Carbon and water fluxes from the European terrestrial biosphere:
Reducing uncertainty through combining monitoring with process based modelling

Ph. D. Thesis
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Carbon and water fluxes from the European terrestrial biosphere

Reducing uncertainty through combining monitoring with process based modelling

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To my family

If I could not be there, many times I have wished ye could be here
Si el futuro ya ha pasado,
Y el pasado ya no existe,
Solo nos queda...
Agraïments

It has been a pleasure. In the three and a half years I have spent at CREAF, I have really many reasons to be thankful. Many people and many experiences. I have never worked in such an agreeable environment, or had the opportunity to work with such inspiring people. I get the feeling that there are not many research institutes of its sort. If I ever go I will most likely come back, and if I ever come back I will probably never go.

First and foremost I would like to thank my supervisors: Santi Sabaté and Carlos Gracia, for their patience and understanding, and for giving me the liberty to explore.

My place in the GREENCYCLES project was made possible by Andrew Friend, who not only obtained the funding, but along with Soenke Zaehle, pointed out (to a very confused student) some of the more complicated and interesting things that could be done with field data. Raquel García was also a great help in getting to understand what happens when models meet data, and the three together helped lay the foundations of much of this thesis, for which I am very grateful. In fact, I really appreciate the inspiration I gained from all of the GREENCYCLES members, and the students at the QUEST summer school and the ERCA course.

Thanks also to the other inspirational researchers I have had the pleasure to work with, in particular to Josep Peñuelas, Ülo Niinemets and Reudiger Grote. Their encouragement and professionalism was key not only to my work, but also to feeling good about my work. I am not sure which is most important, but I was more than happy to receive both from them.

Last, but definitely not least were those who collaborated in spirit – from the CREAFeens and adopted CREAFeens, for their endless warmth and friendship, to the non-academic friends, half of whom still think modelling involves catwalks, flashes or toy train sets. In particular, to Lasse for shouting in the hallway and blaring classical
music, to Vincenz for giving the perfect example of calm (particularly in the face of shouting and blaring music), to Pep for taking me out every now and again for the odd drink and passing by the Sunday night sessions. To Leire, la Borrachina, Mireia, Rebeca, Helena and even Benja, wherever he may be. I am not capable of expressing how much you have all meant to me during these years (or at least not here!). If I left my family a long time ago, together you are the closest I have found since.

There were many ups and downs, from times when nothing seemed to make sense to times when sense made itself. After 3 years of banging my head against the wall, I think I have managed in the end to see some light through the cracks – and I have actually come to the conclusion that research, when seen in the right light, is actually a very nice thing to do.
“So close -- the infinitesimal and the infinite. But suddenly, I knew they were really the two ends of the same concept. The unbelievably small and the unbelievably vast eventually meet, like the closing of a gigantic circle.

...And in that moment, I knew the answer to the riddle of the infinite.

I had thought in terms of Man's own limited dimension.

I had presumed upon Nature.

That existence begins and ends is Man's conception, not Nature's.

And I felt my body dwindling, melting, becoming nothing.

My fears melted away, and in their place came -- acceptance.

All this vast majesty of creation -- it had to mean something.

And then I meant something too.”

The incredible shrinking man (1957)
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General Introduction
“The journey of a thousand miles begins with a single step”
Lao Tzu (~1570-490BC)
Chinese Philosopher
A Living Planet in Change

Every year, every day, every second – indeed, continuously, the Earth is breathing. Carbon, oxygen and water, cycle continuously between the atmosphere and the biosphere. Not only is it breathing carbon and oxygen, as we humans, but there are numerous biogeochemical cycles between the atmosphere, the oceans, and the terrestrial biosphere which constantly renew the vital elements necessary for life. Everything is connected in this huge dynamical system, and, over time, somewhat incredibly, a certain balance has been reached.

The atmosphere is of particular importance due to its crucial role in mediating all energy that enters and leaves earth. Overall, the atmosphere is the component that controls the dominant energy flow in the earth's climate system, and solar radiation from the sun provides the energy to make the weather machine work. Embedded in this process are the biogeochemical cycles that operate on a variety of temporal and spatial scales and help to regulate flows of energy and materials throughout the earth system.

The atmosphere is mainly made up of Nitrogen, Oxygen and various trace gases. These trace gases, although they make up only 1% of the total atmosphere, play very important roles in regulating life on earth, in particular, Carbon dioxide. Carbon is a key element of all life on earth, and has a complicated biogeochemical cycle of great importance to global climate change, contributing to about 50% of the total greenhouse gas forcing at present (Arrhenius, 1896; Ramaswamy et al., 2001). It is found in four main reservoirs of stored carbon: as carbon dioxide (CO₂) in the atmosphere; as organic compounds in living things; as dissolved carbon dioxide in the oceans; as calcium carbonate in limestone and in dead and buried organic matter (e.g. natural gas, peat, coal, and petroleum). Ultimately, the cycling of carbon through each of these reservoirs is tightly tied to living organisms.
With the evolution of the human species and, particularly, contemporary social trends of consumption, the stage has been reached where the global biogeochemical balance is being disturbed. The result of unregulated fossil fuel burning, high industrial activity, and widespread deforestation and land use change, has led to an increase in global atmospheric CO$_2$ concentrations, currently at levels which surpass anything experienced within the last 420,000 years (Petit et al., 1999; Falkowski et al., 2000). The global atmospheric CO$_2$ concentration has risen from 280 ppm in pre-industrial times to about 370 ppm in 2000 (McGuire et al., 2001). At the writing of this thesis (2009) the concentration has reached 386 ppm, which represents even a bigger increase than that predicted in the “worst case scenario” (A1fi) estimate made for 2009 by the International Panel on Climate Change in 2001 (IPCC, 2007). Atmospheric concentrations of CO$_2$ and other greenhouse gases continue to rise, and are expected to do so for the rest of the 21st century, mainly as a consequence of increasing anthropogenic fossil fuel use under a wide range of plausible scenarios of socio-economic development (Nakicenovic et al., 2000). Climate change calculations based on these scenarios suggest that the global mean temperature will most probably rise by about 2 °C relative to pre-industrial levels (Ramaswamy et al., 2001) due to the increase in atmospheric CO$_2$, though if current trends continue the global mean temperature is likely to rise even higher, with knock on effects on rainfall distribution and extreme weather events.

