-03 July 2008 and 20-22 July 2009 at the highland site. Similar measurements for the lowland site were made on 20-28 June 2008 and 05-14 July 2009. During these measurement periods, gas exchange was measured for every experimental tree in the morning (0800-1100h), around noon (1200-1400h) and in the afternoon (1500-1700h) on a single leaflet, using an open portable gas exchange system, LcPro (ADC, Bioscientific, Hoddesdon, UK). On each measurement day, one tree was randomly selected from each tapping treatment and gas exchange measured on a well-expanded leaflet.

In addition to these in-situ measurements under ambient conditions, we established light responses curves for five leaves in each site. For this purpose, photosynthesis was measured for a range of light values, using a detachable mixed Red/Blue LED light source chamber (2x3 cm) on top of the LcPro leaf chamber. Leaves were enclosed in a leaf chamber without any light for 30 minutes and, consequently, light levels were increased progressively over a realistic light intensity range, *i.e.* from 0, 50, 100, 200 to 400 and then up to 2000 using steps of 200  $\mu\text{mol}/\text{m}^2/\text{sec}$ . For each measured leaf, a light response curve was established using the non-rectangular hyperbola (Thornley and Johnson 1990).

$$A = \frac{\phi I + A_{\max} - \sqrt{\{(\phi I + A_{\max})^2 - (4\theta\phi I A_{\max})\}}}{2\theta} - R_d \quad (1)$$

In this model, the parameters are the irradiance ( $I$ ), the light saturated photosynthetic rate ( $A_{\max}$ ), dark respiration rate ( $R_d$ ), the quantum yield ( $\phi$ ), and the curvature factor ( $\theta$ ) per selected tree.

### Scenarios

We estimated the daily photosynthetic rates by integrating the photosynthetic measurements under ambient light conditions over the day. Since we took three periodic measurements during the day (each assumed to represent 1/3 of the day), we integrated each measurement for four hours. Subsequently, we estimated the wet season annual carbon gain (dry season is leafless) of leaves per tree by integrating the daily photosynthetic rates to the leaf lifespan. Annual crown carbon gain is the product of the site-specific crown leaf lifespan, leaf specific daily photosynthetic rate, and tree specific total leaf area.

Many studies used vertical integration against radiation gradient to determine total crown carbon gain (Baldocchi 1993, Bonan 1995, Nasahara et al. 2008), and distinguished between sunlit and shaded leaf parts (De Pury & Farquhar 1997, Wang & Leuning 1998). Because frankincense trees exhibit little self-shading under natural conditions, we assumed that all leaves in the canopy were exposed to similar light level, which is a reasonable assumption for the relatively small and open crowns of the study species. Moreover, leaves were selected randomly during gas exchange measurement to account for any possible shedding effect.

Moreover, we could compare the ambient light-based estimated annual carbon gain described above, with estimations assuming either entirely clear or entirely overcast days. For the latter two contrasting light scenarios, diurnal leaf light interception was calculated as a function of time, using  $I_t = I_{t=12} \sin(\pi(t-6)/12)$ , where time  $t$  was entered as hour from 0600 h until 1800 h. Here the noon irradiance  $I_{t=12}$  was assumed to be  $2000 \mu\text{mol}/\text{m}^2/\text{sec}$  light for clear sky conditions and  $500 \mu\text{mol}/\text{m}^2/\text{sec}$  light for cloudy day conditions. Leaf photosynthetic rates were subsequently calculated using a non-rectangular hyperbolic light response curve of leaf photosynthesis for each site (equation 1), and integrated over the day. But, ambient light was used for first field based measurement. In all scenarios, the day length (h) was taken as 12 h. Subsequently, we estimated the (wet season) annual carbon gain as the product of leaf daily photosynthetic rate, leaf lifespan, and total leaf area.

### Data analysis

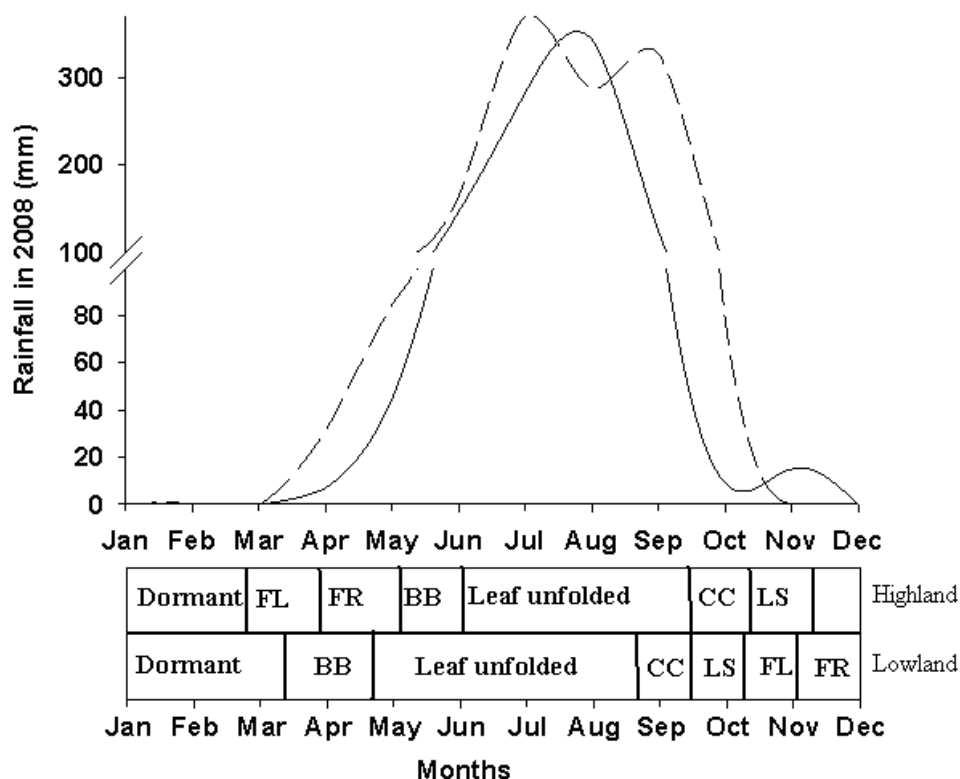
A general linear model with univariate analysis and Tukey *post-hoc* multiple comparisons was used to test the effect of tapping intensity on plant traits driving annual carbon gain. The analysis was done by including the interaction between sites and tapping intensity as a fixed factor. The

relationship between photosynthetic rates or crown leaf area with crown carbon gain and frankincense yield was tested by linear regression models. Data was analyzed using SPSS (PASW 17.0 for Windows statistical software package).

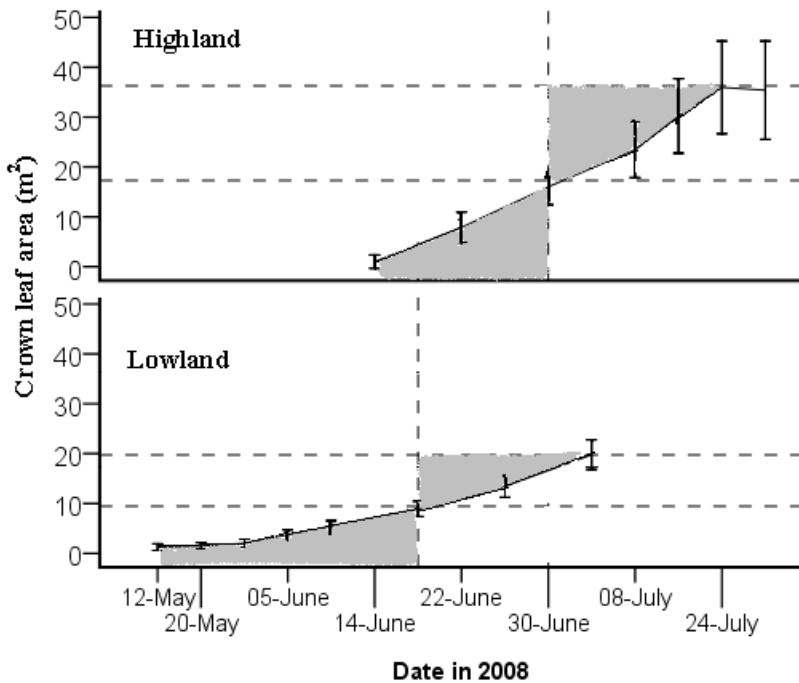
## Results

### Leaf phenology

Leafing and senescence started earlier in the lowland than in the highland (Figure 1). Nevertheless, the estimated effective crown leaf lifespan was 81 days in the lowland and only 69 days in the highland. In both sites, leaf bud burst already started before the actual onset of the first rains. Flower and fruit production occurred during the leafless dry period, but sites differed in their timing (Figure 1). Fruit bud initiation started early during the dry season shortly after leaf shedding in the lowland, whereas it occurred at the end of the dry season in the highland. Tapping had no significant effect on the timing of leafing (Figure 2).



**Figure 1.** Phenological patterns of *Boswellia papyrifera* in relation to rainfall in Metema (lowland, dashed line) and Abergelle (highland, solid line) sites in Ethiopia. Codes for successive phenological periods include: FL= flowering, FR= fruiting, BB= leaf bud breaking, CC= Pre-leaf fall color change and LS= leaf shedding.



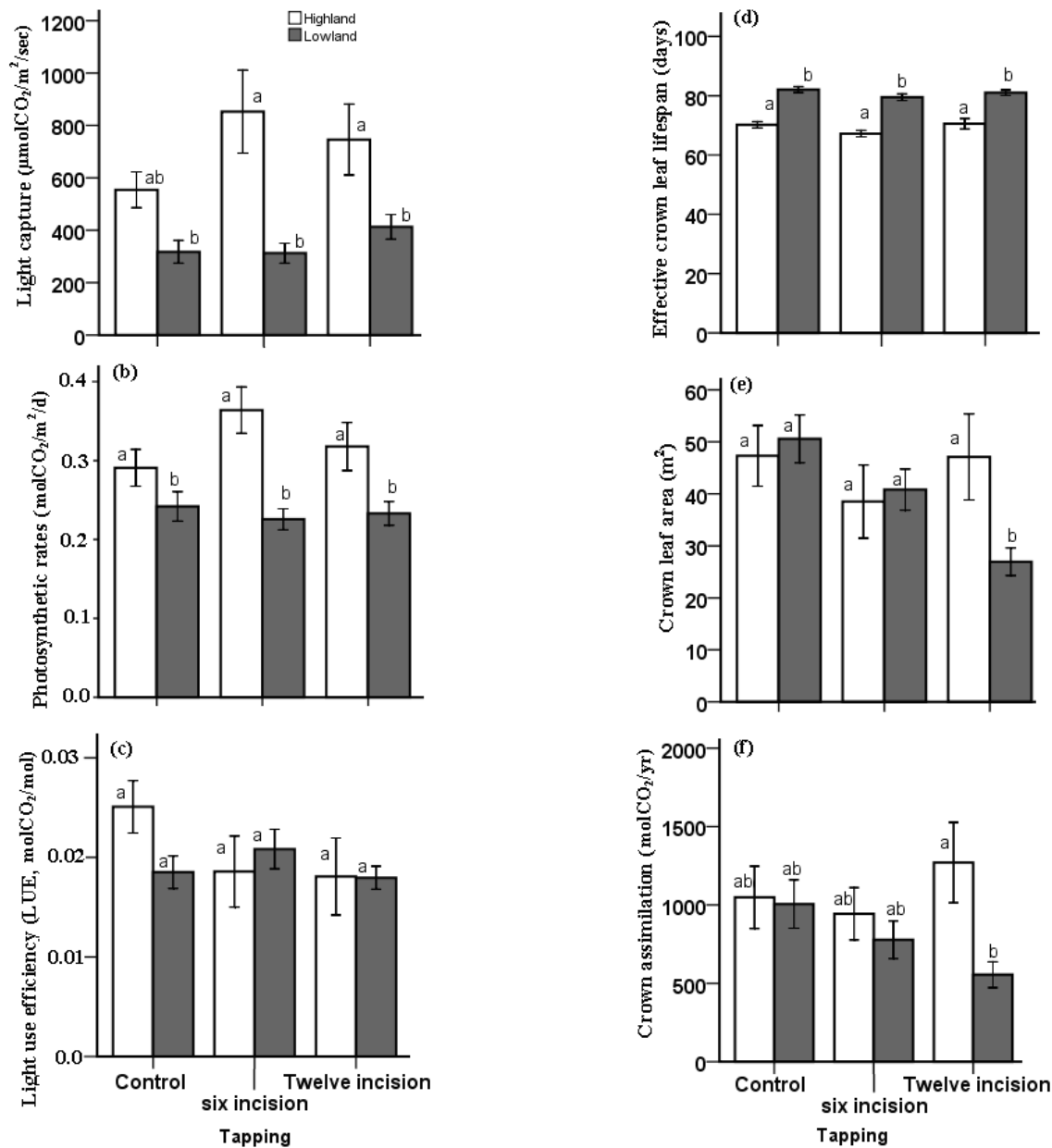
**Figure 2.** The expansion in mean crown leaf area during the start of the wet season in the lowland site (Metema) and the highland site (Abergelle). Mean values of the three tapping treatments are plotted because there was no significant difference between tapping treatments (ANOVA: Abergelle,  $P = 0.33$  and Metema,  $P = 0.13$ ). The horizontal broken lines are the maximum and half expansion values while the vertical broken lines marked the beginning of effective crown leaf lifespan (a point where half of the crown leaf area expanded, see methods). The upper and lower colored regions of each site are assumed to be equal.

#### *Tapping Effects on Leaf Area and Carbon Gain*

Tapping reduced crown leaf area in the lowland, but not in the highland. Because of the reduction in crown leaf area, estimated annual crown assimilation was lower for heavily tapped trees in the lowland (Figure 3; Table 1).

Estimated annual crown assimilation (Table 2) of trees was higher in the highland ( $1081 \text{ molCO}_2 / \text{yr} \pm 118$ ) than in the lowland ( $776 \text{ molCO}_2 / \text{yr} \pm 72$ ), resulting from the higher daily photosynthetic rate in the highland ( $0.35 \text{ molCO}_2 / \text{m}^2 / \text{d}$ ) than the lowland ( $0.23 \text{ molCO}_2 / \text{m}^2 / \text{d}$ ). With similar average crown leaf area between the two sites, the shorter crown leaf lifespan of trees in the highland apparently was more than compensated by their higher photosynthetic rates

(Figure. 3 B, D, E). Higher light interception together with high photosynthetic capacity resulted in higher photosynthetic rates in the highland compared to the lowland.



**Figure 3.** Light capture (a), daily photosynthetic rates (b), light use efficiency (c), effective crown leaf lifespan (d), crown leaf area (e) and estimated annual crown assimilation (f) in relation to different levels of tapping intensity. Tapping included 6 or 12 incisions, and these treatments were compared with the control (without tapping) across sites. Different letters indicate across site differences after *post-hoc* test.

**Table 1.** Leaf and crown traits of *Boswellia* trees under the different tapping levels in the study sites. Mean values are given and different letters along the row indicate significant differences.

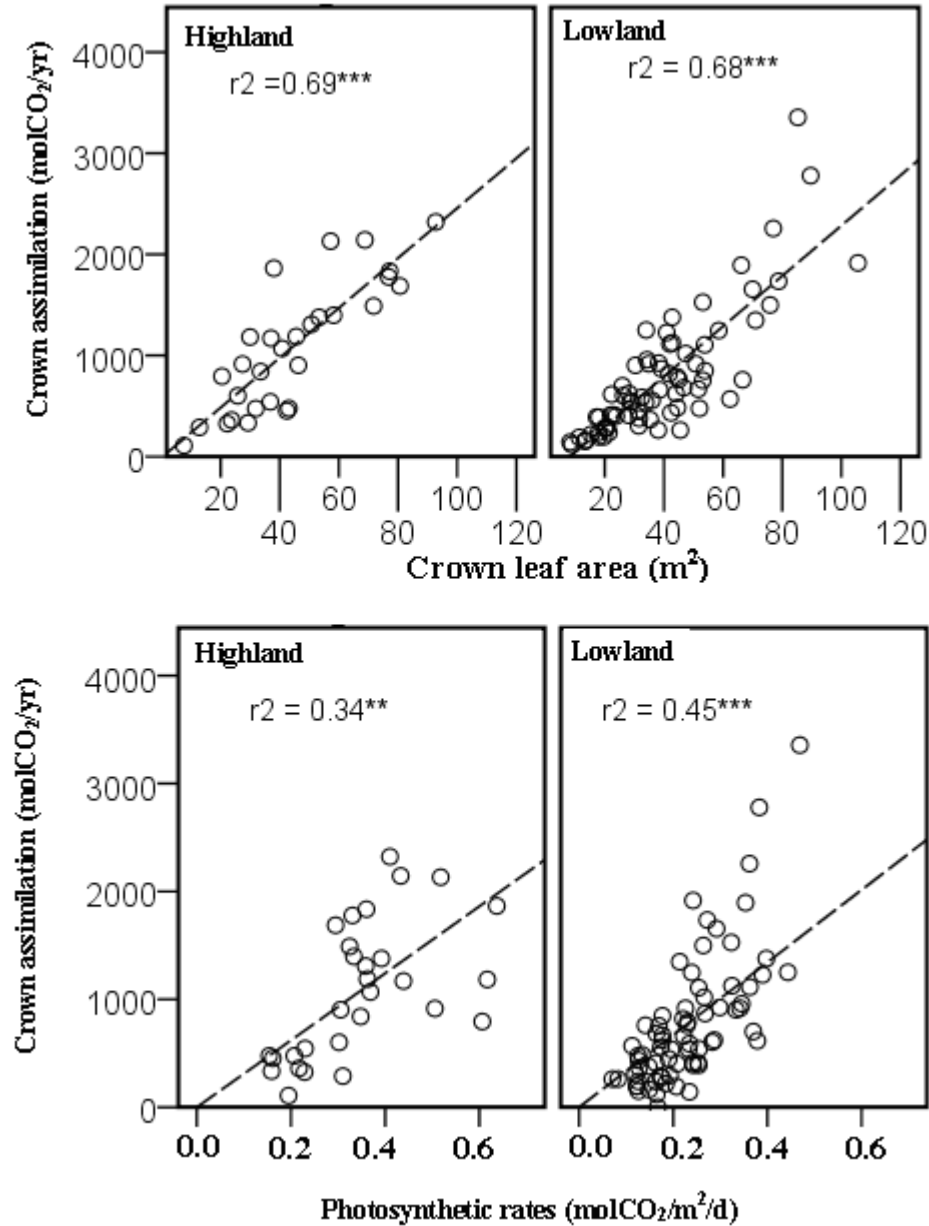
Plant Traits	Units	Highland			Lowland		
		Tapping			Tapping		
		0	6	12	0	6	12
Tree DBH	cm	18.16 <sup>a</sup>	18.84 <sup>a</sup>	18.61 <sup>a</sup>	19.66 <sup>a</sup>	19.26 <sup>a</sup>	18.72 <sup>a</sup>
Number of apex/tree	number	63 <sup>a</sup>	75 <sup>a</sup>	80 <sup>a</sup>	118 <sup>b</sup>	86 <sup>ab</sup>	88 <sup>ab</sup>
Number of leaf/apex	number	11 <sup>a</sup>	11 <sup>a</sup>	10 <sup>a</sup>	10 <sup>a</sup>	9 <sup>a</sup>	9 <sup>a</sup>
Number of leaflet/leaf	number	16 <sup>a</sup>	16 <sup>a</sup>	19 <sup>a</sup>	19 <sup>a</sup>	19 <sup>a</sup>	17 <sup>a</sup>
Effective leaf lifespan	days	70 <sup>a</sup>	67 <sup>a</sup>	70 <sup>a</sup>	82 <sup>b</sup>	79 <sup>b</sup>	81 <sup>b</sup>
Leaflet area	cm <sup>2</sup>	42.9 <sup>c</sup>	29.4 <sup>b</sup>	31.8 <sup>bc</sup>	22.6 <sup>a</sup>	26.5 <sup>ab</sup>	19.5 <sup>a</sup>
Light use efficiency	molCO <sub>2</sub> /mol	0.025 <sup>a</sup>	0.019 <sup>a</sup>	0.018 <sup>a</sup>	0.019 <sup>a</sup>	0.021 <sup>a</sup>	0.018 <sup>a</sup>
Photosynthetic rates	mol CO <sub>2</sub> /m <sup>2</sup> /d	0.29 <sup>abc</sup>	0.38 <sup>c</sup>	0.36 <sup>bc</sup>	0.22 <sup>ab</sup>	0.23 <sup>a</sup>	0.23 <sup>ab</sup>
Crown leaf area	m <sup>2</sup>	47.31 <sup>a</sup>	38.51 <sup>a</sup>	47.11 <sup>a</sup>	50.57 <sup>a</sup>	40.81 <sup>a</sup>	26.94 <sup>b</sup>
Crown assimilation	mol CO <sub>2</sub> /yr	1048 <sup>ab</sup>	944 <sup>ab</sup>	1270 <sup>b</sup>	1005 <sup>ab</sup>	777 <sup>ab</sup>	555 <sup>a</sup>
Whole plant carbon gain	Kg /yr	31.5 <sup>ab</sup>	28.3 <sup>ab</sup>	38.0 <sup>a</sup>	30.2 <sup>ab</sup>	23.3 <sup>ab</sup>	16.6 <sup>b</sup>

**Table 2.** Comparison of plant traits between lowland Metema and highland Abergelle and among the tapping treatments. Tapping levels are compared for both sites combined.

Plant traits	Units	Sites			Tapping			Interaction	
		Highland	Lowland	F	Control	Six incision	Twelve incision	F	F
Number of apex/tree	number	73	98	11.68**	102	83	86	3.1*	6.3***
Number of leaf/apex	number	11	9	7.13**	10	9	9	0.6ns	3.6**
Number of leaflet/leaf	number	17	19	6.1*	18	18	18	0.1ns	2.8*
Effective leaf lifespan	days	69	81	119.1***	79	76	78	1.4ns	25.7***
Leaflet area	cm <sup>2</sup>	34.8	22.8	30.3***	28.6	27.4	22.8	2.6ns	10.8***
Light use efficiency	molCO <sub>2</sub> /mol	0.021	0.019	0.3ns	0.021	0.020	0.018	0.9ns	0.9ns
Photosynthetic rates	mol CO <sub>2</sub> /m <sup>2</sup> /d	0.35	0.23	26.33***	0.25	0.27	0.27	0.6ns	6.03***
Crown leaf area	m <sup>2</sup>	44.2	39.8	0.9ns	49.6	40.1	33.3	5.8**	3.7**
Crown assimilation	mol CO <sub>2</sub> /yr	1081	776	4.95*	1018	826	744	1.7ns	2.6*
Frankincense yield	g	464	342	1.9ns		332	423	1.3ns	1.3ns

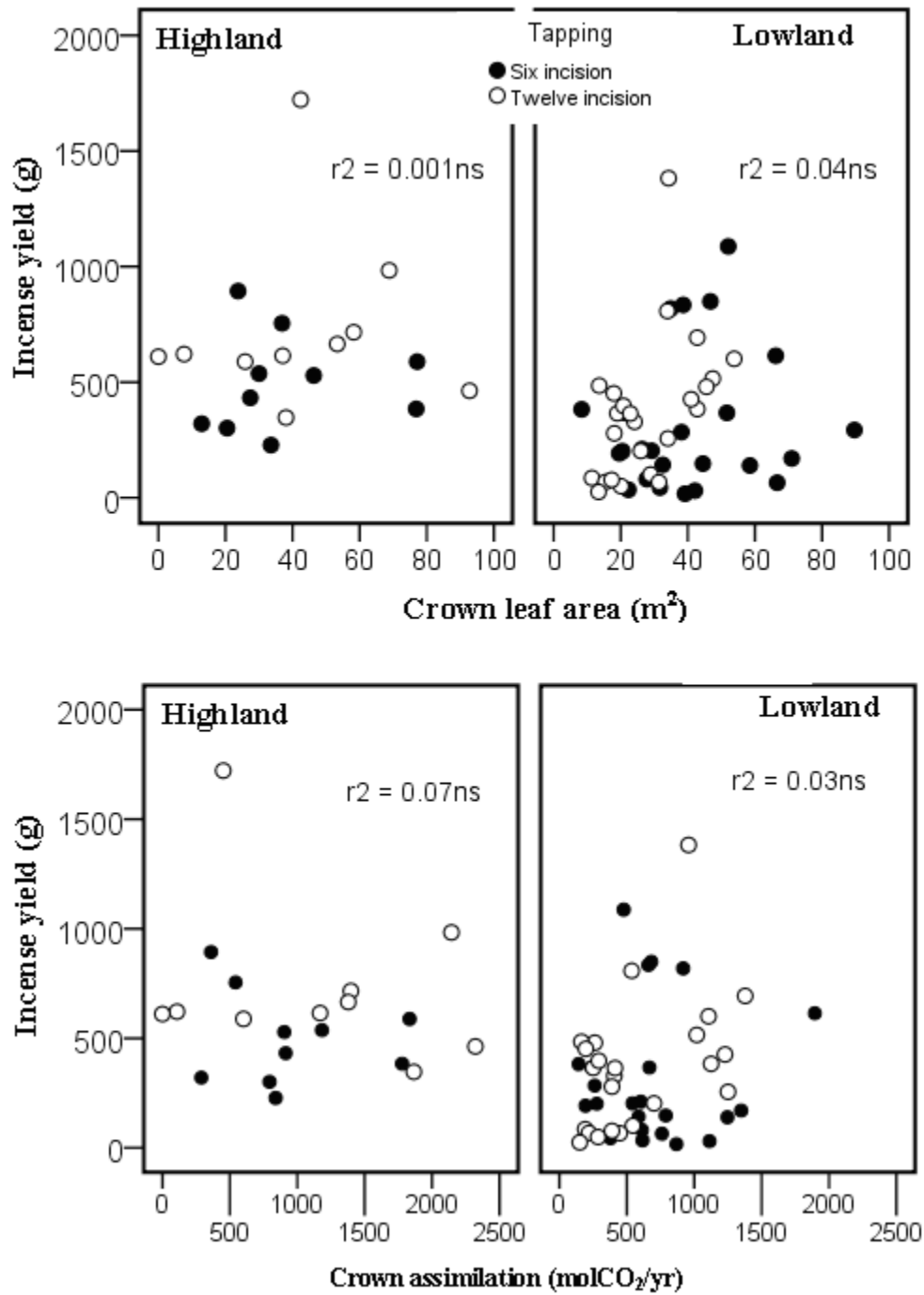
\*, 0.05-0.01; \*\*, 0.01-0.001; \*\*\*, &lt;0.001

Both leaf photosynthetic rate and crown leaf area positively correlate with crown assimilation (Figure 4). However, neither crown assimilation nor crown leaf area leads to higher frankincense yield (Figure 5) in both sites.



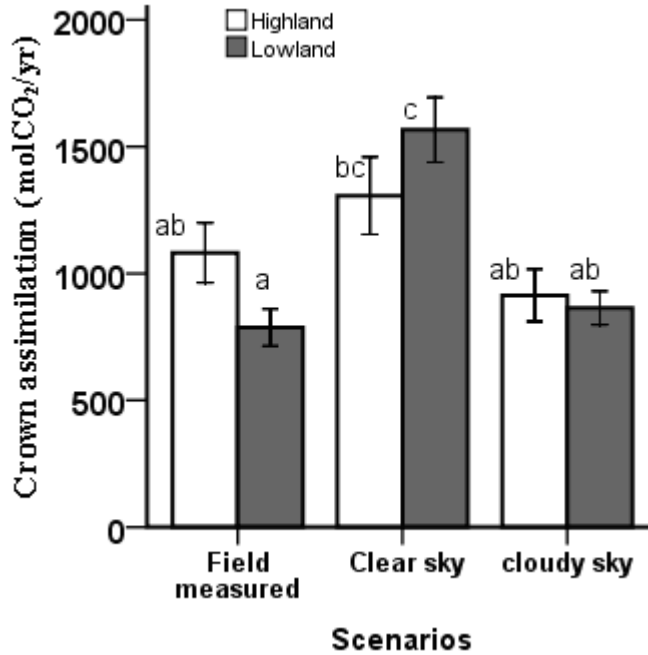
**Figure 4.** Linear regressions of annual crown assimilation with crown leaf area (upper panel) and photosynthetic rate (lower panel) for lowland Metema and highland Abergelle. Crown assimilation showed significant  $r^2$  values for both photosynthetic rates and crown leaf area (\*\*,  $0.01 > r^2 > 0.001$ ; \*\*\*,  $r^2 < 0.001$ ).





**Figure 5.** Linear regression to predict frankincense yield from the two tapping levels based on crown leaf area (upper panels) and crown assimilation (lower panels) is shown. Frankincense yield shows non-significant  $r^2$  values (ns) for both crown parameters.

Despite differences in light capture, the light use efficiency (LUE-the ratio of assimilation to absorbed light), was the same across treatments (Figure 3C; Table 1). Moreover, our predictions for light scenarios showed better performance for clear sky conditions especially in the lowland (Figure 6). Based on our prediction for annual crown carbon gain under the light scenarios, highland trees achieved 86 percent of the potential carbon gain ( $0.86 \pm 0.02$ ), while lowland trees achieved only 56 percent ( $0.56 \pm 0.03$ ).



**Figure 6.** Predicted crown assimilation is compared among ambient light, clear sky and cloudy sky conditions. The predicted values are based on the light response curves for clear sky conditions (a saturating mid-day light value of  $2000 \mu\text{mol}/\text{m}^2/\text{sec}$ ), for cloudy sky conditions (a mid-day light value of  $500 \mu\text{mol}/\text{m}^2/\text{sec}$ ) and field conditions (under ambient light).

### Discussion

We determined the effect of light, frankincense tapping and functional traits on crown carbon gain and subsequent resin yield for two *Boswellia* tree populations at contrasting sites. Earlier, the impact of resin tapping on reproductive effort was clearly shown (Rijkers et al. 2006), but not on crown leaf area. We expected a higher tapping intensity to reduce crown leaf area and hence crown carbon gain. Indeed, heavy tapping reduced annual carbon gain, but only in the lowland. Moreover, we did not observe any effect of carbon gain or site on the annual resin yield.

Timing of leaf and flower bud initiation, and crown leaf longevity were important phenological differences between the two areas (Figure 1 & 2). In both study sites, leaf bud burst started before the first rain of the wet season. This phenomenon was earlier recorded for tropical dry forest trees (Rivera et al. 2002, Elliott et al. 2006, Williams et al. 2008), and it has been suggested that such trees either have access to deep soil moisture or have a large stem water storage (Borchert 1994, Elliott et al. 2006, Williams et al. 2008, Kushwaha et al. 2010).

We expected trees in the drier highland area, with a shorter rainy season, to be restricted in crown carbon gain as a result of limited crown leaf lifespan (Suárez 2010), and also to be more affected by tapping. This was clearly not the case. Despite their shorter leaf lifespan, trees of the drier highland attained substantially higher annual crown assimilation. This difference was the product of the greater light availability (less cloud cover) and the larger photosynthetic capacities of trees at the highland site. These two factors more than compensated for the shorter leaf lifespan at this site. Previously (T. Mengistu et al. unpublished data), it was also shown that the photosynthetic capacity was higher in the highland than the lowland (highland =  $22.14 \pm 1.3$ ; lowland =  $14.89 \pm 0.98$ ;  $P = 0.008$ ). In the lowland, with higher rainfall and a longer wet season, trees achieved a lower annual crown carbon gain. Thus, surprisingly, rainfall differences alone could not explain the observed difference in annual carbon gain. It seems that light limitation has a more significant effect on annual carbon gain differences between these two sites. While the light limitation by clouded weather has been demonstrated for trees of rain forests (Clark & Clark 1994), this is as far as we know the first study that demonstrates such strong light limitation by persistent cloudiness for a dry woodland system.

Leaf area growth and carbon gain of lowland trees were negatively affected by intensive tapping. This suggests a trade-off between tapping and leaf formation: the carbohydrate used for resin production was at the cost of the carbohydrate to be invested in leaf area, comparable to the resin production to reproduction trade-off (Rijkers et al. 2006) and rubber production to growth trade-off (Chantuma et al. 2009). However, tapping effects on leaf area or annual carbon gain were not observed for highland trees. Possibly, these highland trees buffered the impact of tapping by their higher annual carbon gain.

Despite the variation in environmental conditions between sites and the individual tree variation in annual carbon gain, trees achieved similar resin yields. None of the considered functional traits (crown leaf area and assimilation) had immediate impacts on resin yield. This is

remarkable, given the large difference in annual carbon gain across trees. Since the measured trees had a similar stem diameter, we propose that the size of bark and the amount of resin secretory structures and canals could be a stronger driver for frankincense yield than the constraints set by annual carbon gain. Moreover, maximum yield of resins from plants depends on the kind of duct, the location of ducts in plants and how ducts are influenced by wounding (Langenheim 2003). On the other hand, frankincense is primarily to defend damage (Langenheim 2003) and that allocation to this function might have preference over other sinks even when trees have reduced carbon gain.

*Boswellia* trees thus seem acclimated or adapted to local conditions through changes in functional crown traits. Moreover, carbon gain of trees responded strongly to the variation in light intensity associated with the degree of cloudiness during the rainy season. Because rain is of course another important climatic factor, setting limits to leaf lifespan, the possible consequences of increased drought and associated weather conditions under climate change (Vanacker et al. 2005, Hély et al. 2006, WWF 2006, Butterfield 2009) suggest difficulty of predicting future sustainability of *Boswellia* trees, both for their annual carbon gain and resin productivity.

## Conclusion

The impact of tapping *B. papyrifera* on annual carbon gain was site specific. Heavy tapping negatively affected leaf area production and annual crown assimilation in the lowland. In the highland, trees are less affected by tapping due to better light conditions and photosynthetic capacity that give better annual carbon gain advantage. Thus, the combined effect of higher photosynthetic rate and shorter leaf lifespan resulted in more carbon gain advantage than the combined effect of long leaf lifespan and lower photosynthetic rate. We conclude that *Boswellia* trees are differentially acclimated to their local environmental conditions within the tropical woodland systems they live in. However, the impact of future climate change may alter the length of the leaf bearing period with a possible effect on crown carbon gain and resin productivity of the species.

## *Chapter 4*

### *Frankincense tapping reduces the carbohydrate storage of Boswellia trees*

Tefera Mengistu, Frank J. Sterck, Masresha Fetene and Frans Bongers

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*Abstract*

Carbohydrates fixed by photosynthesis are stored in plant organs for future use mainly in the form of starch or sugars. Both starch and sugars form the total non-structural carbohydrates (TNC) and serve as intermediate pools between assimilation and utilization. We examined the impact of tapping on TNC concentrations and its seasonal variation in the wood, bark and root tissues of *Boswellia papyrifera* in two natural woodlands of Ethiopia. Tapping is expected to reduce carbon from the plant organs. We expected “exhaustion” of storage carbon during the leafless dry season and “re-fill” during the wet season when crowns are in full leaf. We also expected well-protected roots to have higher TNC concentrations than wood and bark.

As predicted, tapping reduced TNC concentrations and pool sizes in the plant system. Given the distinct seasonal changes in phenology of this deciduous tropical woodland tree, a significant difference was observed between end of wet season maxima and end of dry season minima in TNC concentrations. Moreover, *Boswellia* trees appear to have more starch stored in the stem and more soluble carbon stored in the bark. Evidently, reduced TNC concentrations after tapping in all tissues and the seasonal dynamics of carbohydrate reserves appear relevant parameters to cautiously evaluate the long-term sustainability of tapping *Boswellia* trees in the dry woodlands systems.

**Keywords:** non-structural carbon, *Boswellia papyrifera*, frankincense, starch, sugar

## Introduction

Plants acquire carbon when they have a full green canopy and directly use or store it until required for future use (Chapin et al. 1990, Newell et al. 2002, Bansal and Germino 2009, Chantuma et al. 2009, Regier et al. 2010). Storage carbohydrates will then be allocated to growth, maintenance, reproduction and defense. While new foliage on evergreen plants could initially be supported by carbohydrates supplied 'online' from pre-existing shoots (Hoch et al. 2003, Bansal and Germino 2009), this benefit is hardly possible for deciduous species. Therefore, tropical deciduous species should accumulate carbon when canopies are in full leaf and completely depend on their stored carbohydrates while leafless.

Storage is in the form of non-structural carbohydrates, largely starch and sugars (Würth et al. 2005, Raessler et al. 2010), which form the Total Non-structural Carbohydrates (TNC). TNC and its seasonal variations in trees reflect the source-sink balance (Würth et al. 2005, Bansal and Germino 2009) and enhance plant survival as it allows plants to overcome periods of stress (Poorter and Kitajima 2007). It is well established that a TNC pool becomes larger when sinks are reduced (Chapin and Wardlaw 1988) and vice versa. Therefore, on whole tree basis, the stored TNC should reflect not only the supply but also the potential for future demands.

Trees may accumulate reserves in different tissues, i.e. leaves, stems and roots. The storage in such tissues depends on the size of these compartments and resource concentrations in each of them. Most studies of seasonal TNC patterns in tropical trees concentrated on one compartment, while TNC has rarely been examined in multiple compartments simultaneously (Newell et al. 2002). Moreover, knowledge of storage carbon reserves for resin and gum producing tropical dry woodland trees is lacking.

In this study, we determined how frankincense tapping influences the non-structural carbohydrate content of the frankincense tree, *Boswellia papyrifera*, and to what extent the carbohydrate storage changes with season, in two contrasting natural *Boswellia* populations of Ethiopia. For Abergelle and Metema populations, we determined (1) how frankincense tapping influences the total non-structural carbohydrate content of trees (2) the extent to which *Boswellia* trees re-charge their storage carbon during the shorter growing season after dry season exhaustion by reproductive effort and tapping and (3) how concentrations differ in plant organs.

Tapping is expected to drain carbon from the plant and reduce storage carbon concentration in the plant organs during the dry season. We also expect trees to face

“exhaustion” of storage carbon after competing carbon sinks during the long dry season and possible “re-fill” when canopies are in full leaf during the short wet season. Finally, we expect higher storage carbon concentration in the well protected root (Hoffmann et al. 2003, Regier et al. 2010) followed by the wood and bark sections, the latter commonly affected by fire, tapping and herbivory.

In order to test these hypotheses, we quantified the non-structural carbohydrate concentrations in the wood, bark and root compartments of a dry woodland frankincense tree for a period of two years (2007-2009) in two contrasting areas in Ethiopia. The species differed in leaf and reproductive phenology in the two areas (Chapter 3). TNC concentrations were determined after the long dry season and after the short wet season to elucidate whether *Boswellia* trees are able to “re-fill” their storage carbon during wet season and “exhaust” during the dry season. For this purpose, we harvested sliced samples of wood, bark and root tissues in October (wet season end) 2007 and 2008 and June (dry season end) 2008 and 2009 from tapped and control trees to determine their TNC concentrations per unit dry mass. Pool sizes were extrapolated using data on whole tree biomass. This study is pioneer in evaluating the influence of tapping on storage carbohydrates in multiple organs of the frankincense producing tree in a tropical dry woodland system. However, there are prior studies for rubber (Chantuma et al. 2009, Silpi et al. 2007), Mango (Mialet-Serra 2006) and temperate deciduous taxa (Li et al. 2002, Hoch et al. 2002, Hoch et al. 2003).

## Methodology

### *Study site and species*

Adult trees of *Boswellia papyrifera* (Burseraceae) were selected at Metema, a low altitude area (810-990 meters) still with vast *Boswellia* populations and at Abergelle, a high altitude area (1400-1650 meters), with more fragmented *Boswellia* populations. The Abergelle site is drier and has erratic rainfall with the wet season shorter than Metema. The less dry Metema site has a relatively better rainfall distribution.

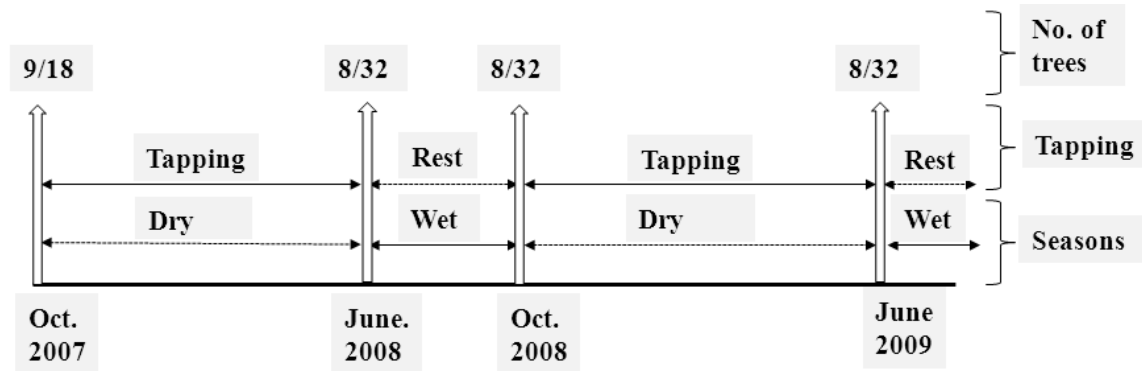
*B. papyrifera* is a deciduous tree up to 13m tall, and grows mainly in tropical dry woodlands with the centre of geographic distribution in the Horn of Africa (Lovett and Friis 1996). Flowering and fruiting occurs during the dry leafless season. Upon wounding, the tree



produces frankincense (a water-soluble gum and alcohol soluble resin) with distinctive fragrance and this is expected to drain the carbon pool from the plant system.