The terrestrial biosphere is currently thought to sequester up to 30% of the fossil-fuel based carbon emissions (Ciais et al., 1995; Prentice et al., 2001; House et al., 2003), the majority of which is due to forest ecosystems. The exact figure is unknown (House et al., 2003), nevertheless, global carbon fluxes from forest ecosystems have a substantial effect on the current growth rate of atmospheric CO$_2$. The future, however, is much less certain. A changing world can change and be changed by the terrestrial biosphere (Cox et al., 2000). The net flux from forest ecosystems is a delicate balance between carbon captured through photosynthesis, and carbon released through respiration. It is not known with certainty how these two compartments will respond to changing climatic conditions, and indeed, there is a lot left to discover about what drives their responses to current climatic conditions. This dynamic interaction makes it very difficult to predict...
future climatic conditions, and very important to increase our knowledge of the processes that govern the response of terrestrial vegetation to climate.

As terrestrial vegetation interacts with the atmosphere on a global scale through its role in the regulation of the atmospheric CO₂ concentration, it also interacts with the atmosphere on a local scale (Kanakidou et al., 2005). One of the most important interactions of terrestrial vegetation with the local atmosphere is through the emission of biogenic volatile organic compounds (BVOCs). These carbon based trace gases, emitted by most plant species, represent a heterogeneous compound class made up of a wide range of reactive volatile hydrocarbons.

The purpose of BVOC emissions is largely unknown (Peñuelas et al., 2004; Owen & Peñuelas, 2005), but the effect on atmospheric chemistry is well documented (e.g., Fuentes et al., 2000; Gelencser et al., 2007; Kanakidou et al., 2005; Szidat et al., 2006). They play a significant role in the formation of secondary organic aerosols and tropospheric ozone and are also thought to result in an increase of the lifetime of methane, a powerful greenhouse gas, in the troposphere (Poisson et al., 2000; Roelofs & Lelieveld, 2000). The emissions of BVOC have therefore far-reaching implications for air quality (e.g., Bell & Ellis, 2004), and thus human health, both globally and locally. The effects of biogenic emissions on methane lifetime and aerosols can further lead to important feedbacks between emissions and climate change (Kulmala et al., 2004; Peñuelas & Llusíà, 2003).

The forests ecosystems of the Earth dominate the biogeochemical fluxes and storage of trace gases from terrestrial vegetation. Terrestrial ecosystems contain about three times more carbon than is found in the atmosphere, with three quarters in the soil and the rest in the worlds forests. The vast majority of global BVOCs are emitted by tree species. Because of the thick humus layer, loose soil, and soil-retaining powers of the trees' long roots, forests are also vitally important for preserving adequate water supplies (Schröter et al., 2005). In addition, the forest provides shelter for wildlife, recreation and aesthetic renewal for people and irreplaceable supplies of oxygen and soil nutrients. In order to better understand their future, and their potential interactions with future climate, it is necessary to understand their responses to current climatic conditions.
The effects of changing climate on forest ecosystems has already been observed, most clearly in phenology (the timing of bud burst, leaf fall and animal migration), and is expected to continue (Gitay et al., 2001) and perhaps accelerate. Increased temperature and changes in precipitation patterns may reduce the capacity of the terrestrial biosphere to sequester carbon, or even lead to net carbon losses from the terrestrial biosphere thereby providing a positive feed-back to the climate system (Cox et al., 2000; Cramer et al., 2001; Friedlingstein et al., 2003).

For the mediterranean climate regions of the world, changes in temperature are expected to exceed the global average, with an overall decrease in precipitation (Giorgi et al., 2004; IPCC, 2007). As a result, hot summer periods and drought events in the Mediterranean basin are likely to increase in frequency (Giorgi, 2006; Beniston et al., 2007). This is of considerable importance given that water is already the main limiting factor to vegetative growth (Boyer, 1982) and a reduction in precipitation could lead to a decrease in water resources, and strong negative impacts on ecosystem integrity (IPCC 2007, Summary for Policymakers). Currently it is unknown exactly how plants react to soil water stresss, which makes future predictions of responses of terrestrial vegetation to climate subject to great uncertainty.
Forest Modelling: A General Introduction

Models give us a way by which to scale up our understanding of individual process reactions to drivers on a small scale (molecular or chemical) to the ecosystem scale (tree or stand), to the region and finally to the globe. The quantitative predictions have a large range of uncertainty, and are actually by no means predictions, but estimates. However, the qualitative descriptions and general trends of ecosystem responses give a very valuable insight into the functioning and potential response of the ecosystem as a whole.

In this introduction, we first outline the different approaches to forest eco-physiological modeling, with their associated pros and cons, and applications. Through the thesis, the process based ecophysiological model GOTILWA+ is used. Here, the structure and functioning of the model is outlined, and an example application to sites along a latitudinal transect in Europe is presented.

Process Based Models Vs. Empirical Models

There are two main approaches available to modellers: The empirical approach and the process based approach. The choice of approach taken is highly dependent on the problem being addressed. As always, both approaches have valid applications, each with their own strengths and weaknesses. In reality, the two options are not quite independent, with many models containing a synergy of the two approaches.

Empirical models attempt to simplify the system description, by relying purely on known system wide responses to external drivers. They are statistically based, are easy to feed (require less parameters) and generally have faster execution times. This is very useful, making it easy to build a simple and accurate description of a system with very few parameters. As their name suggests, they are based on empirical functions, which attempt to describe direct ecosystem responses. This simplicity and speed also helps in the analysis of model results, and is useful in giving insight into the general functioning of a system, highlighting the key processes and possible reactions. However the applicability of empirical models is restricted and their application as true exploratory
tools is questionable. Limited by their simplicity and their basis of empirical responses, they lack the ability to explore new scenarios and conditions outside of those on which they were built and tested.