#### Field Data Collection

In October 2007, we marked 33 trees in Abergelle and 114 trees in Metema (Figure 1), of similar diameter ( $20 \pm 3$  cm DBH). The sample sizes differ due to the *a priori* assumption of different productivity levels between plots in Metema. The marked naturally grown *Boswellia* trees were randomly subjected to either no tapping (control) or heavy tapping (12 incisions) treatments during the dry season (as practiced locally). Wood, bark and root (including wood and bark) samples were collected from trees to determine the initial stock of storage carbon at the end of the wet season, in October 2007. After a full season of tapping experiment in the dry season (June 2008), a slice of stem wood, bark and root samples of four randomly selected trees were collected. This was repeated after the wet season (October 2008) and after the dry season (June 2009).



**Figure 1.** The design used for sampling plant tissues. The number of trees used for periodic sampling is indicated in the top (numerator for highland and denominator for lowland). In total 147 trees were used for both sites.

Within two hours after collection, samples were dried in a microwave oven at  $90^{\circ}\text{C}$  for 2 hours on site (Hoch et al. 2002) and transferred to Addis Ababa University laboratory for further oven drying to constant weight at  $75^{\circ}\text{C}$ . Samples were then ground to fine powder using a grinding mill. Ground samples were transferred into plastic cups and stored in a freezer until analysis. All samples were analyzed for total non-structural carbohydrates (TNC). TNC is defined here as the sum of free sugars (sucrose, glucose and fructose) and starch. TNC commonly covers more than

90% of mobile carbon in plants (Hoch et al. 2002, Hoch et al. 2003) and other carbohydrates were not included in this analysis because they contribute little (Hoch et al. 2003). Sugar standards were used as controls during the analysis (Hoch et al. 2002, Li et al. 2002).

*TNC analysis*

Total non-structural carbohydrates (TNC) were determined by high-performance liquid chromatography (HPLC; Pump: GS50 Dionex; Detector: PED detector), working with pulsed amperometric mode using high performance anion exchange chromatography (HPAEC). Analytical column was CarboPac PA1 (250x4mm); including guard column with 100 mM NaOH and wash step to 200 mM NaOH. The flow rate in the analytical system was 1ml/minute with a total of 20  $\mu$ l injection volume. Temperature was held constant at 25°C through out the analysis.

At first, 15 mg powdered samples were taken and put in a centrifuge tube. Five milliliter ethanol (80%) was added and mixed before putting the sample in a shaking water bath at 80°C for 20 minutes to extract soluble sugars into aqueous solution. Then the sample was centrifuged for 5 minutes at 8000rpm to separate the supernatant. Only 1ml of the supernatant was separated in an Eppendorf vial and the alcohol evaporated in a SpeedVac for 2hrs. The remaining sample was stored at -20°C for later starch determination. The dried supernatant was diluted by 1ml pure water using a dispenser and then mixed and put in an Ultrasonic bath for 10 minutes. The samples were centrifuged (25000rcf) for another 10 minutes to ensure complete dilution and transferred to a mini-glass vial for glucose, sucrose and fructose determination. HPLC detects electronic signal based on the retention time elapsed for each carbohydrate molecule and the detector converts the signal into numeric soluble sugar concentrations in microgram/ml.

The accurate determination of the high molecular weight polysaccharide starch relies on both its complete extraction from the sample, and its complete hydrolysis into the monomer glucose; the latter being used for analytical quantification of starch. From the original starch sample, the supernatant was removed after diluting with 3ml ethanol (80%) and centrifuged for 5 minutes. We repeated this step three times to ensure that the remaining sample contained only the insoluble part of sugar. The remaining pellet was dried in the SpeedVac for 20 minutes before enzyme digestion. To metabolize the polysaccharides (starch) to glucose units, we used 2ml  $\alpha$ -amylase solution (1mg/ml Rohalase in water) as a reagent. The solutions were put in a shaking water bath for 30 minutes at 90°C. To further break the glucoside bonds in starch, 1ml

amylglucosidase (0.5mg/ml in 50 mM citrate buffer and pH = 4.6) was added and shaken in a water bath for another 15 minutes at 60<sup>0</sup>C. A 1ml sample was then transferred to an Eppendorf vial and centrifuged (25000rcf) for 10 minutes. Then we followed the same procedure described previously for glucose determination. Starch concentrations were then expressed in glucose equivalents. Sucrose, fructose and glucose standards were used in between analyses to check functionality. Results were expressed on a mass per dry mass basis (mg/g).

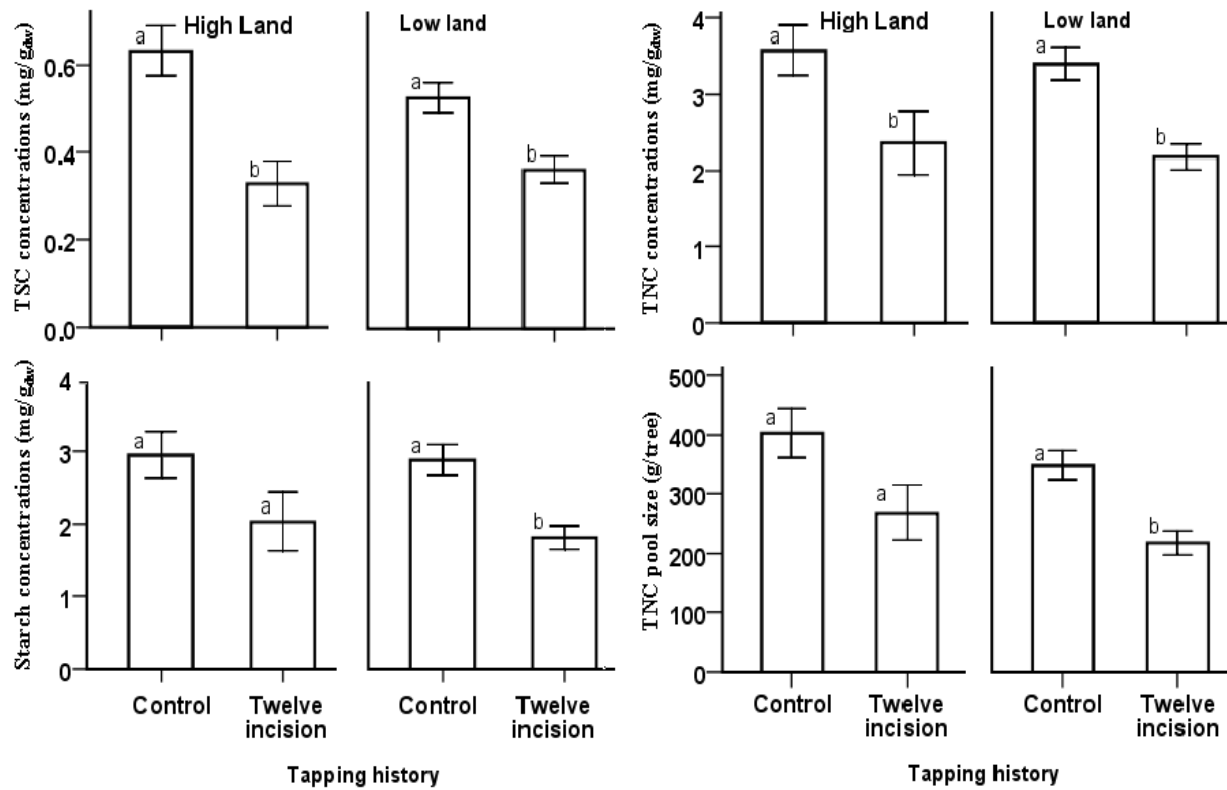
In a separate experiment, we harvested four trees of similar diameter class from each site. Biomass of wood, bark and root portions was measured after carefully separating each compartments. Fresh weight of a sliced sample from each compartment was measured in the field and re-measured after oven drying. The moisture content was then determined for each compartment. From both the concentration and biomass data, we calculated the carbohydrate pool size for each tissue. This was done by multiplying the concentration by the total dry weight of each compartment for a tree from the harvesting experiment (data not shown).

### ***Data Analysis***

We tested our data for homogeneity of variances using Levene's test. Differences between tapping treatments, sites and season in TNC, TSC and starch concentrations of each plant part (wood, bark and root) were analyzed by means of ANOVA. Given that the experimental trees were harvested only once, we used trees as replicate. Tapping effects and seasonal difference in TNC concentration during the study period was analyzed for each site separately. When comparing different compartments, we applied Post-hoc tests; Tukey test was applied for means of equal variances and Tamhane test in case of unequal variances. Data was analyzed using SPSS (PASW 17.0 for Windows statistical software package).

## Results

The mean TNC concentration for *Boswellia* trees was 2.96mg/g<sub>dw</sub>. The highland and the lowland populations did not differ significantly in TNC concentrations (Table 1). About 62% of TNC in the lowland and 70% in the highland consisted of starch and the remaining is soluble sugars (Figure 2). Both concentrations and pool sizes were lower in tapped than untapped trees, although the difference was not always significant (Figure 2, Table 2). Starch was not significantly reduced by tapping in the highland.



**Figure 2.** Reserve carbohydrate concentrations for tapped and untapped (control) *Boswellia papyrifera* trees. Starch, TSC (total soluble carbohydrates) and their sum TNC (total non-structural carbohydrates) concentrations are shown. The TNC pool size is the total amount of TNC in the whole tree. Different letters indicate significant differences between control and heavy tapping treatments for each site. Bars indicate standard error.

**Table 1.** Summarized results of ANOVA showing the effect of site, tapping and season on the concentration of Total Non-structural Carbon (TNC), Total Soluble Carbohydrate (TSC) and starch; *F* values and their significant levels are presented (ns, non significant; \*,  $0.01 < P < 0.05$ ; \*\*,  $0.001 < P < 0.01$ ; \*\*\*,  $P < 0.001$ ).

No	Source of variation	Carbohydrate concentration (mg/g)		
		TNC	TSC	Starch
1	Site	0.81ns	1.97ns	0.47ns
2	Season	17.73***	54.26***	10.50***
3	Tapping	23.15***	21.87***	16.85***
4	Tissue	28.43***	32.71***	38.03***
5	Site X season	9.20***	23.13***	6.30***
6	Site X Tapping	7.81***	8.34***	5.67**
7	Site X Tissue	12.25***	13.55***	15.97***

**Table 2.** Carbohydrate storage in the form of TNC (total non-structural carbohydrates), TSC (total soluble carbohydrates) and starch (mg/g) compared between tapped and non-tapped control trees, and for different plant organs (mean  $\pm$  s.e.).

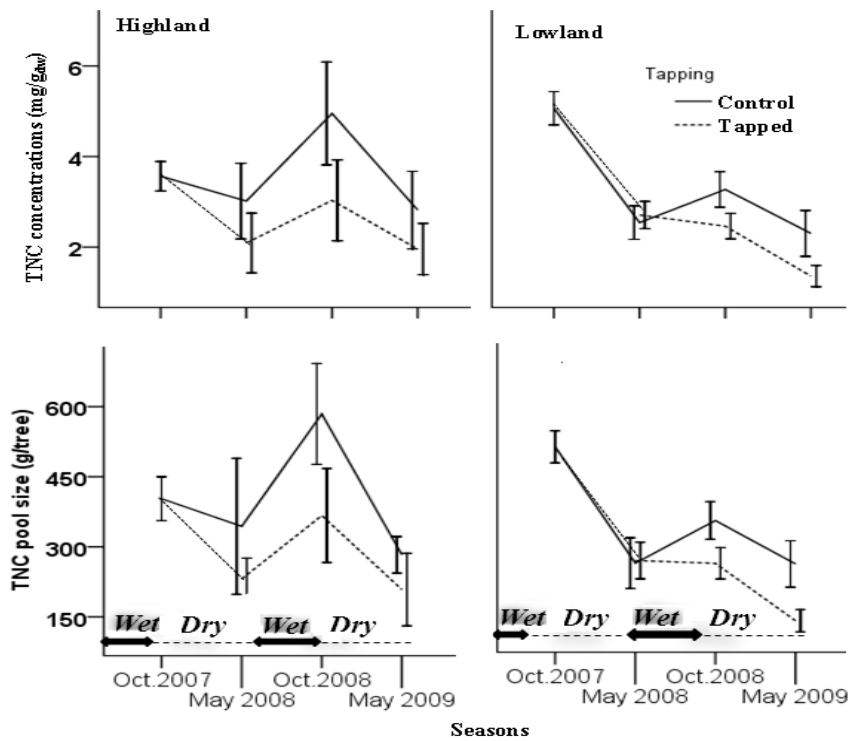
Sites	Plant Tissue	Pool size (g/tree)		Carbohydrate concentrations (mg/g)					
		TNC		TNC		TSC		Starch	
		Control	Tapped	control	Tapped	control	Tapped	control	Tapped
Highland	wood	271.0 $\pm$ 33 <sup>a</sup>	194.2 $\pm$ 40 <sup>a</sup>	5.6 $\pm$ 0.7 <sup>a</sup>	4.00 $\pm$ 0.8 <sup>a</sup>	0.52 $\pm$ 0.1 <sup>a</sup>	0.30 $\pm$ 0.1 <sup>b</sup>	5.07 $\pm$ 0.7 <sup>a</sup>	3.71 $\pm$ 0.8 <sup>a</sup>
	bark	94.2 $\pm$ 12 <sup>a</sup>	50.5 $\pm$ 18 <sup>b</sup>	2.8 $\pm$ 0.3 <sup>a</sup>	1.49 $\pm$ 0.5 <sup>b</sup>	0.89 $\pm$ 0.1 <sup>a</sup>	0.42 $\pm$ 0.1 <sup>b</sup>	1.88 $\pm$ 0.3 <sup>a</sup>	1.07 $\pm$ 0.5 <sup>a</sup>
	root	39.0 $\pm$ 7 <sup>a</sup>	24.2 $\pm$ 8 <sup>a</sup>	2.1 $\pm$ 0.5 <sup>a</sup>	1.31 $\pm$ 0.4 <sup>a</sup>	0.43 $\pm$ 0.1 <sup>a</sup>	0.27 $\pm$ 0.1 <sup>a</sup>	2.56 $\pm$ 0.5 <sup>a</sup>	1.59 $\pm$ 0.5 <sup>a</sup>
Lowland	wood	245.6 $\pm$ 21 <sup>a</sup>	136.69 $\pm$ 15 <sup>b</sup>	5.0 $\pm$ 0.4 <sup>a</sup>	2.75 $\pm$ 0.3 <sup>b</sup>	0.37 $\pm$ 0.0 <sup>a</sup>	0.28 $\pm$ 0.0 <sup>a</sup>	4.58 $\pm$ 0.4 <sup>a</sup>	2.47 $\pm$ 0.3 <sup>b</sup>
	bark	64.0 $\pm$ 6 <sup>a</sup>	46.87 $\pm$ 6 <sup>a</sup>	2.5 $\pm$ 0.2 <sup>a</sup>	1.81 $\pm$ 0.2 <sup>a</sup>	0.79 $\pm$ 0.1 <sup>a</sup>	0.52 $\pm$ 0.1 <sup>b</sup>	1.67 $\pm$ 0.2 <sup>a</sup>	1.29 $\pm$ 0.2 <sup>a</sup>
	root	49.6 $\pm$ 7 <sup>a</sup>	34.67 $\pm$ 5 <sup>a</sup>	2.8 $\pm$ 0.4 <sup>a</sup>	1.96 $\pm$ 0.3 <sup>a</sup>	0.42 $\pm$ 0.0 <sup>a</sup>	0.28 $\pm$ 0.0 <sup>b</sup>	2.41 $\pm$ 0.4 <sup>a</sup>	1.68 $\pm$ 0.3 <sup>a</sup>

Different letters indicate significant differences between tapped and control plants:  $P = 0.05$

Mean TNC concentration ranged from 7.37mg/g<sub>dw</sub> at the end of the wet season to 0.92 mg/g<sub>dw</sub> at the end of the dry season (Table 3), and thus indeed varied over the seasons with generally lower values at the end of the dry season and refilling during the wet season (Figure 3). The TNC pool sizes changed over the seasons in a similar way as the TNC concentrations did (Figure 3).

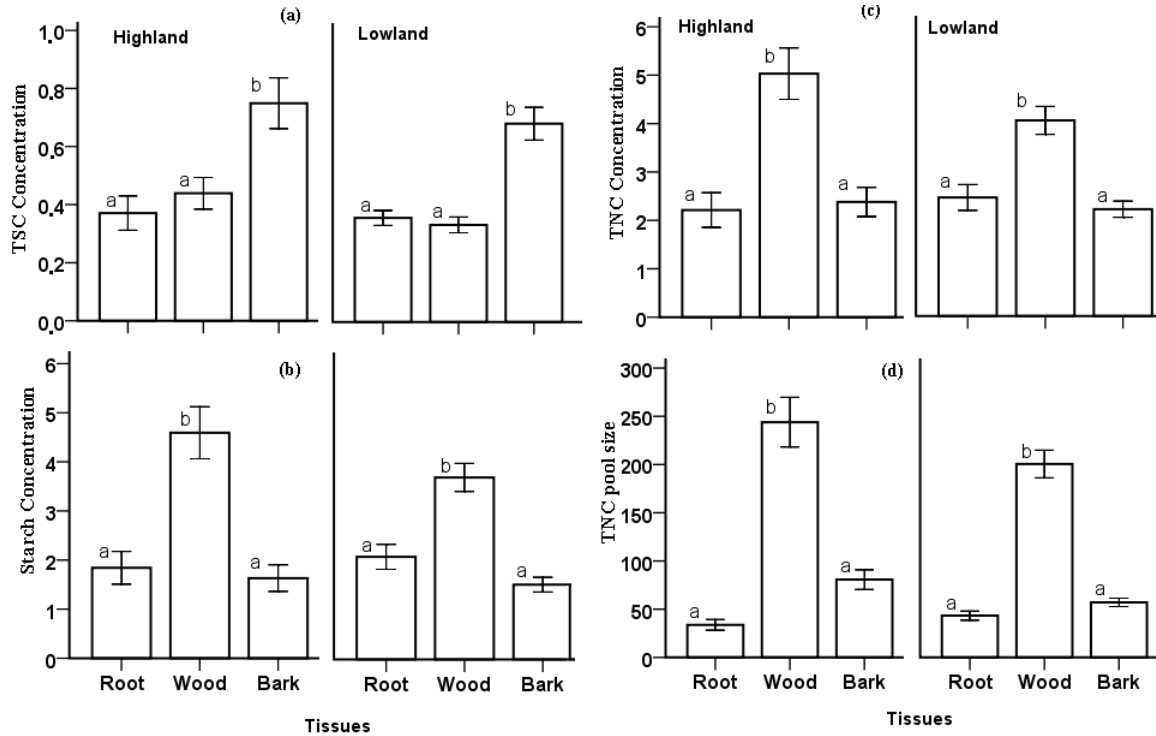
**Table 3.** Seasonal variation of Total Non-structural Carbon (TNC, mg/g) for different compartments. Mean and standard errors are indicated. Different letters indicate significant differences (at  $P < 0.05$ ) among seasons.

Sites	Plant	Oct. 2007	May 2008	Oct. 2008	May 2009	
	Tissue	First fill	First exhaustion	Second fill	Second exhaustion	<i>P</i>
Highland	wood	5.1±0.7 <sup>a</sup>	4.1±1.1 <sup>a</sup>	7.4±1.4 <sup>a</sup>	3.6±0.7 <sup>a</sup>	0.062
	bark	3.3±0.4 <sup>b</sup>	1.9±0.8 <sup>ab</sup>	2.6±0.7 <sup>ab</sup>	0.9±0.3 <sup>a</sup>	0.019
	root	2.4±0.4 <sup>a</sup>	1.7±0.7 <sup>a</sup>	2.0±0.5 <sup>a</sup>	2.7±1.2 <sup>a</sup>	0.81
Lowland	wood	6.9±0.6 <sup>a</sup>	3.3±0.5 <sup>b</sup>	4.3±0.5 <sup>b</sup>	2.7±0.5 <sup>b</sup>	0.000
	bark	3.9±0.4 <sup>a</sup>	2.2±0.3 <sup>b</sup>	1.8±0.2 <sup>b</sup>	1.3±0.3 <sup>b</sup>	0.000
	root	4.6±0.8 <sup>a</sup>	2.4±0.4 <sup>b</sup>	2.3±0.4 <sup>b</sup>	1.6±0.6 <sup>b</sup>	0.008



**Figure 3.** Seasonal variation in total non-structural carbohydrate (TNC) concentrations and pool sizes for the control and tapped frankincense trees. The lower panel shows the estimated total pool size, based on the tree biomass data for the different compartments (data not shown). Climate events are included in the lower panel to show synchrony with reserve “re-fill” and “exhaustion”. Bars indicate standard error of the mean.

Contrary to our expectations, starch and TNC concentration was higher in the wood than in bark or roots ( $P < 0.05$ , Figure 4b,c). Soluble carbohydrates (TSC), however, achieved the highest concentrations in the bark in both areas (Figure 4a). In terms of whole-tree pools, the stem contained the highest reserve pools, while roots and bark had equal amounts (Figure 4d).



**Figure 4.** Reserve carbohydrates in *Boswellia* tree organs for a highland and a lowland site. Total Soluble Carbohydrates (TSC, a), starch (b) and TNC (c) concentrations (mg/g) are shown for root, wood and bark compartments. Total pool sizes of TNC (g/tree) are given in (d). Different letters indicate significant difference between plant organs for a given site ( $P < 0.05$ ). Bars indicate standard error.

## Discussion

We showed the effect of tapping on reserve carbohydrates and its seasonal dynamics for a tropical dry woodland tree growing at different altitudes. Overall, TNC concentrations in the frankincense tree were lower than apple (Naschitz et al. 2010), rubber tree (Silpi et al. 2007, Chantuma et al. 2009) and some Bolivian tropical forest trees (Poorter and Kitajima 2007) but comparable to selected conifers (Bansal and Germino 2009), seasonally dry forest trees (Newell et al. 2002) and temperate tree-line deciduous taxa (Li et al. 2002, Hoch et al. 2002, Hoch et al. 2003) (see also Table 4). As in most other tree species (Hoch et al. 2003, Silpi et al. 2007,

Raessler et al. 2010), in the frankincense tree, starch was by far the dominant contributor to TNC concentration (> 62% of TNC). Soluble sugars had lower concentrations than starch maybe because soluble sugars are more readily available and easily mobilized resources than starch (Poorter et al. 2010). Trees show distinct seasonal variation in response to increased sink demand from tapping and reproductive effort during the dry season.

**Table 4.** Comparison of carbohydrate concentrations (mg/g) in the stem wood section of different trees species/forests.

Species/forest	Location	Stem wood			References
		Starch	Sugars	TNC	
Populus		1.6	0.9	2.5	Landhäuser and Lieffers 2003
Pinus	Switzerland	1.4	0.2	1.6	Li et al. 2002
Tropical trees	Bolivia	3.7	2.9	6.6	Poorter and Kitajima 2007
Evergreen trees	Switzerland	--	---	5.8	Hoch et al. 2003
Pinus	Switzerland	1.07	0.54	1.6	Hoch et al. 2002
Apple tree	Israel	22.6	13.7	36.3	Naschitz et al. 2010
Tropical trees	Panama	6.5	2.6	9.1	Würth et al. 2005
Rubber tree	Thailand	38.5	13.6	52.1	Silpi et al. 2007
Boswellia tree	Ethiopia	2.48	0.54	2.96	This study

Despite differences in leaf phenology, carbon gain, microclimate conditions and altitude (chapter 2), TNC concentrations were similar between the two areas. The higher annual carbon gain at high altitude (chapter 2) thus is not reflected as a higher storage carbohydrate concentration.

#### *Effect of tapping on reserve carbohydrates*

We hypothesized that control plants should have higher TNC concentrations and pool sizes than tapped trees, because tapping requires mobilization of reserve carbohydrates. This hypothesis was confirmed by the data, as tapped trees had indeed lower concentrations and pool sizes than control trees (Figure 2). Chantuma and co-workers (2009) suggested that rubber tapping creates



an additional carbon sink (the latex), which has to be regenerated from stored carbohydrates. In our study, resin production during the dry season reduced the concentration of storage carbohydrates in *Boswellia* trees. Hence, stored carbohydrate is used as a coping mechanism to an increased carbon sink thru tapping. The soluble parts were more reduced by tapping than the insoluble (starch) part (Table 2), probably because the soluble carbohydrates are more immediate sources of carbon for resin production than the insoluble starch.

*Seasonal dynamics of TNC in Boswellia tree*

Stored TNC is supposed to be an intermediate pool between assimilation and utilization (Bansal and Germino 2009), and should therefore change over seasons to reflect sink strength (e.g. frankincense tapping). Hence, we expected the distinctly drought-deciduous *Boswellia* tree to have maximum concentrations at the end of the full canopy season (wet season) and minimum values at the end of the leafless period (dry season). TNC levels in the frankincense tree indeed decreased during the leafless dry season. This is in agreement with results from other studies (Steele et al. 1984, Hoch et al. 2003, Silpi et al. 2007, Chantuma et al. 2009, Bansal and Germino 2009). The seasonal variation in TNC concentration thus reflected the phenomenon of “re-fill” at the end of the wet season and “exhaustion” at the end of the dry season in most of the compartments. This occurred in both highland and lowland *Boswellia* populations. It indicates that carbon demand (e.g. tapping and reproductive effort) during the long dry season is also supplied by reserve carbon that was produced during the wet season. However, dry season costs may also get supply from locally produced carbohydrates thru bark photosynthesis (Pfanz et al. 2002, Pfanz 2008) during the dry season by the green *Boswellia* stem and branches. Indeed, Gebrekidan et al. 2011 (in prep), showed that chlorophyll concentrations in *Boswellia* bark are considerable.

Seasonal changes in total tree pool sizes follow the same pattern as the TNC concentration (Figure 3) and determine the capacity of *Boswellia* trees to invest in the reproductive structures (flowers and fruits), produce frankincense and re-sprout in the following season. The amplitude of TNC variation is irregular and probably depends on inter-annual variability in climatic parameters that either affect carbon gain or sink strength.

*Role of the different compartments*

We expected both storage carbon concentration and pool sizes to be higher in the roots as these are better protected from fire, tapping and herbivory than in the wood and bark tissues (Hoffmann et al. 2003, Regier et al. 2010) and thus may be more secure as a longer term buffer. In contrast to our expectation, starch and total TNC concentration was highest in the wood tissues of *Boswellia*. Bark, however, has the highest soluble TNC. Bark photosynthesis might contribute to higher soluble sugars in the bark. This soluble sugar could also be mobilized as it is the most ready-to-use part in defense after injury, as has been indicated for rubber tree (Chantuma et al. 2009). However, the amount of reserve carbohydrates available for future use depends not only on TNC concentration but also to a large extent on the size of the storage organs (Canham et al. 1999).

Roots of *Boswellia*, like other tropical trees (Würth et al. 2005); contain less TNC than the other two compartments. However, the wood which is protected by the thick bark contains more reserves than the other organs. This is in contrast to woody plants from cold regions; their roots contain higher concentrations of reserve carbohydrates than their stems (Hoch et al. 2002).

**Conclusion**

This study confirmed that tapped trees contain less reserve carbon than control trees. Therefore, tapping indeed drains the carbon storage of the frankincense tree. Although resin production is mainly in the bark, changes related to tapping were also extended in the wood. We also showed that tree organs store reserve carbohydrates in this deciduous woodland tree. *Boswellia* appears to have more starch stored in the stem and more soluble carbon stored in the bark. Given the distinct seasonal changes in climate and phenology of this deciduous tropical woodland tree, periodic depletion and re-fill of TNC concentrations fit our assumption.

Finally, reduced TNC concentrations after tapping in all tissues and the seasonal dynamics of carbohydrate reserves appear relevant parameters to cautiously evaluate the long-term sustainability of tapping *Boswellia* trees in the dry woodlands systems.

## Chapter 5

### *Carbohydrate allocation among competing sinks in the frankincense tree Boswellia papyrifera*

Tefera Mengistu, Frank J. Sterck, Masresha Fetene and Frans Bongers

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*Abstract*

Trees in stressful tropical dry woodlands are characterized by carbohydrates sinks that compete strongly for carbon from of the same pool. Three tapping treatments were applied to naturally grown *Boswellia papyrifera* (Del.) Hochst trees in northern Ethiopia in order to elucidate the annual and seasonal carbohydrate allocation pattern. We estimated annual gross primary productivity (GPP), plant maintenance respiration (R), net primary productivity (NPP), storage carbohydrate (TNC) and frankincense production costs to evaluate carbon allocation at the whole-plant level. We determined the impact of (1) tapping on annual and seasonal carbohydrate allocation pattern to different sinks, and (2) annual cross primary productivity on those allocation patterns. We hypothesized that tapping reduces carbohydrate allocation to other, competing, sinks. We also expected that increased GPP increases the carbon investments to all other sinks.

Generally, the annual GPP was more than sufficient to account for the annual carbon sinks considered in this study. Mean annual carbohydrate costs to sinks per tree were 12 kg (highland) and 16 kg (lowland). Maintenance respiration, foliage establishment and frankincense were the strongest carbohydrate demands. Frankincense tapping decreased foliage production and reproductive effort in the lowland, but not in the highland where trees had higher annual carbon gain or were limited by moisture that hamper growth, decreasing carbon competition with frankincense production. Increasing GPP only leads to an increase in foliage development; other sinks are unaffected. This quantitative analysis gives insight into how allocation patterns change with phenological events and physiological processes and the impact of tapping on foliage production and reproductive effort.

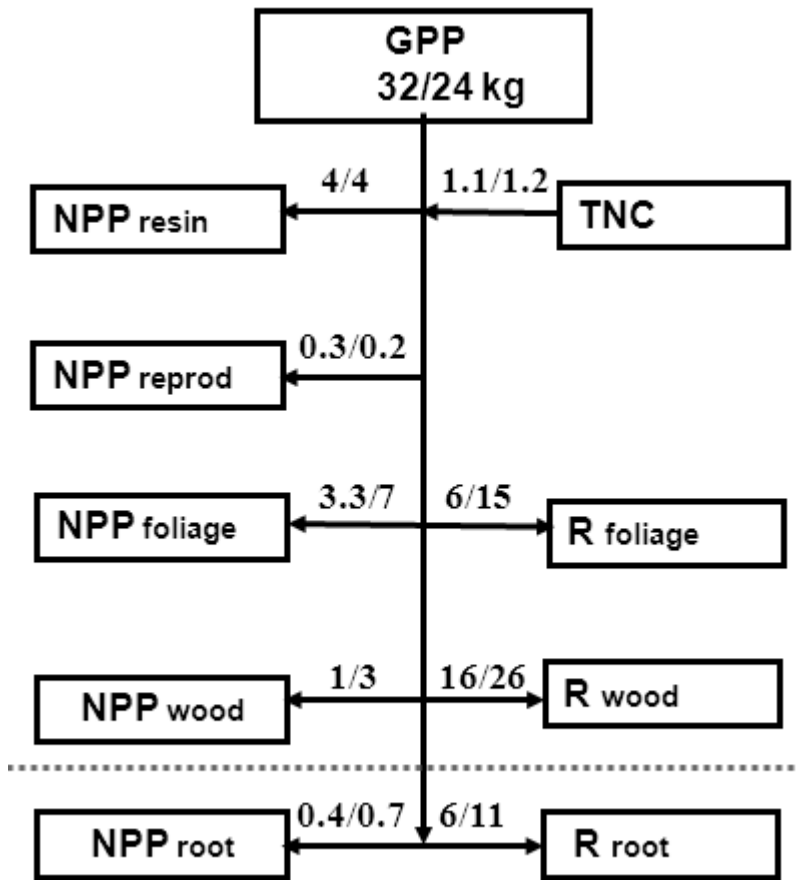
**Keywords:** carbon allocation, frankincense, *Boswellia*, maintenance respiration, Ethiopia

## **Introduction**

When plants are limited by carbon they face trade-offs in sinks that require carbohydrates like defense, vegetative growth, or fruit production (Bazzaz et al., 1987). For example, carbon depletion may negatively affect reproductive output (Stephenson 1981, Ho 1988, Rijkers et al. 2006, Bazzaz et al., 1987, Sterck and Schieving 2007). Similarly, allocation to defense (Poorter and Kitajima 2007) may protect plants against herbivores (Coley 1988, Pare and Tumlinson 1999) and pathogens (Augspurger 1984, Veronese et al. 2003) but might be at the expense of a reduced growth rate (Chantuma et al. 2009, Kleczewski et al. 2010).

Because carbon acts as a major currency in plants, source–sink relationships of trees and forests can be captured in a conceptual carbon balance diagram (Fig 1, see also Ryan et al. 2004, Litton et al. 2007). This diagram simply shows that the carbon costs of growth (NPP), maintenance respiration (R), defense components, reserve carbohydrates and reproductive organs should be balanced by the photosynthetic carbon gain (GPP). However, this scheme is not complete, since it does not show the possible carbon costs of root exudates, mycorrhizal symbiosis, and volatile compound emissions. Nevertheless it implies that carbon consumption by one sink (e.g. growth) trades-off with the activities of other sinks (Lorio and Sommers, 1986).

In resource poor environments, growth may be constrained such that more carbon can be used for secondary metabolites (e.g. tannins, resins) that enhance resistance against herbivores or other stresses (Chapin 1991, Herms and Mattson 1992). On the other hand, in resource rich environments more carbon is allocated to growth setting a carbon limit to production of secondary metabolites (Herms and Mattson 1992, Kleczewski et al. 2010). Sometimes direct competition between secondary metabolism and growth is observed (Herms and Mattson 1992) with growth diverting resources from secondary metabolism or vice versa. Nevertheless, if growth and secondary metabolism are constrained by carbon limitations (Poorter and Kitajima 2007, Kleczewski et al. 2010), it implies that increased carbon availability by assimilation may increase the allocation to all sinks. On the other hand, it is supposed that natural selection will result in sink activities that jointly enhance the growth, survival and reproductive success of plants (Cannell and Dewar 1994, Sterck and Schieving 2007).



**Figure 1.** Metabolic full-year model for carbohydrate sources and sinks in the frankincense tree. GPP values refer to estimated average annual carbon gain (kg. C. y<sup>-1</sup>). Sink values are in percentages. All values are indicated for both highland and lowland trees (highland / lowland). Net primary productivity (NPP) and respiration (R) costs are indicated for each sink components while total non-structural carbohydrate (TNC) pool size is shown for the whole plant.

Seasonality in climate patterns also sets another limit to the plant carbon balance. In most climates, seasonality largely drives the dynamics in resource acquisition and sink activity which are related to phenological phases and physiological processes. While source and sink activity are largely synchronized by seasonality in temperature in the temperate and boreal zones, it is more driven by seasonality in rainfall in the tropics. Generally, trees have a net income of resources under the most favorable conditions and

may then fill resource reserves to maintain living tissues and survive during the less favorable seasons. The resilience of tropical species in such seasonality is largely determined by their plasticity in carbohydrate allocation patterns across such seasonal conditions (Poorter and Bongers, 2006; Poorter and Kitajima, 2007).

Despite significant advances in understanding the terrestrial carbon cycle at global scale (Edwards et al. 1990, Mantlana 2008), limited information exists at plant level concerning the basic process of canopy carbon gain and seasonal allocation of the fixed carbon. Prior carbohydrate allocation studies focused on whole forest stand patterns (e.g. Cairns et al. 1997, Gower et al. 2001, Litton et al. 2007). Moreover, our knowledge on larger trees lags behind other life forms (Veneklaas and Poorter 1998). Information on how annual crown carbon gain is fractioned by all possible sinks at plant level is even more scanty for tropical dry woodland species. The annual and seasonal carbon allocation pattern of trees has yet to be established for most species. Here we present such patterns for an economically important tree in east African woodlands that grows in a climate with a strongly seasonal rainfall pattern.

We selected the frankincense tree *Boswellia papyrifera* (Del.) Hochst for detailed investigation of annual and seasonal carbon allocation patterns. Importantly, frankincense is harvested from this species during a 7-8 month dry period, when the tree has dropped its leaves. Earlier studies have shown that such frankincense harvesting can result in lower fruit production (Rijkers et al. 2006), and smaller leaf area production (chapter 2). Here we present annual and seasonal carbon budgets analysis to show how frankincense harvesting acts as a potential carbon drain and evaluate its impact on other sink activities.

In this study we determined: (1) the impact of tapping on annual and seasonal carbohydrate allocation pattern to different sinks, and (2) the impact of carbohydrate gain on those allocation patterns. We did field studies for two *Boswellia* populations which occur at high and low altitude, which allows us to speculate on the effect of contrasting site conditions on carbohydrate allocation patterns. We hypothesized that tapping reduces carbohydrate allocation to other, competing, sinks. We also expected that increased carbohydrate resource availability increases the carbon investments to all other sinks. We used annual and seasonal estimates of gross primary productivity (GPP) from data on canopy physiology, net primary productivity (NPP) from vegetative and reproductive

phenology, plant respiration (R), storage carbohydrate (TNC) and frankincense costs to evaluate the carbohydrate allocation at the whole-plant level.

## **Methodology**

### *Study areas and species*

The study was conducted for tree populations at a low altitude site (810-990 meters) in the Metema area and higher altitude site (1400-1650 meters) in the Abergelle area of Ethiopia. The high altitude site (Abergelle) has a dry and erratic rainfall with a shorter wet season than the low altitude site (Metema). The study species *Boswellia papyrifera* (Del.) Hochst (Burseraceae) is a deciduous tree up to 13m tall, with stem diameter up to 35 cm (Ogbazghi et al. 2006, Abiyu et al. 2010) and with approximately circular branching crown. In the highland, we established one plot and in the lowland, we established two plots with a priori assumption of site productivity variation.

### *Estimating annual carbon gain (GPP)*

We selected 15 and 36 adult experimental trees of *Boswellia* (DBH =  $20 \pm 3$  cm) in the highland and lowland respectively. Experimental trees were randomly and equally assigned to three tapping treatments, i.e. 0 (control), 6 and 12 tapping incisions. The tapping treatments were applied over two successive dry seasons (2007-2008 and 2008-2009). We estimated annual canopy assimilation from these experimental trees using leaf gas exchange data during the growing season for two years. During the measurement periods, assimilation rates were measured for every tree three times a day on a single leaflet, using an open portable gas exchange system, LcPro (ADC, Hoddesdon, UK.). We estimated the daily photosynthetic rates by integrating the photosynthetic measurements over the day. Since we assumed that each of the three measurements during the day represented one period during a day of 12 sunlight hours, we integrated each measurement over a period of four hours. Subsequently, we estimated the wet season annual carbon gain (dry season is leafless) by integrating the daily photosynthetic rates to total leaf area and leaf lifespan. Annual crown carbon gain is referred here as the gross primary productivity (GPP).



*Estimating carbohydrate sinks*

We distinguish between major sinks that are part of the net primary production (NPP), the respiration costs (R), or storage. The net primary production consist of foliage production ( $NPP_{\text{foliage}}$ ), wood production ( $NPP_{\text{wood}}$ ), root production ( $NPP_{\text{root}}$ ), frankincense and fruits production costs. The respiration costs include the maintenance respiration costs of foliage ( $R_{\text{foliage}}$ ), wood ( $R_{\text{wood}}$ ) and roots ( $R_{\text{root}}$ ). The third category is allocation to storage carbohydrates (TNC), which does not only act as a potential sink of the acquired carbon but also as a potential carbon source for other sinks. The overall carbon budget model is presented in figure 1.

*Net primary productivity (NPP)*

The net primary productivity (NPP) is estimated as the carbon used for new tissue production per year, and is expressed on an annual basis or per wet or dry season. We estimated the annual biomass production in foliage, wood, root, reproductive and resin biomass separately, and converted these values into carbohydrates values, taking the global 50% carbon content assumption of oven-dried weight (Edwards et al. 1980, Litton et al. 2007). The annual foliage carbon mass produced ( $NPP_{\text{foliage}}$ ) was estimated from the product of the total number of apices per tree, the number of leaves per apex the number of leaflets per leaf, the average biomass per leaf, and a carbon-biomass ratio of 0.5 (chapter 3). To estimate the average biomass per leaf, oven-dry biomass of 20 sample leaves and rachis was measured from each experimental tree. The  $NPP_{\text{wood}}$  and  $NPP_{\text{root}}$  were calculated from the product of estimated wood or root volume and a mean specific wood density of  $0.64 \text{ g.cm}^{-3}$  for *Boswellia papyrifera* (<http://cdm.unfccc.int/filestorage> local data for wood density). To estimate wood volume increment, we monitored annual trunk diameter growth for all experimental trees during two years, using diameter dendro-bands. Trunk volume increment was calculated as the difference in trunk volume over the measuring period, and is calculated as:

$$TV = \left( \pi / 4 \times dbh_2^2 \times H \right) - \left( \pi / 4 \times dbh_1^2 \times H \right) \quad (1)$$

Where, TV is the trunk volume increment;  $dbh_1$  is diameter in the first year;  $dbh_2$  is diameter in the second year and H is trunk height.