Process based models, in contrast, are complex simulators that attempt to mimic the real world. The aim is to include mathematical descriptions of both the processes that govern a system, and their interactions, thus recreating the system in a virtual environment. Each process in the system is described separately, and dynamically interacts with other processes. Given an accurate description of each processes separately, it is argued that a better description of the ecosystem in general, through the interaction of these processes, can be achieved. Due to their detail, a large number of parameters are necessary. The parameters determine the response of each function describing an individual process, and are based on detailed field work or lab experiments. This allows an accurate description of all factors affecting a process, but such parameters are not always available. This can be a problem, and a lack of data often leads to assumptions and approximations, but the approach leads to a model with a wide applicability. The detail and dynamic characteristic of process based models allows them, theoretically, to function as effective exploratory tools and they should be fully applicable under new conditions and scenarios.

The scientific community is often somewhat sceptical about the effectiveness of complex process based simulation models, and the role they should play in ecological studies. Many ecologists will laugh if you explain that you are trying to mimic the real world. And indeed they might! The environment is highly variable, and could be said to be the most complex system in existence. However, complex process based models can have a much wider applicability than that of simpler empirical models that are simply designed to fit data. Although far more complicated than empirical models, and much more expensive to build, they give an insight into the internal functioning of the system itself, which could never be achieved with empirical models. For studies involving climate change, this is essential, as complex process are involved in ecosystem wide responses to global change. Unfortunately, our current understanding of many processes is still too limited to allow fully process based modelling, and most so called processed based models use a range of semi to fully empirical equations.
Using models to evaluating present and future terrestrial biogeochemical cycles

Modeling and monitoring the processes involved in trace gas fluxes are often thought to be independent events. In fact, rigorously validated modern modeling techniques are very useful tools in the monitoring of the carbon sequestration potential of an ecosystem through simulation, by highlighting key areas for study of what is a complex dynamical system. There are many possible applications of such models, from studying the responses of individual processes, the interactions of various processes, up to the responses of whole forest stands and ecosystems. This can be performed focusing on the response of forests to climate change (and in turn identifying feedbacks from forest ecosystem responses that may affect the rate of climate change), the effect of climate change on ecosystem service supplies which are necessary for societies well being (such as water supply, soil fertility and productivity), the effect of management on forest productivity, or in assessing the suitability of a certain site for plantation.

Forest Services

Modelling can prove a useful tool in assessing the expected future state of forest ecosystem services (e.g. water availability, soil fertility, wood production, fire hazard reduction etc.) that are vital for human well being. This is of particular interest in the light of climate change. Anthropogenically driven global change is continually altering such services, and is expected to do so to an even greater extent in the future. Previous Europe wide studies have applied terrestrial ecosystem models such as those described here to assess the expected future status of services such as soil fertility, water availability and the risk of forest fires (Schröter et al. 2005).

Applications in Forest Management

Forest management practices aim to optimise the productivity of the forest and minimise the risk posed by environmental stresses. The suitability of a management strategy is highly dependant on site characteristics and the general state of the forest stand. It is a difficult balance to achieve, where over-harvesting can lead to serious damage to a forest ecosystem, whilst under-harvesting can fail to make full use of the ecosystems potential, or indeed lead to a significant loss of aboveground biomass (e.g.
in the case of fire). Models allow for the evaluation of many alternative strategies, and the effectiveness of each can thus be tested based on the requirements of the manager. This is relevant both in the maximising of the potential for the ecosystem to sequester carbon, and in protecting ecosystems which are threatened by changing environmental conditions (with the aim to be to give the system more time to adapt naturally, and avoid threshold limits).
GOTILWA+: A process based ecophysiological forest model

GOTILWA+ (Growth of Trees Is Limited by WAter), (Gracia et al., 1999; Sabate et al., 2002), is a process based forest growth model that has been developed to simulate the processes underlying carbon and water fluxes from forest ecosystems and to explore how these processes are influenced by climate, tree stand structure, management techniques, soil properties and climate change. The GOTILWA+ model simulates carbon and water fluxes through photosynthesis, respiration and growth, for forests in different environments, for different tree species, and under changing environmental conditions, either due to climate or to management regimes.

Figure 1. A schematic graph of processes and interactions accounted for in the GOTILWA+ model (courtesy of S. Sabaté)

How GOTILWA+ copes with processes.

The processes in GOTILWA+ are described with different sub-models that interact and integrate the results of simulated growth and the evolution of the whole tree stand through time (hourly calculations integrated at a daily time step) (Fig. 1). The three
main compartments are the climate, the tree structure and the soil. Each interacts to varying degrees and is represented to a varying degree of mechanistic detail.

Process based models start at the very basic physiological leaf level, combining and describing the different processes involved. GOTILWA+ comprises of a two layer canopy photosynthetic model, coupled with a carbon allocation and growth model and a soil respiration and hydrology model. It describes monospecific stands, which can be even or uneven aged. The key environmental forcing factors taken into account are precipitation, air temperature, vapour pressure, global radiation, wind speed, and atmospheric carbon dioxide concentration. Using this data, the response of ecosystem processes is calculated to estimate the carbon and water fluxes in a forest ecosystem. It is an individual based model, where individual trees in the forest are grouped into 50 DBH (Diameter at Breast Height) classes, with calculations being performed separately for each class. Stand characteristics are taken into account and species specific parameters, to give highly accurate predictions of forest growth and carbon or water fluxes in the system.

Figure 2. A schematic diagram of the representation in GOTILWA+ of the photosynthetic assimilation rate.
One of the most fundamental compartments is the leaf (Fig. 2). Here, photosynthesis is calculated dynamically, based on internal and external conditions. Transpiration, photosynthesis, respiration, leaf internal CO\textsubscript{2} concentrations and leaf temperature are all coupled and interdependently driven by the canopy microclimate. The two layer canopy photosynthesis submodel splits the available leaf area index into sun and shade leaves, depending on the time of the day, leaf area angle and the canopy’s ellipsoidal distribution. Assimilation rates depend on the direct and diffuse radiation intercepted, the species specific photosynthetic capacities, leaf temperature, available carbon, the extent of stomatal opening, and the availability of soil water.

Growth and the allocation of mobile carbon for tree maintenance are considered through three compartments: Leaf respiration, Sapwood respiration, and Fine roots respiration. Fine litter fall (e.g. leaves), gross litter fall (e.g. bark, branches) and the mortality of fine roots add to the soil organic carbon content. The soil in GOTILWA+ is divided into two layers, an organic layer and a mineral layer, with a rate of transfer between them. Soil organic carbon is decomposed depending on to which layer it belongs, with both decomposition rates depending on a Q\textsubscript{10} function taking into account soil water content and soil temperature. Soil temperature is calculated from air temperature as the average of the past 11 days. The amount of soil water available for organic layers is calculated taking into account the cumulated rainfall of the previous 30 days and soil water availability for mineral layers depends on the soils water filled porosity which in turn is a function of the organic matter present in soil.

Soil water content is described as one layer, taking inputs though precipitation less leaf interception, which is evaporated, (stem interception, or stemflow, is not evaporated, but directed to the soil), and outputs though drainage, runoff, and transpiration. Surface evaporation only occurs when the canopy is not closed. Flux calculations are performed hourly, whereas slower processes such as growth and other state variables are calculated daily. Horizontal space is assumed homogeneous and the vertical profile distinguishes two canopy layers (sun and shade conditions).
Figure 3. A map of selected pixels with *Pinus sylvestris* stands in Europe along a latitudinal gradient.

*The future of European Forests – An example model application on a Latitudinal Gradient*

As an example, simulations at select sites along a latitudinal gradient (Fig. 3) are presented. Theoretically, any tree species can be simulated by the GOTILWA+ model, given the availability of species specific parameters. Here, we report on the modelling of *Pinus sylvestris*, as the predominant species along the gradient. Each site in Fig. 3 is the site of a current *Pinus sylvestris* stand. Site locations and climatic conditions are given in Table 1.
Table 1. Pixel climate details – Current Yearly Averages. Column Headings are: Lon – Longitude, Lat – Latitude, Q – Global Radiation (MJ m\(^{-2}\) yr\(^{-1}\)), P – Annual Precipitation (mm yr\(^{-1}\)), PET – Potential Evapotranspiration (mm yr\(^{-1}\)), Min T/Max T – Minimum/Maximum Temperature (\(\degree\)C), VP – Vapour Pressure (kPa), dP – Number of days with precipitation

<table>
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<th>Lat</th>
<th>Q</th>
<th>P</th>
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</table>

*Future Climate Data – A Multi GCM Ensemble*

Climate data is fundamental to any modeling study. For small scale site modelling, it is often locally available, from weather stations or measurements taken *in situ*. When moving to a bigger scale in time and space, large databases are available. The most commonly used climate record for the 20\(^{th}\) century comes from the Climate Research Unit (CRU) at Norwich. This is an extensive database of reconstructed climate from various sources (Mitchell *et al.* 2004).

The best tools available for predicting future climate evolution are Global Climate Models or General Circulation Models (GCMs). GCMs aim to describe climate behavior by integrating a variety of fluid-dynamical, chemical, or even biological equations that are either derived directly from physical laws (e.g. Newton’s Law) or constructed by more empirical means. A large number of GCMs exist for projecting future climate evolution. Each applies the laws of physics and mathematical descriptions of atmospheric interactions to varying degrees to give a projection for the evolution of future climate.

A range of socio-economic scenarios has been developed to explore future paths of carbon emissions related to the burning of fossil fuels. These can be used to force
GCMs. This approach is currently used by the IPCC (Inter-governmental Panel on Climate Change) is used as a driver for the GCMs, giving various possible future greenhouse gas emissions, depending on the economic model applied and the resulting changes in population, land use change and energy consumption. Four emissions scenarios are derived from the IPCC’s SRES1 (The global Intergovernmental Panel on Climate Change Special Report on Emissions Scenarios): A1 Fossil-Intensive, A2, B1, and B2, ranging from pessimistic to optimistic regarding future anthropogenic impact on the climate system.

A large difference exists between the projections of each of the GCMs, and each of the scenarios. They differ in: a) their climate sensitivity and b) the spatial pattern of change, making multi model assessments essential for a good understanding of potential changes.

Here a multi model ensemble was used to illustrate the inherent variability for future per pixel climate evolution. By using an ensemble, inter-model and inter scenario uncertainty can be assessed, and the effect of this uncertainty on terrestrial eco-physiological models can be gauged. Data from four GCMs were used, with each one qualified through its use by the IPCC Data Distribution Centre. The specific data used was compiled through the ATEAM (Advanced Terrestrial Ecosystem Analysis and Modelling, www.pik-potsdam.de/ateam) project, and ALARM project (Assessing Large-scale Risks for biodiversity with tested Methods, www.alarmproject.net/alarm).

Climate data from the following four GCMs were applied along the latitudinal gradient:

- The HadCM3 model from the Hadley Centre in England. (Mitchell et al., 1998)
- The NCAR-PCM model from the National Centre of Atmospheric Research, USA, which has the smallest sensitivity of all models compared to forcing at the global scale. (Washington et al., 2000)
- The CSIRO2 model from the Commonwealth Scientific and Industrial Research Organisation, Australia, which has above-average climate sensitivity, a little higher than HadCM3. (Flato & Boer, 2001)
- The CGCM2 model from the Canadian Centre for Climate Modelling and Analysis. (Laprise et al., 2003)
The multi model ensemble for the latitudinal gradient gives the mean projected values for the range of ecosystem indicators available, but also illustrates a measure of the uncertainty associated from the choice of climate model and scenario (Fig. 4). Although the climate models and scenarios vary in their projections under a given socio-economic scenario, they agree in qualitative terms and each ecosystem in this example is predicted to become a net source of carbon, thus constituting a positive feedback on the climate system. The amount of uncertainty in this projection is broken down into two categories: the uncertainty that derives from the choice of emissions scenario, and the uncertainty that derives from the choice of GCM to supply the climatic variables.