Wood volume increment was calculated as the sum of trunk volume increment and branch volume increment, where branch volume increment was considered as trunk volume increment times the branch to trunk mass fraction. Root volume increment was calculated as the product of the trunk volume increment times the root to trunk mass fraction. The biomass values were converted to carbohydrate costs by multiplying with the 50% conversion factor. To estimate the resin production ( $NPP_{resin}$ ), experimental trees were tapped during the dry season (starting from October-May) for two years. The amount of frankincense collected (in grams) was then measured every week until the end of the dry season. The annual frankincense biomass harvested from each tree was used to estimate the carbon cost of frankincense production. The dry weight of the pure incense was estimated as 85% of the harvested biomass (Chantuma 2009), the rest being moisture and other impurities. However, the energy cost of resin production required a conversion factor of 3.26 gram glucose per gram of resin (Gershenzon 1994; Zavala and Ravetta 2001). To estimate reproductive sinks, we counted the annual fruit biomass produced as the product of the number of apices with fruits, the number of fruits per fruit bearing apex, and the average fruit biomass as calculated from 10 randomly collected and oven-dried fruits. Eventually, reproductive carbon cost were calculated from the product of this total fruit biomass per tree times 0.5 to account for the 50% carbon content per unit biomass, and 6.25 to account for the carbon cost of fruit production (Loomis and Connor, 1996).

*Total non-structural carbohydrates (TNC cost)*

Additional trees of similar diameter class were subjected to either no tapping (control), or heavy tapping (12 incisions) treatments in both sites. Wood, bark and root samples were collected from these trees at the end of the dry season and end of the wet season to determine storage carbon changes. This was done for two years (October 2007 - June 2009). All samples were analyzed for TNC (total non-structural carbohydrates). TNC is defined here as the sum of free sugars (sucrose, glucose and fructose) and starch. Total non-structural carbohydrates (TNC) were determined by high-performance liquid

chromatography (HPLC; Pump: GS50 Dionex; Detector: PED detector). For each plant tissue, we calculated the carbohydrate pool size by multiplying the concentration by the mean total dry weight of each compartment for a tree from a separate tree harvesting experiment. Mean total dry weight for the wood, bark and root compartments was 48.5, 33.9 and 15.3 kg/tree in the highland and 49.6, 25.8 and 17.6 kg/tree in the lowland from a separate harvesting experiment. Assuming that the TNC losses during the dry season are refilled during the wet season, we estimated the TNC loss over the dry season as the annual TNC cost of a tree. The energy cost of total non-structural carbohydrates was obtained using a conversion factor of 1.21 g glucose/g of TNC (Loomis and Connor 1996, Zavala and Ravetta 2001).

#### *Respiration sinks (R)*

Estimates of respiration costs are still uncertain (Teskey et al. 2008). We estimated annual respiration costs for each tree based on estimates of measured biomass (crown, wood and root), tissue nitrogen content and temperature averages (Ryan 1991). Based on personal observation, I considered 90% of the stem active, and thus respiring, sapwood. Following earlier studies (Jones et al. 1978, Ryan 1991, Ryan 1996), we calculated the maintenance respiration costs ( $R$ , in g. C y<sup>-1</sup>) as:

$$R = N \times LS \times 0.059^{0.07T} \quad (2)$$

Where  $N$  is the total nitrogen content (g);  $T$  stands for the temperature (°C), and  $LS$  represent the leaf lifespan (days). For wood and roots a lifespan of 365 days was assumed. This equation thus empirically accounted for the temperature effects on nitrogen rich proteins.

Crown maintenance respiration was estimated for only leaf lifespan (days) while the stem and root maintenance respiration was estimated on annual (365 days) basis (Ryan 1991). The nitrogen content of the crown biomass is based on the measured leaf nitrogen content (chapter one) while the nitrogen content of the stem is estimated as 0.2 % of the biomass (Martius 1992), but varied between our sites based on the nitrogen content variation of the leaf (Ryan et al. 1997). Moreover, the growth respiration was

estimated using construction costs (Table 1). Finally, we estimated respiration for foliage ( $R_{\text{foliage}}$ ), wood ( $R_{\text{wood}}$ ) and root ( $R_{\text{root}}$ ) tissues.

**Table 1.** Conversion factors used to estimate carbohydrate costs that include intermediate costs, and costs of biosynthesis.

<i>Parameter</i>	<i>Unit</i>	<i>Value</i>	<i>Source</i>
Frankincense dry mass ratio	g pure incense/ gram biomass	0.85	Chantuma 2009
Frankincense carbon mass ratio	g carbon/gram pure incense	3.26	Gershenzon 1994; Zavala and Ravetta 2000
Carbon dry mass ratio	g carbon/gram biomass	0.5	Yang and Midmore 2005; Carely et al. 1996; Litton et al. 2007
Fruit carbon mass ratio	g carbon/gram fruit	6:25	Loomis and Connor, 1996
Construction cost ratio	g carbon/gram crown mass	0.25	Ryan 1991, Ryan et al. 1994
Energy content of TNC	g carbon/gram TNC	1.21	Loomis and Connor, 1996
Wood nitrogen content	g nitrogen/ gram biomass	0.2%	Martius 1992

N.B: TNC = Total non-structural carbohydrates.

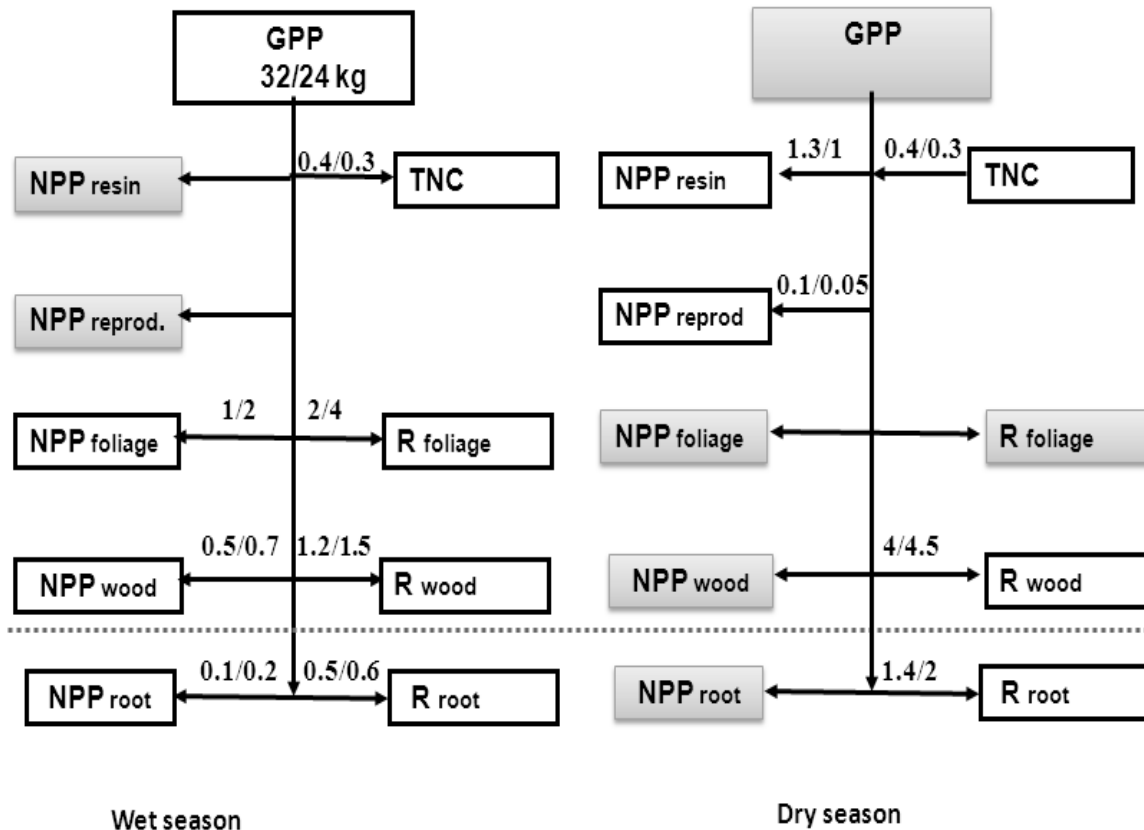
### *Data analysis*

A general linear model with univariate analysis and Tukey post-hoc multiple comparison was used to test if tapping reduces carbohydrate allocation to other sinks. The analysis was done by including the interaction between sites and tapping as a fixed factor and tree as a random factor. We used F-test to compare contrasting sites. The relationship between GPP and the other carbohydrate sinks was tested by linear regression models. Data was analyzed using SPSS (PASW 17.0 for Windows statistical software package).

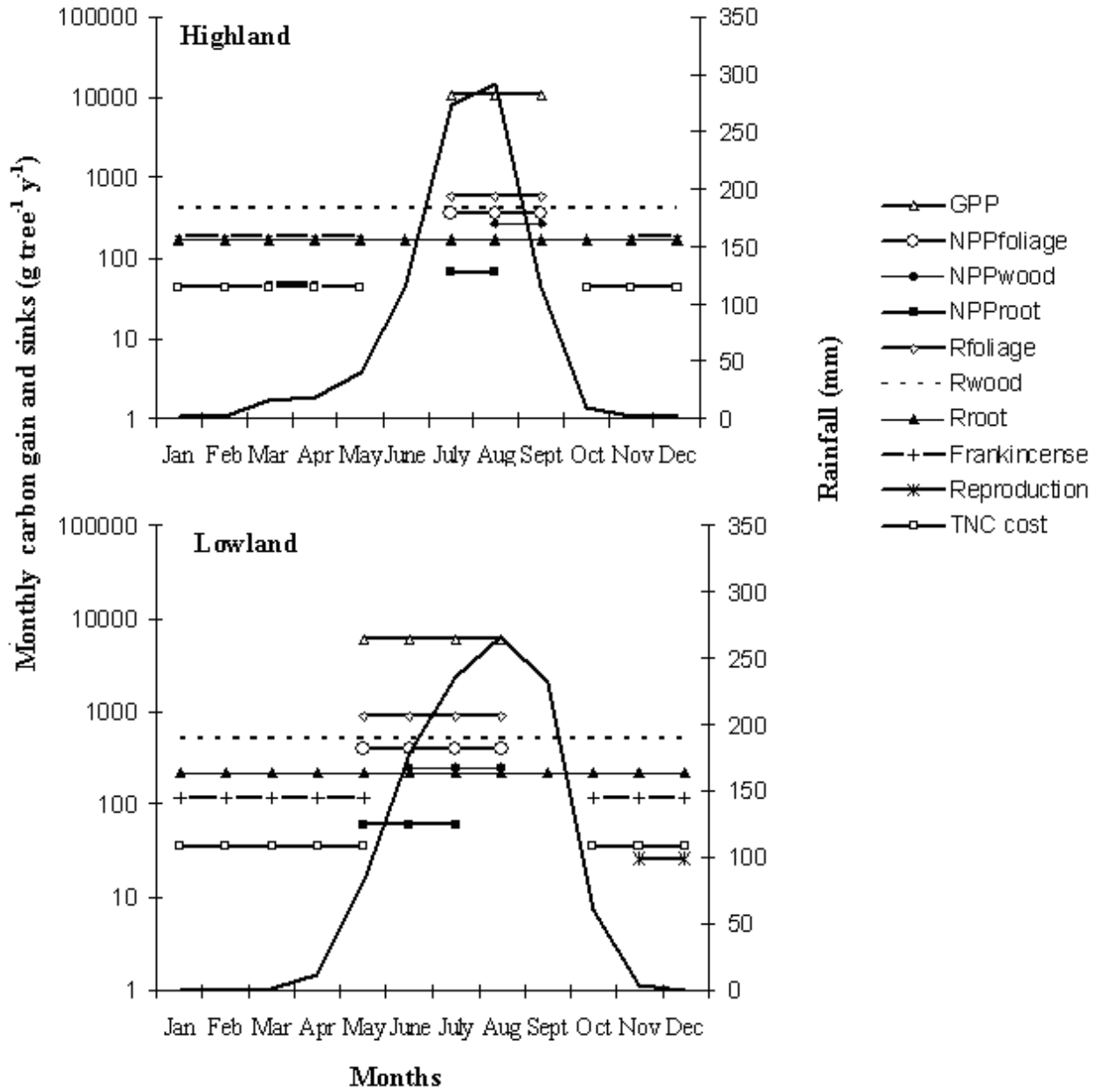
## Results

### Annual patterns

The estimated average annual GPP per tree was higher than the estimated annual sum of carbon consumption by the different sinks. On average, the sinks were estimated to consume 38% (12 kg) and 68% (16 kg) of the GPP in the highland and lowland, respectively (Figure 4 and 5; Table 2). Carbohydrate sinks overlapped throughout the year (Figure 3). For both sites, most carbon was used for maintenance respiration (8.8 kg in the highland site and 12.1 kg in the lowland site). Annual crown net primary production is the second biggest sink (1 kg and 2 kg) and the frankincense production the third biggest sink, consuming on average 1.3 kg.y<sup>-1</sup> and 1 kg.y<sup>-1</sup> of carbon, respectively (Figure 1, 2, 4, 5). Wood and fruit production were the least carbon demanding sinks in *Boswellia* trees.



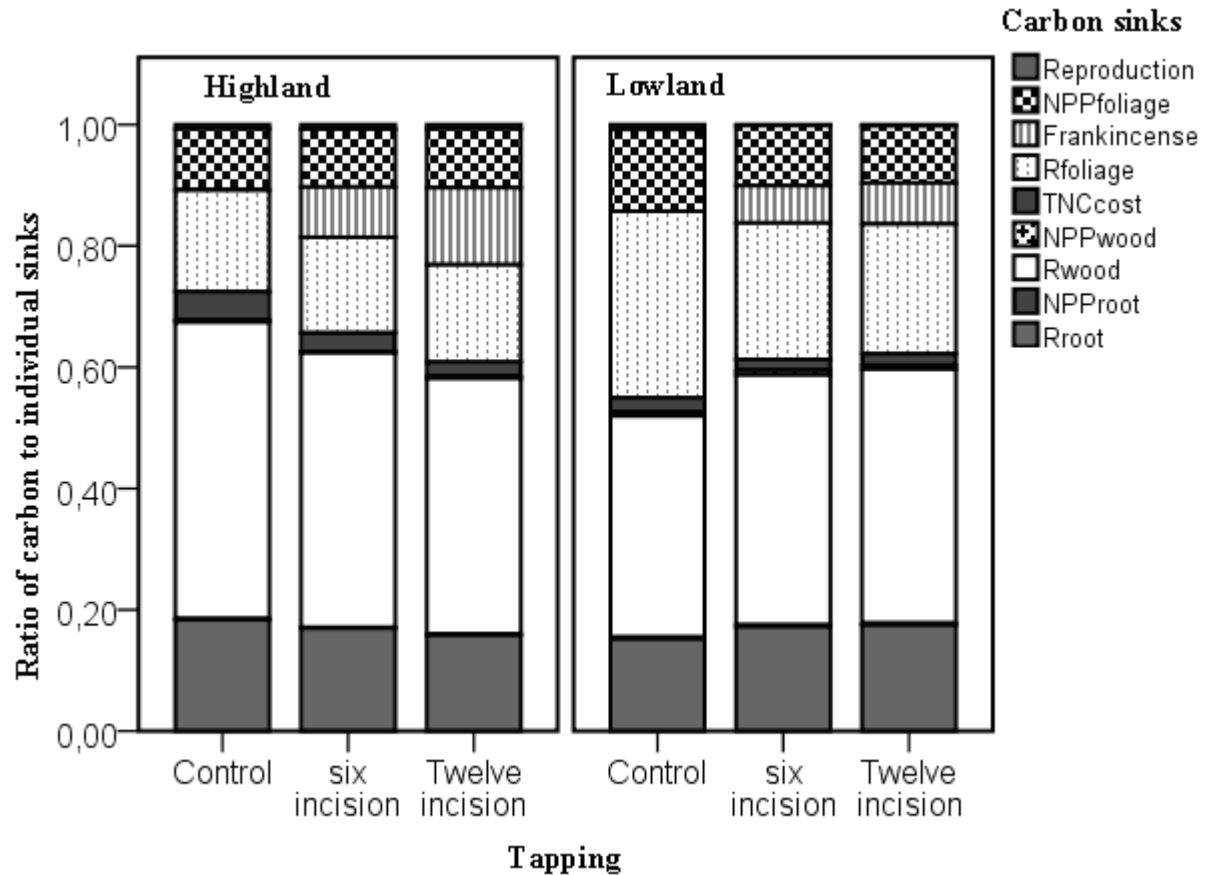
**Figure 2.** Metabolic season-based (i.e. wet and dry season) model for carbohydrate sources and sinks in the frankincense tree. Values indicate carbohydrates (kg.C) for both sites (highland / lowland). Highlighted boxes are inactive sinks during either the wet or dry season.



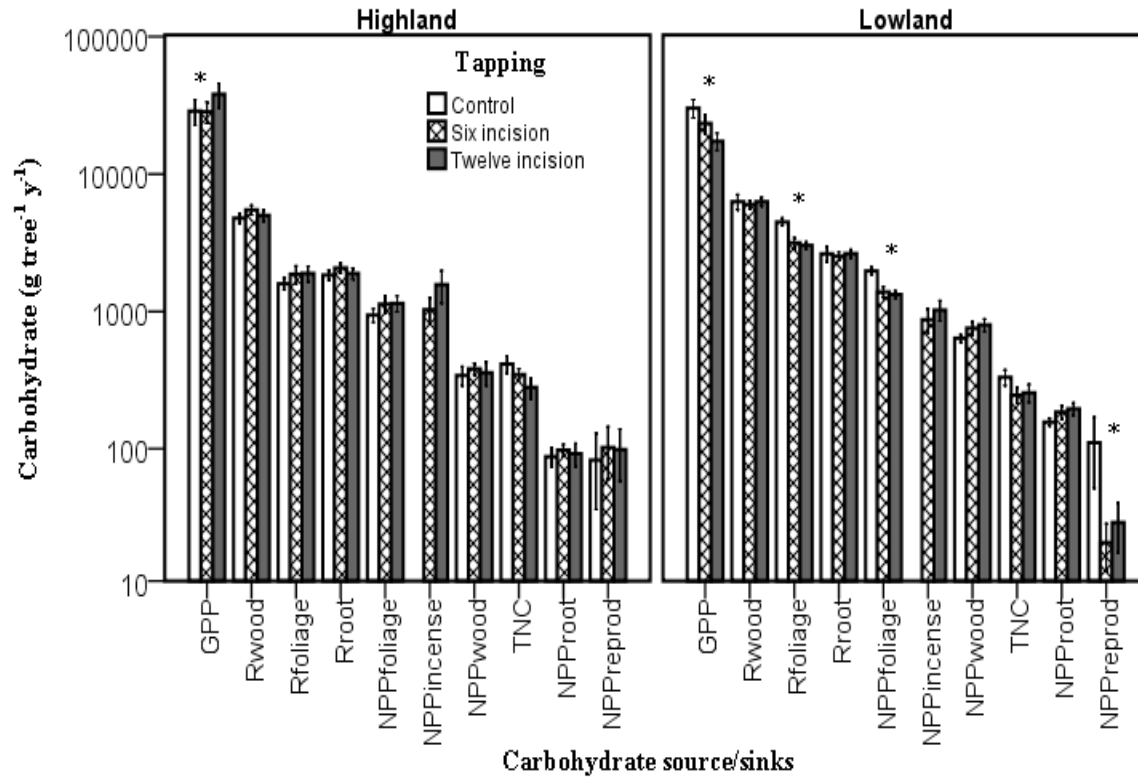
**Figure 3.** Simplified scheme of monthly carbon gain and losses during a year for *Boswellia* trees in Ethiopia (note: carbon gain and sinks is in log scale). The highland is Abergelle and the lowland is Metema site. The solid graphs are rainfall patterns with scale on the right side. On how these values are estimated, see Method section.

Intensive tapping reduced the amount of carbon allocated to foliage ( $\text{NPP}_{\text{foliage}}$ ,  $\text{R}_{\text{foliage}}$ ) and reproductive sinks ( $\text{NPP}_{\text{reprod}}$ ) in the lowland (Figure 5; Table 2), but this was not observed

for the highland. In contrast to our expectation, trees with higher GPP only invested more in foliage production ( $NPP_{\text{foliage}}$ ) and foliage maintenance ( $R_{\text{foliage}}$ ) but not in the other sinks (Figure 6). Remarkably, GPP was higher in the highland but the total carbon consumption by sinks was lower compared to the lowland site (Figure 5; Table 2). However, TNC, fruit production and frankincense yield did not vary significantly between these two sites (Table 2).