As can be seen from Fig. 4, the choice of which GCM to use accounts for almost as much variability as the choice of socio-economic scenario. That the variation due to the choice of emission scenario used is only slightly greater than that associated with the choice of GCM, highlights that important climate processes are imperfectly accounted for by these climate models. This is something that should be taken into account when considering any climate driven prediction for the future.
Figure 4. Left: The evolution of GOTILWA+ modelled annual values of Net Ecosystem Exchange at each site, with CRU climatology until 2000 and an ensemble of GCMs using the A2 Climate scenario, from 2000 to 2001. Right: The evolution of NEE at each site, with CRU climatology until 2000, and an ensemble of climate change scenarios with the HadCM3 GCM from 2000 to 2100. The grey lines represent the maximum and minimum range of NEE, and the blue line gives the 10 year running average of the maximum and minimum range.
Stand performance at the selected sites

Given the computational cost of performing simulations with an entire multi model ensemble, most studies choose a particular model and scenario. Here, we present examples from the HadCM3 model predictions with the A2 emission scenario as our description of future climate (this gives mid-range levels of future climate change). Fig. 5 shows the Gross Primary Production and Net Primary Production predicted by the model for each pixel of the transect using the HadCM3 models climatic variables. Here an increase in GPP can be observed, resulting from higher temperatures and CO₂ fertilisation. This trend is followed by all sites, except the more northern Spanish site, which suffers high mortality. This increase in GPP leads to only a slight increase in NPP, as higher production is balanced by higher respiration rates.

Figure 5. The GOTILWA+ projection of annual Gross Primary Production (GPP) and Net Primary Production (NPP) at each site, between 1960 and 2100, using HadCM3 – A2.
The Thesis: An Overview

General Overview

This thesis is designed to reduce uncertainty in modelling carbon and water fluxes from the terrestrial biosphere, through challenging key weak points in our current knowledge, from leaf scale processes to the calculation of fluxes on regional scales, both in the present and under potential future climate change. We focus on two key issues of uncertainty: 1) The understanding of leaf and canopy level responses of photosynthesis and transpiration to natural cycles of soil water stress as encountered in the field, focusing on the processes involved and our ability to model them, and 2) Species specific BVOC emissions from European forest canopies, focusing on the comparison of predictions from various modelling approaches, both under present conditions and under projected future climate change.

Specific Overview

Chapter 1: Improved understanding of drought controls on seasonal variation in Mediterranean forest canopy carbon and water fluxes through combined in situ measurements and ecosystem modelling.

Water stress is the main limitation to plant growth in the Mediterranean. Such uncertainty in our knowledge of leaf level responses to seasonal drought, and how to model them, is a large source of error when modelling in Mediterranean-type forest ecosystems. In this chapter, we address this problem from the pragmatic perspective of determining the most straightforward additional parameterisations to include in the models in order that they accurately reproduce observed seasonal cycles of carbon and water fluxes across a range of different forest ecosystem types. Using observations of carbon and water fluxes made over four different Mediterranean forest ecosystems, we investigate the relationships between the fluxes and calculated seasonal soil moisture variations. These data are further analysed to determine relative stomatal and non-stomatal limitations to photosynthesis. Simulations are performed using two contrasting
forest models at each of the four sites in order to test the hypothesis provided by the
data analysis and to evaluate modelled responses of photosynthesis and conductance to
changes in soil water.

Chapter 2: The importance of mesophyll conductance in regulating forest
ecosystem productivity during drought periods.

In this chapter, we further explored forest canopy responses to soil water stress,
examining the potential role of mesophyll conductance. We used data from multiple
FLUXNET (http://daac.ornl.gov/FLUXNET/) sites to quantify the role of both stomatal
conductance and mesophyll conductance in regulating forest ecosystem productivity
during drought periods. Both bulk canopy stomatal and mesophyll conductance were
calculated from the FLUXNET data, using an inversion of the McNaughton and Black
equation (McNaughton & Black, 1973), the Harley variable J method (Harley et al.
1992), and an ecophysiological process based photosynthesis-conductance model
coupling (Farquhar et al. 1980; Leuning et al., 1995) together with a canopy distribution
model (Campbell, 1986; Dai et al., 2004; Keenan et al., 2009a), thus describing foliar
photosynthesis and conductance’s as distributed through the canopy. The resulting
responses of the bulk canopy leaf intercellular and chloroplast carbon pool
concentrations to changes in soil water availability were thus assessed. A quantitative
analysis (Grassi & Magnani, 2005) allowed for the relative soil water related responses
of stomatal limitations, mesophyll conductance limitations and biochemical limitations
to productivity to be gauged.

Chapter 3: Mesophyll conductance, soil water stress and coupled photosynthesis-
conductance models: Bridging the gap between conflicting reports on the relative
roles of stomatal, mesophyll conductance and biochemical limitations to
photosynthesis.

In this chapter, different hypothesis on the role of stomatal conductance, mesophyll
conductance and biological limitations to photosynthesis during water stressed periods
are tested, reflecting the different limitations reported in chapter 2. We modify the
traditional Farquhar et al. (1980) model to incorporate mesophyll conductance, $C_c$, and
thus to calculate the subsequent rates of photosynthesis on a $C_c$ basis. This is coupled to
the widely used Leuning et al. (1995) version of the Ball-Berry conductance model (Ball, Woodrow, and Berry 1987), and embedded in the biogeochemical forest growth model GOTILWA+ (Gracia et al. 1999; Keenan et al. 2009; www.creaf.uab.es/GOTILWA+). The effectiveness of modelling canopy level photosynthesis based on the concentration of carbon in the chloroplast is examined with particular attention paid to water stressed periods. Different hypothesis are tested, challenging current assumptions regarding the relative roles of $SC_L$, $MC_L$ and $B_L$ to photosynthesis and conductance, and how to model them, during seasonal water stressed periods in the field. Carbon and water eddy-covariance flux data from the five year period from 2001 to 2005 at the Puechabon Mediterranean Quercus ilex forest site in France are used to asses the results.

Chapter 4: Process based estimates of isoprenoid emissions inventory from European forests: a model comparison, current knowledge and uncertainties.

Most forest species emit trace gases known as Volatile Organic Compounds. These VOCs are involved in many chemical reactions in the atmosphere, and are important factors in local air pollution, ozone formation and the development of clouds. Different methods exist for predicting forest canopy VOC emissions, but they are subject to large variation and have never been compared on a canopy or regional level. In this chapter, we address these uncertainties by embeding three different isoprenoid emission models into a process based terrestrial biogeochemical model, thus providing a bottom up approach both to quantify the isoprene and monoterpenes emissions being released into the atmosphere under present day and potential future climate change. With these new tools we tested the possible variability due to differences in leaf level models. For this, isoprene emission flux measurements were used to compare diurnal and seasonal emission predictions at two forest sites with contrasting forest structure and species composition, one in the south of France and the other in Michigan, USA. We further revised the emission potentials for 80 dominant European forest species both occurring naturally and in forest plantations. Using these basal emission potentials we derive an emission inventory for both isoprene and monoterpenes emissions from European forests under current climatic conditions using the three different modelling approaches. Finally, European forests were used as an example to analyse and compare large scale
model performance of the different modelling approaches under future climatic conditions.

Chapter 5: Seasonality of monoterpene emission potentials in *Quercus ilex* and *Pinus pinea*: Implications for regional BVOC emissions modeling

The overall uncertainty in our knowledge of the drivers of seasonal dynamics of BVOC emissions, and how to model them, is potentially a large source of error when modeling isoprenoid emissions from terrestrial vegetation. In this chapter, we addressed the problem of seasonal dynamics of monoterpene emission potentials by developing seasonal response functions for two key species in Mediterranean forest ecosystems: the broadleaved evergreen sclerophyll *Quercus ilex* and the evergreen conifer *Pinus pinea*. The response functions were integrated into the Niinemets *et al.* isoprenoid emission model coupled to the process-based terrestrial biogeochemical model GOTILWA+. Simulations were run for these two dominating species over the European Mediterranean region to quantify the effect of the consideration of a seasonally dynamic emissions potential on the total emissions budget for these two species. The simulations were further compared with simulations coupling the commonly used Guenther *et al.* model development, MEGAN, both with and without its seasonal modifications of emissions, to the GOTILWA+ model.
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General Introduction


Chapter 1

Drought Controls on Forest Canopy Carbon and Water Fluxes in the Mediterranean
Improved understanding of drought controls on seasonal variation in Mediterranean forest canopy carbon and water fluxes through combined in situ measurements and ecosystem modelling

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Abstract

Water stress is a defining characteristic of Mediterranean ecosystems, and is likely to become more severe in the coming decades. However, our current understanding of how soil moisture controls ecosystem functioning is not sufficient to adequately constrain model parameterisations.

Canopy-scale flux data from four Mediterranean forest ecosystems were analysed in order to determine the changing controls on carbon and water fluxes through the year. Stomatal and non-stomatal limitations on photosynthesis were separated, and new parameterisations were derived and implemented in two independent modelling approaches.

The effectiveness of the two approaches to ecosystem process-based modelling, one a dynamic global vegetation model (ORCHIDEE), and the other a forest growth model
(GOTILWA+), was assessed, and modelled canopy responses to seasonal changes in soil moisture were analysed with respect to \textit{in situ} flux measurements.

In contrast to commonly held assumptions, we find that stomatal control does not dominate photosynthesis under natural seasonally-developing soil moisture stress. However, applying a soil moisture dependency to photosynthetic capacity results in accurate prediction of both carbon and water fluxes under all soil moisture levels encountered in the field. The new parameterisation has important consequences for simulated responses of carbon and water fluxes to seasonally varying soil moisture stress, and will greatly improve our ability to anticipate future impacts of climate changes on the functioning of Mediterranean ecosystems.
1.1 Introduction

The Mediterranean region contains a highly distinctive endemic flora and is characterised by warm wet winters and hot dry summers, with ecosystem functioning dominated by the seasonal cycle of water availability (Allen, 2001). Soil water availability is the main limiting factor vegetation growth in the Mediterranean (Boyer, 1982) and changes in regional temperatures and precipitation are placing increasing stresses on these vulnerable ecosystems. Climate models predict further increases in temperature, and changes in rainfall patterns in this region in the future (Giorgi et al., 2004; Giorgi, 2006), leading to a decrease in water resources and negative impacts on ecosystem integrity (IPCC 2007, Summary for Policymakers).

However, our ability to predict with confidence the impacts of changing climate on these ecosystems is poor due to a lack of understanding concerning ecophysiological responses to soil moisture stress and consequent effects on the cycling of carbon and water (Loreto and Centritto, 2008). This is confounded by the uncertainty concerning the role of Mediterranean forests as either sinks or sources of CO₂ due to wide variation in published data and their interpretation, particularly with respect to inventory-based estimates (Schimel, 1995). Correctly understanding current, and thus future, land-atmosphere exchanges of water and carbon in the Mediterranean region is essential for predicting water resource availability and the future functioning of ecosystems, which provide services such as the production of raw materials, sequestration of carbon (Schröter et al., 2005), and numerous recreational benefits.

A thorough assessment of the vulnerability and likely future development of Mediterranean ecosystems requires the application of climate-driven, process-based models. Such models should incorporate the relevant ecosystem processes to successfully simulate the sensitivity of ecosystem functioning to soil moisture stress at all relevant time scales. However, existing models consistently perform worse for Mediterranean ecosystems than in any others, mainly due to difficulties in reproducing the effects of seasonal droughts on carbon and water fluxes (Krinner et al., 2005;
Morales et al., 2005; Reichstein et al., 2007; Jung et al., 2007). This study is a response to this challenge.