**Figure 4.** Average fractions of the estimated annual carbon costs allocated to different carbon sinks for *Boswellia* trees under different tapping levels.



**Figure 5.** Estimated annual carbon gain and costs for different sinks for the frankincense tree at Abergelle, highland and Metema, lowland site in Ethiopia. GPP is the gross primary production (carbon gain), NPP the net primary production in terms of carbon costs, R is the maintenance respiration costs and TNC is the carbon costs in non-structural carbohydrates. (note: the Y-axis is on log scale). Significant differences between tapping treatments is indicated by \* (*F*-test,  $P < 0.05$ ).

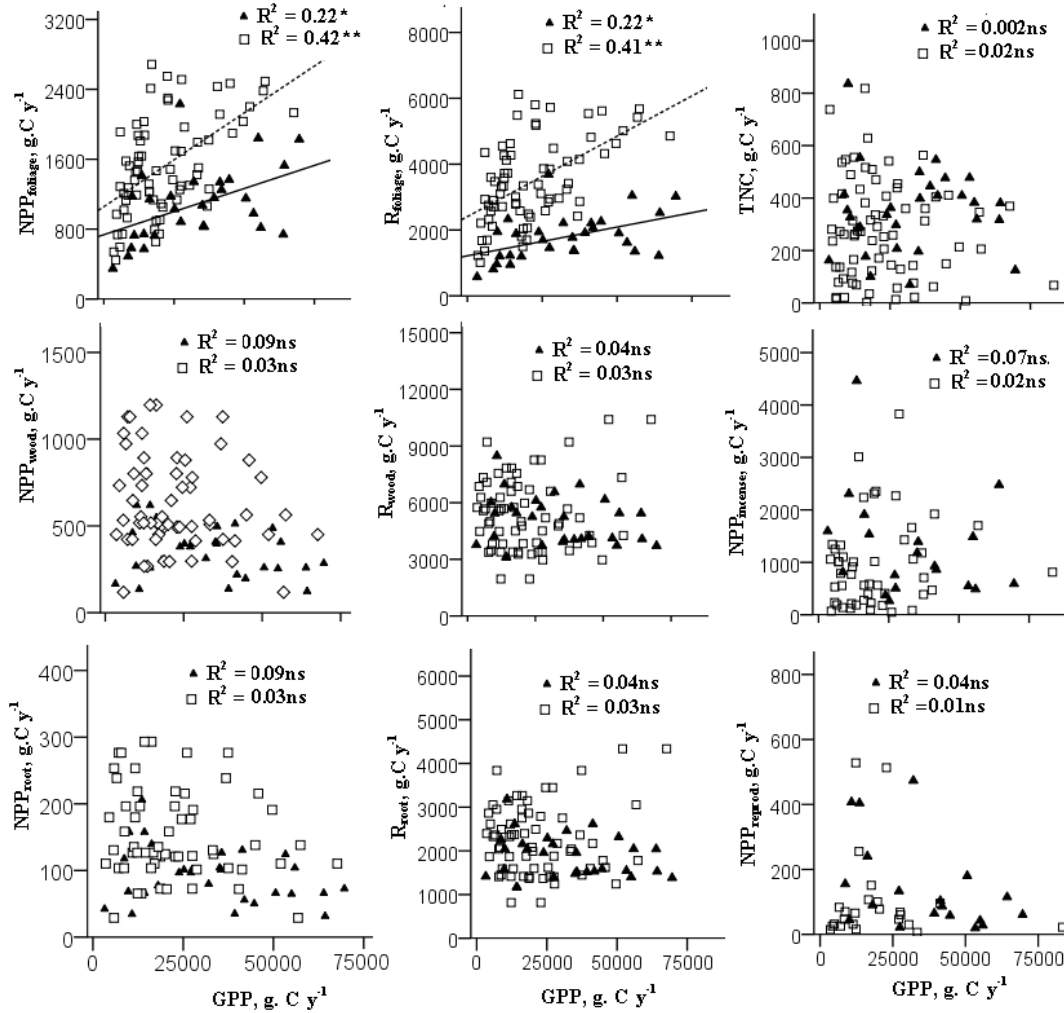


**Table 2:** Carbohydrate sources and sinks in *Boswellia papyrifera* trees. The first part shows the effects of site, tapping and their interaction on carbohydrate sources and sinks using *F*-test. *F* values and their significant levels are presented (ns, non significant; \*, 0.01<*P*<0.05; \*\*, 0.001<*P*<0.01; \*\*\*, *P*<0.001). The second part shows mean annual carbohydrate source and sinks in *Boswellia* trees under different tapping treatments (control, six and twelve incisions); mean values (g. C y<sup>-1</sup>) are presented after separate post-hoc, Tukey tests for each parameter. Total carbohydrate costs are the sum of all carbohydrate sinks. Total NPP is the sum of all sinks except respiration costs; autotrophic respiration is the sum of foliage (R<sub>foliage</sub>), wood (R<sub>wood</sub>) and root (R<sub>root</sub>) respiration costs.

Parameters	Site	Tapping	Site×tapping	Highland			Lowland		
				Control	Six incision	Twelve incision	Control	Six incision	Twelve incision
GPP	4.6*	1.5ns	2.4*	31451 <sup>ab</sup>	28314 <sup>ab</sup>	38115 <sup>a</sup>	30174 <sup>ab</sup>	23326 <sup>ab</sup>	16566 <sup>b</sup>
NPP <sub>foliage</sub>	14.9***	4.7*	8.1***	966 <sup>a</sup>	1133 <sup>a</sup>	1145 <sup>a</sup>	1971 <sup>b</sup>	1390 <sup>a</sup>	1329 <sup>a</sup>
NPP <sub>wood</sub>	28.2***	1.1ns	6.3***	342 <sup>a</sup>	383 <sup>a</sup>	359 <sup>a</sup>	639 <sup>b</sup>	756 <sup>b</sup>	798 <sup>b</sup>
NPP <sub>root</sub>	25.8***	1.2ns	5.8***	87 <sup>a</sup>	98 <sup>a</sup>	92 <sup>a</sup>	157 <sup>b</sup>	185 <sup>b</sup>	196 <sup>b</sup>
NPP <sub>incense</sub>	1.9ns	1.3ns	1.4ns	415 <sup>a</sup>	1036 <sup>a</sup>	1568 <sup>a</sup>	332 <sup>a</sup>	874 <sup>a</sup>	1026 <sup>a</sup>
TNC	3.3ns	2.7ns	1.9ns	82 <sup>a</sup>	348 <sup>a</sup>	280 <sup>a</sup>	111 <sup>a</sup>	247 <sup>a</sup>	256 <sup>a</sup>
NPP <sub>reprod</sub>	1.3ns	2.1ns	1.2ns	1892 <sup>a</sup>	102 <sup>a</sup>	99 <sup>a</sup>	3210 <sup>ab</sup>	20 <sup>b</sup>	28 <sup>b</sup>
NPP <sub>total</sub>	2.8ns	2.7*	1.8ns	1892 <sup>a</sup>	3100 <sup>b</sup>	3543 <sup>b</sup>	3210 <sup>ab</sup>	3473 <sup>b</sup>	3634 <sup>b</sup>
R <sub>foliage</sub>	42.1***	4.3*	15.2***	1595 <sup>a</sup>	1872 <sup>ab</sup>	1892 <sup>ab</sup>	4489 <sup>d</sup>	3165 <sup>c</sup>	3027 <sup>bc</sup>
R <sub>wood</sub>	4.3ns	0.01ns	0.9ns	4777 <sup>a</sup>	5487 <sup>a</sup>	5008 <sup>a</sup>	6296 <sup>a</sup>	6020 <sup>a</sup>	6307 <sup>a</sup>
R <sub>root</sub>	8.8**	0.02ns	1.8ns	1844 <sup>a</sup>	2075 <sup>a</sup>	1891 <sup>a</sup>	2635 <sup>a</sup>	2522 <sup>a</sup>	2643 <sup>a</sup>
R <sub>total</sub>	18.5***	0.5ns	4.4**	8215 <sup>a</sup>	9434 <sup>a</sup>	8792 <sup>a</sup>	13421 <sup>b</sup>	11708 <sup>ab</sup>	11977 <sup>ab</sup>
Total carbohydrate costs	23.2***	1.1ns	43.0***	10108 <sup>a</sup>	12534 <sup>b</sup>	12335 <sup>b</sup>	16631 <sup>cd</sup>	15181 <sup>c</sup>	15611 <sup>c</sup>

*Seasonal patterns*

The carbon allocation switched between the wet and dry season (Figure 2). During the wet season, GPP supplied carbon to all carbohydrate sinks except reproduction and resin export, which occurred during the dry season only. While it was assumed that the storage carbon (TNC) acted as the major carbon source during the dry season, the TNC storage did not fully account for the estimated carbon costs by tapping, reproduction, and maintenance respiration costs. Remarkably, the carbon consumption by resin production only was even higher than the carbon provided by TNC, suggesting that there were additional carbon sources.



**Figure 6.** The relation between carbon gain and carbon investments into different sinks across different *B. papyrifera* trees. Allocation patterns to foliage production (NPP<sub>foliage</sub>), wood production (NPP<sub>wood</sub>), root production (NPP<sub>root</sub>), foliage respiration (R<sub>foliage</sub>), wood respiration (R<sub>wood</sub>), root respiration (R<sub>root</sub>), frankincense production (Frankincense), reproduction costs (NPP<sub>reprod</sub>) and non-structural carbohydrate storage costs (TNC) with increasing gross primary production (GPP) for the *Boswellia papyrifera* tree. All values are in g C. tree<sup>-1</sup> y<sup>-1</sup>. Triangle symbols and solid lines are for the highland trees, and squares and dotted lines are for the lowland trees. The coefficient of determination (R<sup>2</sup>) values are indicated with ns =  $P > 0.05$ ; \* =  $0.01 < P < 0.05$ ; \*\* =  $0.001 < P < 0.01$ .

## Discussion

In this study, we quantified the carbon budget of *Boswellia* trees on an annual and scale, and also estimated seasonal budget to compare the wet and dry seasons (Figure 2). In this model, not all possible sink categories were included and estimations of some of the cost categories involve critical assumptions, particularly the maintenance cost estimates. However, the estimated carbon budget shows some interesting trends, and allowed us to explore our hypotheses. Overall, we showed that the estimated GPP was more than sufficient to account for the annual carbon sink consumption (Figure 1). This was not the case during the dry season separately (see Figure 2 dry season) when the carbohydrate supply is considered to come from the storage pools only.

The total of the estimated annual carbon sinks to the different components were 38-68% of the annual carbon gain in both study sites but these sinks did not include sinks for root exudates, export for mycorrhiza, resin stock increment, volatile organic compound emission and herbivory (e.g. insect on leaf and bark). However, *Boswellia* trees establish mycorrhizal association (Birhane et al. 2010) and the consumption of carbon by the fungal symbiont can be stronger (Corrêa et al. 2011). Mycorrhiza may consume up to 20% of the total fixed carbon (Smith and Read 2008). Therefore, assuming 20% mycorrhizal cost, the total carbon cost can reach 68-88% of the GPP. And yet the extent of root colonization is found three times higher in the highland than the lowland (Birhane et al. 2010), which implies more carbon cost for mycorrhizal symbiosis in the highland. Like in most other studies (Edwards et al. 1980, Ryan et al. 1997, Lambers et al. 1998, Kim et al. 2007), a large proportion of GPP is expended for respiration but the costs for critical stages like reproduction is minimal. The reproductive outcome seems low compared to other estimates from theoretical studies (Sterck and Schieving 2007). The percentage of total autotrophic respiration in this study was 28% and 52% of GPP in the highland and lowland. There is evidence that annual cost of respiration is between 30 and 70% (Edwards et al. 1980, Ryan et al. 1994, Ryan et al. 1997) of GPP. Considering the strong dependence of maintenance respiration rates on temperature (Ryan et al. 1990) and given the high temperature averages of our sites, the annual cost of respiration for *Boswellia* may indeed be higher than in other studies. However, annual aboveground wood maintenance respiration costs fell within the range of other studies (Table 3).

**Table 3.** Comparison of above ground woody tissue maintenance respiration estimated at various forest stands. Taking density of *Boswellia papyrifera* as 200 trees/ha (Eshete et al. 2011).

Species	Temperature (°C)	Precipitation (mm y <sup>-1</sup> )	LAI (m <sup>2</sup> m <sup>-2</sup> )	R <sub>wood</sub> (t C ha <sup>-1</sup> y <sup>-1</sup> )	Base for estimation	References
<i>Pinus contorta ssp. latifolia</i>	2	740	12.3	0.61-0.79	Sapwood volume	Ryan and Waring 1992
<i>Chamaecyparis obtusa</i>	15	1543	—	2.4	Dry weight	Yokota and Hagiwara 1995
<i>Pinus resinosa</i>	3	804	6.2	0.7	Sapwood volume	Ryan et al. 1995
<i>Quercus alba</i>	13	1400	5	0.83-1.10	Sapwood volume	Edwards and Hanson 1996
<i>Picea mariana</i>	—	542	4.9	0.57	Surface area	Ryan et al. 1997
<i>Pseudotsuga menziesii</i>	22	1180	2.2	1.46	Sapwood volume	Ryan et al. 1995
<i>Fagus sylvatica</i>	9.2	820	5.6	1.54	Sapwood volume	Damesin et al. 2002
Sterculiaceae, Leguminosae	23-26	1520	4.4	2.06-2.18	Surface area	Meir and Grace 2002
<i>Pinus densiflora</i>	13.5	1494	—	2.55	Sapwood volume	Kim et al. 2007
<i>Boswellia papyrifera</i>	22-28	796-953	3	1.0-1.2	Sapwood volume	This study

LAI : estimated leaf area index ; R<sub>wood</sub>: annual above ground woody tissue maintenance respiration rate

*Is there seasonality in allocation patterns?*

During the dry season, carbohydrate sinks were expected to be covered by the storage carbon (TNC). Higher carbon costs than provided by TNC during the dry season imply that trees do not fully depend on TNC for reproductive costs, frankincense production and maintenance respiration during this period. It can be speculated that the dry season frankincense is either constitutive frankincense (i.e. synthesized in the secretory structures) produced during the wet season, induced frankincense produced from TNC in the dry season or induced frankincense produced from direct carbohydrate supply by bark photosynthesis during the dry season (*Boswellia* bark contains chlorophyll, Gebrekidan et al. 2011 in prep.). Whereas it can be speculated that the white frankincense is the product of both wet season and dry season physiological activity, the carbohydrate demand by tapping remains a critical drain to the carbon budget of a tree during the dry season. During the wet season, the growth sinks (NPP) were largest but were largely covered by the carbon supply coming directly from the leaves. Nevertheless, at the onset of leaf expansion, TNC and bark photosynthesis are probably major carbon sources to initiate foliage development. Later, the newly established shoots may act as their own carbon sources (Whiley and Wolstenholme 1990, Yang and Midmore 2005,).

*Does tapping affect carbohydrate allocation pattern?*

Overall, we hypothesized that tapping will reduce carbohydrate allocation to all the other competing sinks. For example, we expect carbohydrate depletion by tapping to negatively affect fruit/seed setting (cf. Stephenson 1981, Ho 1988, Rijkers et al. 2006). In general, this prediction is partially supported in our study, because tapping traded-off with fruit production in the lowland only. Despite the fact that tapping is out of phase with foliage development, tapping also reduced the amount of carbohydrates allocated to foliage growth ( $NPP_{\text{foliage}}$ ) and respiration ( $R_{\text{foliage}}$ ) in the lowland. This may have a negative effect for the next season survival and growth. In the highland, however, the impact of tapping on carbohydrate allocation to other sinks was not significant. Trees in the highland have higher annual carbon gain (GPP) that may help to buffer the impact of tapping. Alternatively, following the arguments based on growth differentiation balance hypothesis (Herms and Mattson 1992, Kleczewski et al. 2010), it might be that the highland trees are constrained by other resource limitations (e.g. moisture) such that carbohydrates become available for frankincense production.

*Does allocation to sinks increase with GPP?*

We also investigated the impact of total carbohydrate gain by trees on subsequent carbon allocation patterns, and hypothesized that carbohydrate resource availability increases the carbon investments to all other sinks. Increasing GPP, however, does not lead to an increase in carbon supply to most, but not all sinks. Our expectation is thus only partially supported. Only partitioning to foliage production indeed increased with carbohydrate resource availability in the plant system. In our data set, the link of GPP to foliage production is weaker than in other studies (Litton et al. 2007). Here, GPP explained up to 42% of the variability in foliage carbohydrate allocation (Figure 6), while in other studies this was up to 71% (Litton et al. 2007). Remarkably, other carbon sinks such as frankincense production, were not affected by carbon gain. This suggests that for example trees produce similar amount of resin, irrespective of the total carbon available. This means that trees with low carbon gain (little leaf area) might suffer sooner from carbon starvation by tapping.

**Conclusions**

This study gives insight into carbon allocation patterns at tree level and how allocation patterns change seasonally driven by phenological events. The carbohydrate consumption by tapping *Boswellia* trees remains a critical drain of up to 4% of the tree carbon budget. Although tapping competes with growth and reproductive sinks, the extent of competition for carbohydrates between frankincense production and other costs was site specific. Only comparative studies on whole-tree level carbohydrate allocation of other species will reveal if the patterns that we found here are general and how tapping will impact the carbohydrate allocation patterns across the large diversity of gum-resin producing species.

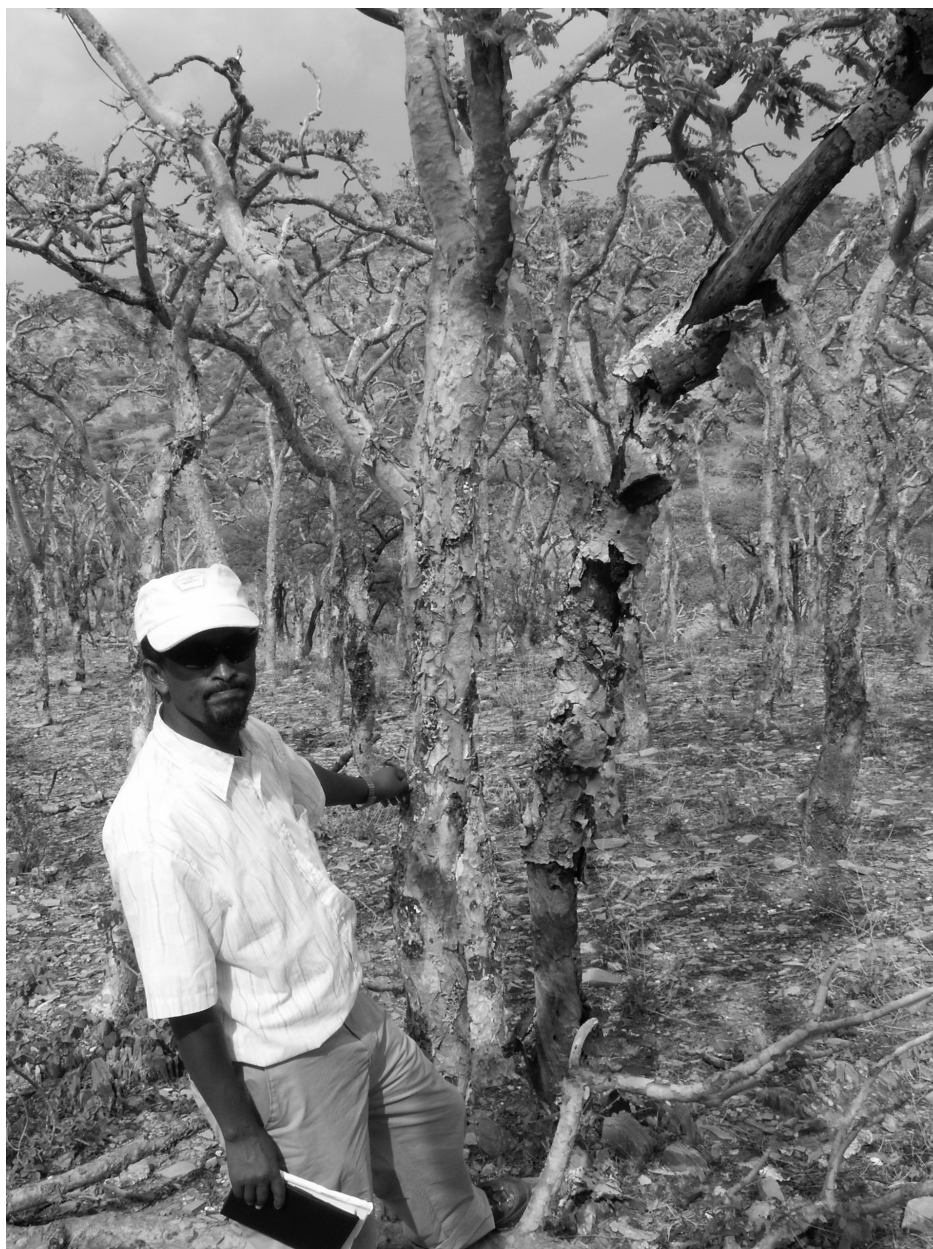




## *Chapter 6*

### *Synthesis*

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## Introduction

African drylands are among the most exploited systems (Campbell 2000), and are being degraded or transformed to agricultural lands at an increasing spatial scale (Bongers and Tennigkeit 2010). Furthermore, the people that inhabit dry woodlands of Africa are often poor and overexploit the remaining resources, resulting in a resource decline and risk for peoples' livelihood. On the other hand, erratic rainfall and high temperatures, characteristic for these drylands, also play an important role. Recurrent drought episodes challenge plant survival in these regions. Therefore, plants face seasonal water deficit making drought stress a recurrent phenomenon. Strong seasonality in rainfall and thus water stress has significant effects on the annual carbon gain and allocation patterns of plants. In this study, I selected the frankincense producing tree, *Boswellia papyrifera* (Del.) Hochst, for detailed tree carbon balance studies.