A dominant approach to modelling stomatal conductance is to use a linear relationship with leaf- or canopy-level photosynthesis, the so-called “Ball-Berry” (BB) and “Ball-Berry-Leuning” (BBL) parameterisations (Ball et al., 1987; Leuning et al., 1995; Field et al., 1995). Such relationships underpin the coupling of water and carbon cycles in many process-based ecosystem models. Recent studies have suggested, however, that such an empirical relationship does not hold under conditions of soil moisture stress (Reichstein et al., 2003; Misson et al., 2006). The most appropriate manner in which to incorporate a moisture stress response into the BB or BBL photosynthesis model coupling is not at all clear. The effect of water stress on plant photosynthesis and stomatal conductance has been widely studied (Wilson et al. 2000; Chaves et al., 2002), but there is little consensus as to which processes govern responses over seasonal time-scales (Warren, 2008). The most widely accepted hypothesis for the control on photosynthesis during water-stressed periods is a reduction in the supply of CO₂ to the carboxylation sites through stomatal closure (e.g. Chaves et al., 2002).

Short-term studies suggest that stomatal conductance during water stress can be effectively included in the BB and BBL (BB-type) models by reducing the ratio of conductance to photosynthesis with increasing soil moisture stress (Tenhunen et al., 1990; Harley and Tenhunen, 1991; Sala and Tenhunen, 1994). Both models also take into account a residual, or base conductance level, which has also been reported to change under water stress (Misson et al., 2004). Such functions imply that only stomatal aperture is affected by soil moisture levels, whereas there is substantial evidence that photosynthetic capacity is directly affected by soil moisture (Medrano et al. 1997; Parry et al., 2002). The importance of each process on different timescales has been the subject of much debate (Lawlor, 1995; Tezara et al. 1999; Cornic 2000; Lawlor and Cornic 2002; Flexas and Medrano 2002). Stomatal conductance and photosynthetic capacity tend to be closely correlated (Wong et al., 1979; Hetherington and Woodward, 2003), leading to the suggestion that it is in fact photosynthetic activity that determines stomatal aperture in order to maintain biochemically optimal rates of CO₂ supply (Flexas and Medrano, 2002; Galmes et al., 2005).
There has been much discussion concerning the relative roles of stomatal and non-stomatal limitations during drought periods (e.g. Jones, 1985; Ni and Pallardy, 1992; Kubiske and Adams, 1993; Wilson et al., 2000). The existence of non-stomatal limitations to photosynthesis have been detected under a variety of conditions (Wilson et al., 2000; Grassi and Magnani, 2005), which have been hypothesised to relate to either changes in photosynthetic capacity (Medrano et al. 1997; Parry et al., 2002), or the conductivity of the mesophyll cell walls to CO₂ (Warren, 2008). These studies give weight to a number of studies showing that non-stomatal limitations come into play during seasonal soil water stress (e.g. Colello et al., 1998; Xu and Baldocchi, 2003). However, conflicting results, often depending on the strength and duration of the water stress, have resulted in great uncertainty as to which limitation is strongest under natural water-stressed conditions (e.g. Lawlor, 1995; Tezara et al., 1999; Lawlor and Cornic, 2002; Flexas and Medrano, 2002; Breda et al., 2006).

Such uncertainty in our knowledge of leaf level responses to seasonal drought, and how to model them, is a large source of error when modelling in Mediterranean-type forest ecosystems. In this study, we address this problem from the pragmatic perspective of determining the most straightforward additional parameterisations to include in the models in order that they accurately reproduce observed seasonal cycles of carbon and water fluxes across a range of different forest ecosystem types. Using observations of carbon and water fluxes made over four different Mediterranean forest ecosystems, we investigate the relationships between the fluxes and calculated seasonal soil moisture variations. These data are further analysed to determine relative stomatal and non-stomatal limitations to photosynthesis.

The understanding derived from the observations is then incorporated into two independent process-based ecosystem models: GOTILWA+, a biogeochemical forest growth model (Gracia et al., 1999; Keenan et al., 2008; www.creaf.uab.es/GOTILWA+), and ORCHIDEE, a dynamic global vegetation model (Krinner et al., 2005; http://ORCHIDEE.ipsl.jussieu.fr). Simulations are performed at each of the four sites in order to test the hypothesis provided by the data analysis and to evaluate modelled responses of photosynthesis and conductance to changes in soil water.
1.2 Materials and Methods

1.2.1 FLUXNET Site Data and Data Manipulation

Measurements of ecosystem carbon and water fluxes were obtained from the FLUXNET database (www.fluxnet.ornl.gov) for three sites in Mediterranean Europe (Puechabon, France; Roccarespampani, Italy; Collelongo, Italy.), maintained under the CarboEurope-EUROFLUX project (Allard et al., 2008; Kowalski et al., 2004; Valentini et al., 1996), and one site at a location with a mediterranean-type climate in California (Blodgett, California), maintained under the AMERIFLUX project (Goldstein et al., 2000). Together, these data from the four sites amount to a total of eleven measurement years (Table 1). These sites cover a range of species, including temperate broadleaf deciduous, temperate needleleaf evergreen, and temperate broadleaf evergreen types, and varying levels of water stress in summer. They include the dominant species Quercus ilex, Quercus cerris, Fagus sylvatica, and Pinus ponderosa. FLUXNET datasets include measurements of carbon dioxide and water fluxes at half-hourly time steps (Wofsy et al., 1993). We used the level-4 datasets, in which flux separation techniques for splitting the observed net carbon fluxes into assimilation and respiration have been employed (Reichstein et al., 2005). Gap-filled data were excluded.
Table 1. Characteristics of the FLUXNET sites chosen. Plant functional types (PFTs) considered are temperate broadleaved evergreen (TeBE), needleleaved evergreen (TeNE) and broadleaved-summergreen (TeBS). Max LAI - Maximum Leaf Area Index; SD – Soil Depth; SWHC – Soil Water Holding Capacity