Of the several species of the genus *Boswellia* (family Burseraceae) few can be regarded as sources of the classical frankincense, and the one from *Boswellia papyrifera*, has been prized for commercial importance. This dissertation is on the physiology and carbon balance of this frankincense producing tree species; *Boswellia papyrifera*, which grows in dry woodlands of the Sudano-Sahelian region including Ethiopia, Somalia and Eritrea (White 1983, Lovett and Friis 1996, Ogbazghi et al. 2006). The species is indigenous and occurs in the northern, western and central parts of Ethiopia (Tengnas and Azene 2007, Tadesse et al. 2007). When *Boswellia* trees are tapped, the frankincense (mainly carbon-based secondary compounds) exudes from the tree. Frankincense harvesting occurs during the dry season, when trees are without leaves while there is also carbohydrate demands for maintenance and reproduction. Therefore, tapping causes the tree to divert carbohydrate to resin at the expense of carbon investment in reproductive organs (Ogbazghi et al. 2006, Rijkers et al. 2006). Continuous tapping is therefore expected to have implications for the carbon budget of *Boswellia* trees, and for their ability to provide sufficient carbon to future growth, survival and reproduction.

Nowadays trees are more continuously and indiscriminately tapped for their frankincense than before, putting an extra pressure on the remaining populations. There are clear indications of export expansion by expanding the harvest of this export item in the future while harvesting techniques remain inert and only maximize short-term economic gains. Eventually, current intensive frankincense production could affect the whole tree carbon balance. During the last decade, only few studies are done on the ecology and reproductive effort of *Boswellia*

(Gebrehiwot 2003, Rijkers et al. 2006, Ogbazghi et al. 2006, Abiyu et al. 2010, Eshete et al. 2011) but not on the impact of frankincense production on the tree carbon balance. Understanding the implications of intensive frankincense tapping and of climate for the carbon balance of *Boswellia* trees will provide a solid basis for understanding the consequences for growth, survival and reproduction.

In this thesis, I showed the impact of tapping and climate factors on the carbon budget of *Boswellia* trees and how competing demands by different sinks are balanced by this tree species. Carbon allocation studies are increasingly important as they allow us to investigate tree-level source-sink dynamics and physiological plant responses in relation to biotic, abiotic, and anthropogenic stresses. Studies such as this allow us to quantify source-sink dynamics and construct a more precise tree-level carbon budget from the bottom up. Furthermore, this study represents as far as I know the first *in-situ* mature tree carbohydrate analysis for a tree inhabiting dry woodlands.

The objectives of this research include understanding the impact of tapping on leaf gas exchange properties, tree annual carbon gain and carbon allocation pattern of *Boswellia papyrifera* in northern Ethiopia. First, the diurnal gas exchange pattern of *Boswellia* leaves is evaluated in two populations. Second, the annual carbon acquisition of *Boswellia* trees is assessed in relation to tapping and climate parameters. Third, the impact of tapping on the storage carbohydrate dynamics is investigated. Finally, the impact of tapping on the tree carbon balance and carbohydrate allocation patterns in high and low altitude areas is evaluated. I studied these carbon gain and allocation patterns in relation to tapping by collecting field and laboratory data for a period of two years (2007-2009) from naturally grown trees in woodlands of north Ethiopia (Chapter 2 until 5).

The main results showed that *Boswellia* trees adapt to local conditions. Moreover, results on the impact of tapping clearly indicate that continuous tapping depletes storage carbon, diminishes leaf growth and reproductive effort and these negative effects will be apparent sooner for smaller trees than for larger ones. Thus guidelines for tapping intensity and frequency should be formulated considering these effects on *Boswellia* populations.

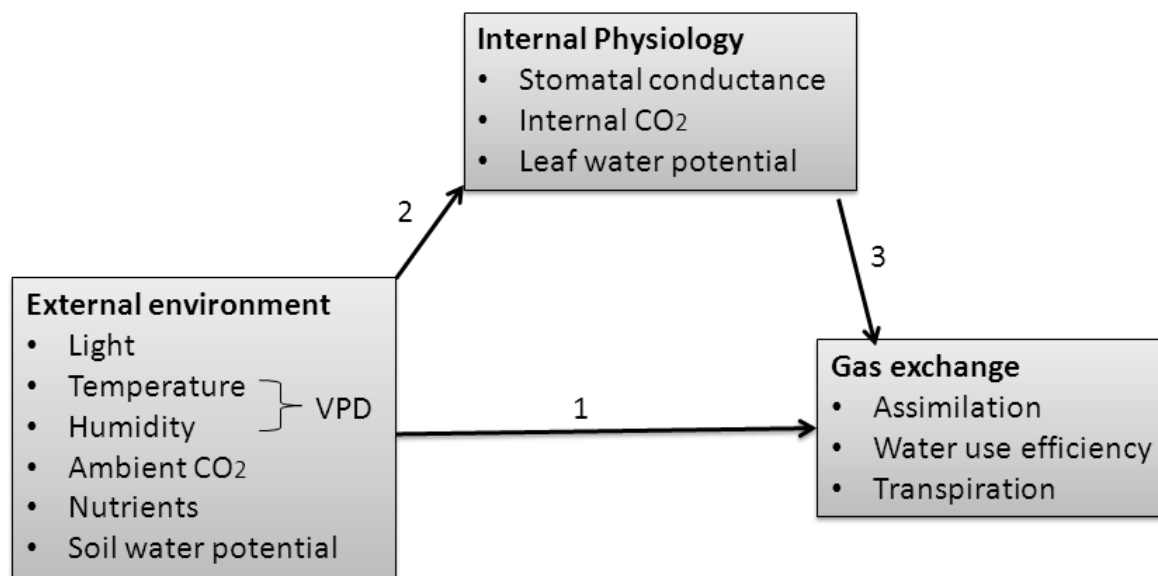
The research questions include:

**(1) How do external climate factors and physiological mechanisms explain the variation in leaf gas exchange characteristics of the *Boswellia* tree?**

The relationship of environmental conditions on leaf gas exchange traits like photosynthesis, transpiration and water use efficiency was clearly outlined from other empirical studies in Mediterranean (e.g. Tuzet et al. 2003, Zweifel et al. 2007, McDowell et al. 2008) and Neotropical (e.g. Goldstein et al. 2008, Bucci et al. 2008) systems. In this dissertation, I tested the relationship between environmental and physiological parameters on leaf gas exchange in the deciduous dry tropical woodland tree (Chapter 2, Figure 1) using path analysis. Furthermore, diurnal pattern of leaf gas exchange was evaluated in comparison with either the classical hump-shaped pattern of some temperate and wet tropical forest trees (Weber and Gates 1990, Ishida et al. 1996, Souza et al. 2008) or a gradual decline after morning peak like other temperate (Bassow and Bazzaz 1998) and dry tropical savanna trees (Eamus et al. 1999).

*Gas exchange patterns*

In order to assess the combined contribution of the environmental and physiological parameters on leaf gas exchange, the research model in chapter 2 is shown in a more simple way as in figure 1 here. In this model, environmental variables are expected to affect leaf gas exchange either directly (Figure 1, arrow 1), indirectly (Figure 1, arrow 2 & 3) or both.



**Figure 1.** Interacting environmental, internal physiological and gas exchange parameters. Numbers indicate possible direct (1) and indirect (2, 3) paths of relationships. VPD is vapor pressure deficit.

Leaf carbon gain is more challenged by atmospheric drought (VPD) than by soil water deficit during the wet growing season. Moreover, light availability during the wet season had a strong impact on leaf assimilation rate. Saturating light and higher photosynthetic capacity gave highland trees a higher daily photosynthetic rate and higher annual carbon gain than lowland trees (Chapter 3, Figure 3).

#### *Diurnal pattern*

Leaves increase assimilation in the morning during the period of least atmospheric drought, followed by a decrease due to gradual closure of stomata as the transpiration demand increases (see also Zweifel et al. 2007, Bucci et al. 2008). This diurnal pattern of *Boswellia* leaf gas exchange differs from wet tropical and temperate tree species (Weber and Gates 1990, Mulkey et al. 1996, Pathre et al. 1998) but, resembles to other temperate (Bassow and Bazzaz 1998), Mediterranean (Gatti and Rossi 2010) and tropical savanna (Eamus et al. 1999) trees. The leaf water potential varied relatively little and did not influence gas exchange during the measurement period.

**(2) How do climatic factors link to crown functional traits to affect annual carbon gain and resin yield?**

Erratic rainfall patterns may create limiting conditions for plant physiology, carbon gain and growth (Murphy & Lugo 1986, Bullock et al. 1995, Vanacker et al. 2005). Such conditions are expected to become more severe on tree carbon gain in the face of global warming (Lacointe 2000, Hély et al. 2006, Bolte et al. 2010). For a tropical deciduous dry woodland tree, it is even a major challenge to maintain sufficient annual carbon gain within the limited leaf lifespan. In this part of the study, I linked environmental conditions, plant traits and tapping intensity with the annual crown carbon gain of *Boswellia* trees in two populations. Scaling-up these factors to determine crown carbon gain in the field is scarce. This information is especially limited for tropical dry forests and dry woodland trees (Yoshifugi et al. 2006, Kushwaha et al. 2010). Previous studies suggest that tapping creates a carbon sink that is at the cost of growth, including vegetative growth and reproduction (Cannell & Dewar 1994, Rijkers et al. 2006, Chantuma et al. 2009).

*Comparing the two study sites*

Higher light interception together with high photosynthetic capacity (Chapter 3, Figure 3) resulted in higher photosynthetic rates in the highland compared to the lowland. Apparently, shorter crown leaf lifespan of trees in the highland was more than compensated by their higher photosynthetic rates. Therefore, the radiation during the wet season had a stronger impact on tree carbon balances than wet season length. Despite the variation in environmental conditions and annual carbon gain between the two sites, trees achieved similar resin yields. Moreover, neither high crown assimilation nor larger crown leaf area lead to higher frankincense yield. This could indicate that frankincense production is not directly coupled to carbon gain, but also implies that smaller trees may suffer sooner from carbon starvation by tapping. Carbon gain is related to crown leaf area and if both smaller and larger crown leaf area are providing similar resin yield (within the diameter class studied), smaller crown trees are also making unreserved effort to provide incense, which eventually implies that their smaller carbon stock will deplete sooner eventually leading to carbon starvation and mortality.

*Tapping effect on crown carbon gain*

Tapping frankincense reduced the leaf area production and annual assimilation in the low altitude area but not in the high altitude area (Chapter 3, Figure 3). This was comparable to the resin production to reproduction trade-off (Rijkers et al. 2006) and rubber production to growth trade-off (Chantuma et al. 2009). The fact that tapping did not impact on trees of the highlands implies that these trees are either buffered by their higher annual carbon gain, or face less competition between carbon sinks. The lack of concomitant decrease in foliage mass after tapping does however not necessarily mean that tapping has no effect. In line with the growth differentiation balance hypothesis (Herms and Mattson 1992, Kleczewski et al. 2010), it can be argued that at limited resource availability, growth is more constrained making carbohydrates available for secondary metabolism. Alternatively, foliage production in the highland could be constrained by moisture, such that competition for carbon between tapping and other sinks is low. Generally, I concluded that the impact of tapping was site specific.

**(3) How does frankincense tapping influence the concentration and seasonality of non-structural carbohydrate storage in the frankincense tree?**

Carbohydrates fixed by photosynthesis are stored in plant organs mainly in the form of starch or sugars for future use (Newell et al. 2002, Würth et al. 2005, Bansal and Germino 2009, Chantuma et al. 2009, Regier et al. 2010). Both starch and sugars form total non-structural carbohydrates (TNC). These carbohydrates are intermediate between assimilation and utilization (Chapin et al. 1990) and are used to support maintenance respiration or other metabolic processes under low photosynthetic periods. Deciduous species mainly depend on their TNC while leafless during the dry season. TNC may thus allow plants to survive periods of stress (Poorter and Kitajima 2007). Although trees may accumulate their TNC in different compartments (e.g. leaves, stems and roots), TNC have rarely been examined for multiple compartments simultaneously (Newell et al. 2002). Research on TNC for resin and gum producing tropical dry woodland trees is lacking.

In this part of the research, I determined (1), how frankincense tapping influences TNC content of trees; (2), the seasonal dynamics of TNC owing to exhaustion during the dry season

and; (3), the variation in TNC concentrations in different plant organs. This marks one of the first, if not the first, study on storage TNC in different organs for a dry tropical woodland tree.

I found that TNC in *Boswellia* trees consist mainly of starch and far less of soluble sugars (Chapter 4, Figure 2). Tapped trees have lower TNC concentrations than untapped trees in all compartments. Because tapped trees face declining carbon storage pools during the dry tapping season and these pools are not fully replenished during the wet season, they face higher risks of carbon starvation compared to untapped trees. Furthermore, the higher annual carbon gain in the highland site (Chapter 3, Figure 3), does not correspond with higher storage TNC concentrations. The fact that higher annual carbon gain is not reflected in none of the carbohydrate sinks including storage, points out that there are additional underground sinks to be investigated further. Generally, TNC concentrations in the *Boswellia* tree was lower than in the apple tree (Naschitz et al. 2010), the rubber tree (Silpi et al. 2007, Chantuma et al. 2009) and some Bolivian tropical forest trees (Poorter and Kitajima 2007) but comparable to some conifers (Bansal and Germino 2009), seasonally dry forest trees (Newell et al. 2002) and temperate tree-line deciduous taxa (Li et al. 2002, Hoch et al. 2002, Hoch et al. 2003).

Given the distinct seasonal changes in climate and phenology of this tree, periodic depletion and re-fill of TNC concentrations are in line with our expectations. This was also in agreement with results from other studies (Steele et al. 1984, Hoch et al. 2003, Silpi et al. 2007, Chantuma et al. 2009, Bansal and Germino 2009) where TNC levels were depleted during the dormant leafless dry season.

#### **(4) How do the multiple carbon sinks respond to tapping and to seasonal variation?**

Carbohydrates fixed by photosynthesis are the sources of energy for growth and metabolic processes in the plant system. If these carbohydrates are limited, competition could occur among different demands for the same carbohydrate resource (Bazzaz et al., 1987, Stephenson 1981, Ho 1988, Rijkers et al. 2006, Poorter and Kitajima 2007). Allocation patterns may shift seasonally challenged by climate and anthropogenic factors. In the past decades, the study of carbon cycles has received increasing attention due to the crucial role of carbon in global warming. However, a necessary pre-requisite yet to be studied in CO<sub>2</sub> flux is to understand the basic mechanism of carbon allocation pattern at the whole plant level (Litton et al. 2007).



In this part of the dissertation, I capture the findings of my thesis in terms of the annual carbon gain (Chapter 3) and the allocation to competing sinks (chapter 4 and 5) while tapping is considered as an additional drain like in rubber trees (Chantuma et al. 2009). This is as far as I know the first attempt to understand annual and seasonal carbohydrate allocation pattern at tree level. Frankincense harvesting will reduce the allocation of carbohydrates to the remaining sinks. Increased annual carbohydrate gain is expected to increase carbohydrate resource availability to individual sinks. To demonstrate this, I used a carbon flow scheme (see also Ryan et al. 2004, Litton et al. 2007) to evaluate annual and seasonal source-sink balances. In this scheme, GPP (gross primary production) is considered as the carbon source during the wet season and TNC is considered to serve as carbon source during the dry season (Chapter 5, Figure 2).

The total of the estimated annual carbon sinks to the different components were 38-68% of the annual carbon gain in both study sites. However, *Boswellia* trees also establish mycorrhizal association (Birhane et al. 2010) and the consumption of carbon by the fungal symbiont can be strong (Corrêa et al. 2011). Mycorrhizal symbiosis can consume up to 20% of the total fixed carbon (Smith and Read 2008). Therefore, assuming 20% cost of mycorrhiza, the total carbon cost can reach 68-88% of the GPP. And yet the extent of root colonization is found three times higher in the highland than the lowland (Birhane et al. 2010), which implies more carbon cost of mycorrhiza in the highland. Contrary to our expectation, the sum of all dry season costs was found above the total available TNC stock, and the cost of frankincense production alone was higher than TNC. Such high carbon costs during the dry season imply that trees do not fully depend on TNC for dry season costs. This suggests that trees use additional sources of carbohydrates, for example carbohydrates that are produced by bark photosynthesis. *Boswellia* trees indeed have considerable amount of bark chlorophyll (Gebrekidan et al. 2011 in prep). Our results indicate that the additional carbon cost by tapping reduced subsequent foliage development and reproductive effort especially in the lowland. This was not the case in the highland. Trees in the highland have a higher annual carbon gain (GPP) that may help to buffer the impact of tapping. An alternative argument is based on the growth differentiation balance hypothesis (Herms and Mattson 1992, Kleczewski et al. 2010). The implication of this hypothesis for our case is that *Boswellia* trees may respond to the gradient of resources: the growth of highland trees may be constrained by other resource limitations (e.g. moisture) in such a way that carbohydrates become available for alternative use such as frankincense production. A

good indication for this is that although GPP was lower in the lowland, annual carbohydrate sinks in the lowland exceeded those of the highland (Chapter 5, Figure 5 and Table 2).

I also demonstrated that, with the exception of carbon allocation to foliage production ( $NPP_{\text{foliage}}$ ) and maintenance ( $R_{\text{foliage}}$ ), increasing carbon gain is not accompanied by an overall increase in carbohydrate allocation to the other sinks. Therefore, the carbon allocation pattern is constrained not exclusively by the absolute amount of carbon gained but also by other factors for example environmental factors such as moisture, temperature and vapor pressure deficits.

## Conclusions

**Climate impacts:** The drier highland *Boswellia* populations occur at higher light levels and achieve higher annual carbon gain during a shorter growing season than the less dry lowland. However, more carbohydrates are invested in sinks in lowland trees than highland trees. Therefore, beyond carbon gain, environmental factors like moisture stress may be additional important elements affecting the carbon allocation patterns. In the highland, the short wet season and the long period of moisture stress may constrain tree growth to a greater degree than overall assimilation. However, in the less dry lowland, the better tree growth (see Chapter 5 table 2, higher NPP values for the lowland than the highland) requires high carbon demands. I therefore conclude that radiation during the wet season is the most important factor affecting *Boswellia* tree carbon gain. But rainfall amount and wet season length is more important in the allocation of the acquired carbon to the different sinks. Drier and hotter conditions may set limits to existing leaf lifespan, annual carbon gain and thus also to carbon allocation patterns.

**Impact of tapping:** Frankincense production cost (4% of GPP) is the third largest carbon sink in *Boswellia* trees. Tapped trees face declining carbon storage pools that are not fully replenished during the wet season, and may risk carbon starvation sooner than untapped trees. The competition for carbohydrates between frankincense production and other sinks is stronger in the lowland where tapping reduced foliage production and reproductive effort. However, the total amount of frankincense produced is not affected by the total annual carbon gain implying that smaller trees may suffer sooner from carbon starvation by tapping. Therefore, indiscriminate harvesting of frankincense, including small trees, will increase the risk of carbon starvation and

tree mortality. Improved tapping guidelines thus should include a lower tapping intensity and frequency for trees with smaller sizes.

**Carbon budget:** Based on the carbon flow scheme, the total estimated annual carbon sinks to the different components are lower than the total annual carbon gain in both study sites, indicating that additional critical sinks yet have to be explored. However, estimated autotrophic respiration cost (the sum of respiration costs of wood, root and foliage) is uncertain but probably major carbohydrates sink at both sites. Such a quantitative analysis of annual carbon gain and investments into tree carbon sinks shows how allocation patterns change in relation to plant phenological events and physiological processes. The carbohydrate storage reserves (TNC) are not sufficient for paying the sink costs during the leafless dry season, suggesting that other sources are being used. A possible contribution is from the carbohydrates produced by the bark since chlorophyll is amply available. This definitely needs further exploration. But, if the contribution of bark photosynthesis is as high as expected, bark wounding by tapping does have impact on the tree carbon balance not only by creating additional sink but also by reducing the carbon gain from bark photosynthesis.



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## Summary

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African dry forests and woodlands are rich in tree and grass species. Many of these plants have great potential to provide renewable resources of future economic growth. However, several species are lost by increased degradation due to climate and anthropogenic factors. Measures to counteract this problem should be targeted to improved resource management. This study analyses the carbon balance of the economically important frankincense producing tree *Boswellia papyrifera* (Del.) Hochst. This economically important species grows in the dry woodlands of northern Ethiopia. The species is the source of classical frankincense also known as “Olibanum”.

The main research goal of this thesis was to evaluate the impact of frankincense tapping on the carbon balance of the *Boswellia* tree. This research is the first study to analyze the impact of tapping on carbon gain and allocation pattern of a dry woodland tree species. Trees were studied from lowland (Metema) and highland (Abergelle) populations. The research was focused on adult trees of equal size ( $20\pm 3$  cm diameter at breast height).

Leaf gas exchange was measured on naturally grown *B. papyrifera* trees in the morning (8-11 h), around midday (12-14h), and in the afternoon (15-17h) over a series of consecutive days during the wet seasons of two years. The effects of tapping, climate conditions and crown functional traits to annual carbon gain were examined and, in turn, their subsequent impacts on resin yield were evaluated. Furthermore, the influence of frankincense tapping on total nonstructural carbohydrates (TNC) content in wood, roots and bark of trees was evaluated, as well as the seasonal dynamics in TNC as a result of growth in the wet season and exhaustion during the dry season. Finally, the gross primary productivity (GPP), estimated from gas exchange measurements, was related to the carbon allocation to competing sinks using a carbon flow scheme. Tapping was considered as an additional carbon drain.

The results show that *Boswellia* trees adapt to local conditions. Leaf photosynthesis was more limited by atmospheric drought than by soil water deficit during the wet growing season. Radiation during the wet season had a stronger impact on annual carbon gain than wet season length in contrasting environments. Frankincense production costs up to 4% of the annual GPP and is the third largest carbon sink in *Boswellia* trees. However, the yearly production of

frankincense was not affected by the annual tree carbon gain implying that smaller trees may suffer sooner from carbon starvation by tapping. Continuous and intense tapping depletes storage carbon, diminishes total leaf area production and the reproductive effort and these negative effects will be apparent sooner for smaller trees than for larger ones. Guidelines for tapping intensity and frequency should be formulated considering these tapping effects on *Boswellia* populations.

The impact of tapping on the total leaf area production and the reproductive effort was site specific. Our findings have also revealed how frankincense harvesting reduced carbon allocation for reproductive effort. The negative effects of tapping were more apparent in the lowland than in the highland. Trees growing in the drier highland environment achieved better annual carbon gain owing to their higher photosynthetic capacity and higher light conditions and despite a shorter growing season. This possibly allows trees to buffer the negative impact of tapping on leaf area production and the reproductive effort. The higher carbon gain in the highland is however not reflected in larger carbon storage pools, frankincense yield, reproductive effort or growth sinks (net primary production, NPP).

The widely known frankincense is a valuable gift of nature. It has been traded in large quantities for a long time. Frankincense has been a valuable commodity for cultural and religious uses but also gained increasing demand for the production of medicines, cosmetics and chemicals in modern society. Studies in this dissertation showed that the source of frankincense, the *Boswellia* trees, were physiologically capable of acclimating to their natural environmental conditions but also that they are susceptible to intensive tapping. The current practice of harvesting frankincense at high intensity diminishes the tree vitality through decreased carbon storage needed for vital functions and results in a higher chance of tree mortality. Persisting with the current tapping practices will lead to a significant decrease of *Boswellia* populations. Forests with relatively vital *Boswellia* populations will need to receive conservation status, and clear harvesting guidelines for the frequency and intensity of tapping need to be developed and applied. If incense harvesting is based on the understanding of the tree's carbon balance, the future production of frankincense may become sustainable.

## Samenvatting

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Afrikaanse droge bossen zijn rijk aan boom en grassoorten. Veel plantensoorten leveren duurzame hulpbronnen voor toekomstig economische ontwikkeling. Verschillende van deze soorten worden bedreigd door klimaat- en menselijke factoren. Maatregelen om het verlies aan soorten tegen te gaan zouden zich moeten richten op het beheer van de natuurlijke hulpbronnen. Deze studie onderzoekt de koolstof balans van de wierook producerende boom *Boswellia papyrifera* (Del.) Hochst. Deze economisch belangrijke soort groeit in de droge bossen van Noord Ethiopië, en is de bron van de klassieke wierook, ook wel bekend als “olibanum”.

Het belangrijkste doel van het onderzoek in dit proefschrift was het evalueren van de invloed van het tappen van wierook op de koolstof balans van de *Boswellia* boom. Voor de eerste keer is onderzoek verricht naar de invloed van het tappen van hars (wierook in dit geval) op de jaarlijkse koolstofwinst, koolstofopslag en koolstofallocatie van een boom uit droge tropische bossen. Hierbij is gebruik gemaakt van gegevens van populaties uit het laagland bij Metema en het hoogland bij Abergelle. Het onderzoek richtte zich op volwassen bomen van vergelijkbare omvang ( $20 \pm 3$  cm stam diameter op borst hoogte).

Blad fotosynthese en transpiratie zijn gemeten bij *B. papyrifera* bomen in de ochtend (8-11 uur), rond de middag (12-14 uur) en in de namiddag (15-17 uur). Dit soort metingen zijn uitgevoerd voor een aantal dagen in het regenseizoen gedurende twee opeenvolgende jaren. De effecten van tappen, klimaat en boomkroonkenmerken op de jaarlijkse koolstofwinst en wierook productie zijn bestudeerd. Bovendien is de invloed van het tappen van wierook op de totale koolstof reserves in hout, wortels en bast onderzocht. De veranderingen in deze reserves variëren ten gevolge van de groei tijdens het regenseizoen en de wierook productie tijdens het droge seizoen. Ten slotte is de bruto koolstofwinst geschat uit de bladfotosynthese en kroonomvang, en vergeleken met de koolstof uitgaven door verschillende koolstof gebruikende organen. Hierbij werd het tappen van wierook beschouwd als een extra verliespost voor koolstof.

De resultaten geven aan dat *Boswellia* bomen zich aanpassen aan lokale situaties. De blad fotosynthese is meer gelimiteerd door atmosferische droogte dan door een gebrek aan grondwater tijdens de natte groei periode. Zonnestraling tijdens de natte periode had een grote invloed op jaarlijkse koolstofwinst, en die invloed was zelfs groter dan de lengte van de regentijd

voor de twee onderzochte, en sterk contrasterende, onderzoeklocaties. Wierook productie kost tot 4% van de jaarlijkse bruto koolstof winst en staat als koolstof verliespost op de derde plaats in Boswellia bomen. De jaarlijkse productie van wierook wordt echter niet beïnvloed door de jaarlijkse koolstofwinst van de boom. Dit impliceert dat kleine bomen eerder te lijden hebben van koolstof gebrek door tappen dan grote bomen. De resultaten geven duidelijk aan dat frequent en intensief tappen de koolstof opslag uitbuit, en dat het totale bladoppervlak en de vruchtproductie vermindert. De negatieve effecten hiervan zullen sneller zichtbaar zijn bij bomen met kleinere kronen en kleinere koolstofwinst. Er zijn richtlijnen nodig om tap intensiteit en tap frequentie te reguleren waarbij deze effecten in acht genomen worden.

De invloed van tappen op het bladoppervlak en de reproductie verschilde tussen de twee onderzoeksgebieden. Onze bevindingen laten zien dat het oogsten van wierook de koolstof consumptie vermindert voor reproductie. Deze negatieve effecten van tappen waren sterker in laagland populaties dan in hoogland populaties. Bomen die in de drogere hooglanden groeiden verkregen een hogere jaarlijkse koolstofwinst dankzij een hogere capaciteit voor fotosynthese en betere licht condities tijdens een korte groei periode. Dit geeft deze bomen waarschijnlijk de mogelijkheid om een koolstof buffer op te bouwen waarmee de negatieve invloeden van tappen op blad en vrucht productie verminderd kunnen worden. De hogere koolstofwinst in de hooglanden vertaalt zich echter niet in een hogere koolstof opslag, en evenmin in een hogere reproductie, groei of wierook oogst.

Wierook is een waardevol geschenk van de natuur. Het wordt sinds een lange tijd in grote hoeveelheden verhandeld. Wierook is een belangrijk product voor cultureel en religieus gebruik, en het wordt ook steeds meer gebruikt voor medicijnen, cosmetica en chemicaliën in de moderne samenleving. Onderzoeken in dit proefschrift laten zien dat de bron van wierook, de Boswellia bomen, fysiologisch in staat zijn om zich aan te passen aan hun natuurlijke omgeving, maar vatbaar zijn voor het aanhoudend tappen van wierook. Het tegenwoordig zeer intensieve oogsten van wierook leidt tot minder vitale bomen doordat de beschikbaarheid van koolstof voor vitale functies vermindert met toenemende sterfte als gevolg. Dit zal uiteindelijk leiden tot een afname van de vitale Boswellia populaties. Gebieden met vitale Boswellia populaties dienen een beschermde status te krijgen en duidelijke richtlijnen voor de frequentie en intensiteit van het tappen zullen ontwikkeld dienen te worden. Wanneer het oogsten van wierook gebaseerd is op

het begrijpen van de koolstof balans in de boom, dan zou de toekomstige productie van wierook duurzaam kunnen zijn.





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I feel very fortunate to have conducted my PhD program at the Forest Ecology and Management (FEM) group of Wageningen University. I would like to thank all the staff members of FEM group, whose friendship enriched my knowledge and understanding about the Dutch Society. I will never forget the “Dutch Soup” especially with mushroom. Special thanks to Joke, Dr. Jan, Dr. Pieter, Dr. Ute, Dr. Patrick, Dr. Hans, Leo, Ellen, Dr. Mulugeta and Prof. Frits for the support and encouragements.

Also thanks to all PhD studentship mates in Wageningen: Emiru Birhane, Teshale Woldeamanuel, Geovana Carreño, Canisius Mugunga, Jean Damascene, Lennart Suselbeek, Lucy Amissah, Kwame Oduro, Lars Markesteijn, Gabriel Muturi, Yoshiko Iida, Addisalem Ayele, Motuma Tolera, Paul Copini, Corneille Ewango, Peter van der Sleen, Peter Groenendijk, Zenebe Admassu, Abeje Eshete, Atkilt Girma, Birhanu Biazen, Mesele Negash, Girma Kelboro, Tessema Zewdie, Yosias Gandhi, Estella Quintero, Madelon Lohbeck, Cristina Garza-Martinez, Catharina Jakovacs, Mart Vlam, Gustavo Schwartz and Akalu Firew because our discussions and sharing of ideas even during informal talks were valuable to advance and succeed in my PhD. I would like to thank our Sunday school Ethiopian Orthodox Tewahido church members in Wageningen for the incredible moments offered.

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I would like to take this opportunity to thank my close brothers and sisters Tareke Ayalew, Hailu Tessema, Tamene Tessema, Tesfaye Mekonnen, Getaneh Yohannes, Enanu Ahmed, Emebet Damtie, Yimer Adem and Arega Zeru who have been instrumental not only in their permanent advice but also taking care of my family during my absence.

Given that I was born in a rural Ethiopian family, the enormous support of my parents during my early school life has been incredible. My ever strong mother, Mareshet Mekonnen (a monk and close to 80 now) took the lead and replaced the role of my late father during my early education. The spirit of prayer from my mother has been instrumental for me all along my way until now. My elder sister (Zenebech Muhye) and elder brother (Tsegaw Mengistu) deserve special thanks for giving me the privilege of education with their special effort. My other brother Begashaw and sisters Asnakech, Tsehaynesh, Abebech, Tewabech and Atsede also deserve special thanks. They all worked hard to give me the opportunity for education, which they miss. Now, I remain the only family member (out of the dozen others) who happens to get education. This left me with a feeling of more responsibility to get involved in giving access to education for the upcoming generation of Ethiopia as part of my future career.

Finally, my special thanks goes to my wife Asnakech Yohannes, and our children Mahider, Kidist and Dawit, for being the source of love, strength and joy for my life any time. I would like to offer this dissertation to my wife and our children for the infinite love and persistent support. Asni, what I also appreciate about you most is your patience and determination to take the big burden of handling our children and household matters during my absence apart from your strong encouragement to me during tough times. Our children, despite sometimes questioning the long time that I have been away from them, continued to be lovely with incredible patience and understanding beyond their age (now all below 10). My family, the greatest gift I can give for you is my love. I assure you, all I sacrifice is also for you.

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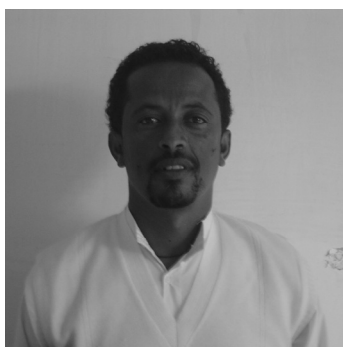


## *Short biography*

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Tefera Mengistu Woldie was born in Kelala of South Wello, on June 10, 1970. He is the youngest son of the farmer Mengistu Woldie and his wife Mareshet Mekonnen. Both the father and mother were hard working and raised their children in a disciplined way. In 1977, he started modern education in the rural area, where he travelled long distances on foot to get elementary and secondary education.

Tefera has got BSc. degree in Forestry in 1992 from Alemaya University of Agriculture. After that, he became district expert and team leader for the Forestry and Agricultural extension of Sekota Zone in the Amhara National Regional State for six years. Big efforts were made to rehabilitate degraded lands and Sekota area. In 1999, he joined education at the Swedish Sciences (SLU) and Wondo post-graduate research was on restoration of degraded lands in Much of his work in the ministry of Agriculture was spent on the rehabilitation of the most degraded dry lands of Northern Ethiopia.



After post-graduate education, Tefera became staff member of Wondo Genet College of Forestry. From 2001-2006, he has been involved in teaching courses like biodiversity conservation and management, forest ecology, restoration ecology, forest influence on climate and ecosystem studies. On March 2007, Tefera joined Wageningen University for his PhD research in the FRAME project (FRAnkincense, Myrrh, and Gum Arabic: sustainable use of dry woodland resources in Ethiopia).

Tefera is married with Asnakech Yohannes in 2001 and has three children. Tefera is a follower of the Ethiopian Orthodox Tewahido Church and likes to participate in church services. In academics, he is interested in teaching and research on issues related to restoration ecology, ecophysiology, and dry land resource management.



## *List of publications*

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1. Mengistu T. 2006. Frontier Community Valuation for Forest Patches: The Case of Wondo-Wosha subcatchment, Southern Nations, Nationalities and Peoples' Region, Ethiopia. **Ethiopian Journal of Natural Resources** 8: 281- 293.
2. Mengistu T., T. Demel, H. Håken and Y. Yonas. 2005. The role of enclosures in the recovery of woody vegetation in degraded dryland hillsides of central and northern Ethiopia. **Journal of Arid Environments** 60: 259-281.
3. Mengistu T., T. Demel, Y. Yonas and H. Håken. 2005. The role of communities in closed area management in Ethiopia. **Journal of Mountain Research and Development** 25: 44-50.



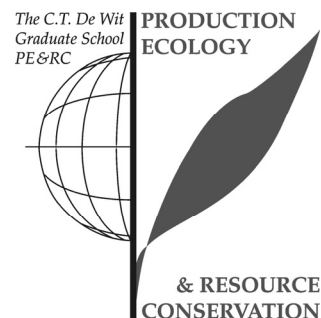


# Education Certificate

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## PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



### Review of literature (5.6 ECTS)

- The ecophysiology of plant carbohydrate production and allocation pattern

### Writing of project proposal (4.5 ECTS)

- Ecophysiological plasticity of *Boswellia papyrifera* (Del.) Hochst. in dry woodlands of Ethiopia under multiple stresses

### Post-graduate courses (7.9 ECTS)

- Multivariate analysis; PE&RC (2007)
- "What is up in tropical community ecology?"; PE&RC (2009)
- The legume-rhizobium symbiosis: from molecules to farmers; PE&RC/PPS (2010)
- Introduction to R; PE&RC (2010)
- Summer school "Rhizosphere signalling"; EPS (2010)
- Plant physiological ecology; Groningen University (2010)

### Laboratory training and working visits (4.1 ECTS)

- Photosynthesis and leaf water potential measurement techniques; Addis Ababa University (2007)
- Total non-structural carbohydrate determination using HPLC; WUR, EPS (2010)

### Deficiency, refresh, brush-up courses (2.8 ECTS)

- Ecological methodology 1 (2007)

### Competence strengthening / skills courses (2.1 ECTS)

- Scientific writing; Language services (2010)
- PhD Competence assessment; PE&RC (2010)

### PE&RC Annual meetings, seminars and the PE&RC weekend (2.4 ECTS)

- PE&RC Weekend (2007)
- PE&RC Day: multiple views in scales and scaling (2007)
- Scenario building for *B. papyrifera* management (2008)
- PE&RC Day: selling science (2010)
- Current PhD researches on tropical ecology (2010)

### Discussion groups / local seminars / other scientific meetings (3.9 ECTS)

- Physiology of flowering (2007)
- Popularization of *B. papyrifera* tapping techniques in Ethiopian (2008)
- Wood biology and dendro-chronology seminar; with presentation; Belgium (2009)
- Functional ecology and sustainable management of mountain forests in Ethiopia (2009)
- Tropical forest trees and climate changes; Utrecht University (2010)
- A world in transition; WUR-mini-symposium (2010)
- How to write a world class paper; WUR-mini-symposium (2010)
- Ecological theory and application discussion group (2010)
- Plant and soil interactions discussion group (2010 & 2011)

### International symposia, workshops and conferences (2.5 ECTS)

- Annual British Ecological Society Meeting; with presentation; Leeds (2010)
- Netherlands Annual Ecology Meeting: NAEM (2010)



## *The FRAME project*

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### **FRAME: Frankincense, Myrrh and gum arabic: sustainable use of dry woodland resources in Ethiopia**

More than half of the total land area in Ethiopia is covered by arid to semiarid woodlands with marginal agricultural potential. These woodlands are commonly overexploited for their natural resources, which reduces the local livelihood options for a rapidly expanding population. Climate change (e.g. drought) may intensify this negative trend. Consequently, there is an urgent need for improved land-use strategies that will make the vast arid and semiarid woodland resources optimally contribute to the livelihoods of local people and national development goals.

The dry woodlands in Ethiopia are not resource poor as they host several woody species that hold economically well recognized aromatic products such as gum arabic, frankincense and myrrh, which are widely used locally and in several of today's commercial industries such as cosmetic, pharmacological and food industries. Frankincense and myrrh are among the oldest internationally traded commercial tree products. Ethiopia is worldwide the main producer of frankincense and myrrh, and exports much gum arabic. Gum/resin production could significantly contribute towards sustainable development of these dry woodland areas. However, the overexploitation of natural resources by intensive grazing and intensive resin/gum harvesting and the lack of land management threatens the sustainability of the woody vegetation, and as a result of that also the long-term gum/resin production. Local communities may also enhance the productive capacity of the natural vegetation by establishing protected enclosures and by cultivation of trees. Such production systems may have a lower status regarding biodiversity and natural ecosystem functioning, but maintain ecological buffering capacity and improve production for human benefit.

The FRAME program addresses the following main research question: in what way dry land forests in Ethiopia can be made productive while maintaining ecosystem integrity in terms of sustainability of production and vegetation cover, with special attention to resin and gum resources?

FRAME uses a multidisciplinary approach involving scientific disciplines ranging from landscape-level geo-information studies to village-level socio-economic studies, plot level ecological and harvesting technology studies to tree-level ecophysiological studies with a strong contribution of local knowledge in answering the central research question. FRAME thus establishes a scientific basis for the sustainable management, including cultivation, of gum and resin yielding tree species and their habitat, the dry woodlands in the Horn of Africa. FRAME is actually involved in development of long-term scenarios for proper use and selection of suitable areas of dry woodland resources in Ethiopia.

The current PhD thesis is part of this FRAME program. A large part of this integrated FRAME research program was financially supported by NWO-WOTRO (Netherlands Organization for Scientific Research- Science for Global Development), grant W01.65.220.00.