<table>
<thead>
<tr>
<th>Site</th>
<th>Period</th>
<th>Longitude</th>
<th>Latitude</th>
<th>Altitude (m)</th>
<th>Max LAI (m²/m²)</th>
<th>SD (m)</th>
<th>SWHC (Kg/m²)</th>
<th>Species/PFT</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Puéchabon, France</td>
<td>2002-2004</td>
<td>3º 35’</td>
<td>43º 44’</td>
<td>270</td>
<td>2.9 - 3.2</td>
<td>4.5</td>
<td>172</td>
<td>Quercus ilex (TeBE)</td>
<td>Allard et al. (2008)</td>
</tr>
<tr>
<td>Roccarespampani, Italy</td>
<td>2003-2004</td>
<td>11º 55’</td>
<td>42º 23’</td>
<td>223</td>
<td>4.0 - 5.0</td>
<td>4.5</td>
<td>485</td>
<td>Quercus cerris (TeBS)</td>
<td>Kowalski et al. (2004)</td>
</tr>
<tr>
<td>Collelongo, Italy</td>
<td>1998-1999</td>
<td>13º 35’</td>
<td>41º 50’</td>
<td>1560</td>
<td>4 – 5.5</td>
<td>4</td>
<td>287</td>
<td>Fagus sylvatica (TeBS)</td>
<td>Valentini et al. (1996)</td>
</tr>
<tr>
<td>Blodgett, California</td>
<td>2001-2004</td>
<td>-120º 37’</td>
<td>38º 53’</td>
<td>1315</td>
<td>2.4 – 4.2</td>
<td>4</td>
<td>583</td>
<td>Pinus ponderosa (TeNE)</td>
<td>Goldstein et al. (2000)</td>
</tr>
</tbody>
</table>
1.2.1.1 Inverting soil moisture, canopy conductance, and leaf inter-cellular CO₂ concentration from FLUXNET data

**Soil moisture**

Interpreting the responses of observed carbon and water fluxes to changes in soil moisture requires the seasonal evolution of soil water content to be known. In the absence of direct measurements in the FLUXNET dataset, daily soil moisture content at each site was reconstructed using a simple water balance model through inverting the measured latent heat flux, and determining the balance with inputs from precipitation and outputs to run-off (calculated as a function of leaf area index and rainfall intensity) and below-ground drainage (calculated as in Gracia et al., 1999 and Honeysett and Ratkowsky, 1989).

**Canopy conductance and leaf inter-cellular CO₂ concentration**

Canopy conductance is the bulk leaf-surface conductance to water vapour, expressed on a ground-area basis. It can be estimated from observed latent heat flux under dry canopy conditions and when soil evaporation is negligible. We inverted the McNaughton and Black equation for canopy latent heat flux (McNaughton and Black, 1973) to estimate bulk canopy conductance, \( Gc \):

\[
Gc = \frac{LH \cdot \varepsilon \cdot \gamma}{(\rho \cdot Cp \cdot vpd)}
\]  
(Eq. 1.1)

where \( LH \) is the observed latent heat flux (W m\(^{-2}\)), \( \varepsilon \) is the coefficient for the conversion of latent heat to its water equivalent (giving actual evapotranspiration \( (E_a) \)), \( \lambda \) is the latent heat of vaporisation of water (2.27 MJ kg\(^{-1}\)), \( \gamma \) is the psychrometric constant (0.66 kPa °K\(^{-1}\)), \( \rho \) is the density of air (kg m\(^{-3}\)), \( Cp \) is the heat capacity of air (1012 J kg\(^{-1}\) K\(^{-1}\)), and \( vpd \) is the observed vapour pressure deficit (Pa).
Using the estimated bulk canopy conductance to carbon $G_{c_{CO2}} (= Gc/1.6)$, observed rates of net photosynthesis from the eddy-covariance measurements, and atmospheric CO$_2$ concentrations, canopy bulk leaf intercellular CO$_2$ concentration ($C_i$) can be calculated using the simple supply and demand algorithm:

$$C_i = C_a - \left(\frac{A_n}{Gc_{CO2}}\right) \quad \text{(Eq. 1.2)}$$

As all measurements used in the calculation of bulk conductance have reference above the canopy, boundary layer effects are assumed to be minimal for the calculation of bulk $C_i$.

### 1.2.1.2 Relationships between bulk canopy conductance, canopy photosynthesis, and soil moisture stress derived from the flux measurements

**Bulk Canopy conductance and soil moisture stress**

The BBL parameterisation of stomatal conductance assumes linear correlations with net photosynthesis and increasing evapotranspiration:

$$G_c = G_{s0} + \left(\frac{m.A_n}{(C_a - \Gamma^*)\cdot(1 + (vpd/D_0))}\right) \quad \text{(Eq. 1.3)}$$

where $G_c$ (mol H$_2$O m$^{-2}$ s$^{-1}$) is bulk canopy conductance to moisture, $G_{s0}$ is the value of $G_c$ at the light compensation point (mol m$^{-2}$ s$^{-1}$), $A_n$ is the rate of net photosynthesis ($\mu$mol m$^{-2}$ s$^{-1}$), $C_a$ is the atmospheric concentration of CO$_2$ at the canopy surface ($\mu$mol mol$^{-1}$), $\Gamma^*$ is the photorespiratory compensation point ($\mu$mol mol$^{-1}$), $D_0$ (unitless) is an empirical coefficient that describes the sensitivity of conductance to $vpd$ (Pa) at the canopy surface, and $m$ is an empirical species specific factor that specifies the baseline ratio between conductance and net photosynthesis (unitless). This is a leaf level model applied at the canopy scale through the use of bulk canopy level variables (Leuning et al., 1995). Using a bulk parameterisation (with one canopy layer) allows for the direct empirical parameterisation of $m$ and $G_{s0}$. 

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This parameterisation does not include a dependency on soil moisture, although conductance will likely fall to some extent during hot dry periods through the effect of increased vapour pressure deficits. To explain the full extent of stomatal closure during moisture stress periods, an empirical dependency of the $m$ or $G_{s0}$ parameter on soil moisture is sometimes employed. This can effectively reduce conductance but any reduction in photosynthesis is then only due to reduced leaf $C_i$ concentrations. We estimated this empirical dependency from Eq. 3. Given $G_c$, the two unknowns in Eq. 3 are $m$, and $G_{s0}$. We therefore calculated $m$, and $G_{s0}$ for each site by applying linear regression to Eq. 3, thus obtaining the slope and intercept of the linear relation. This was performed for each site using data selected as outlined in Section 2.1.3. These values were calculated at different soil water levels to assess possible responses to soil water availability.

**Non stomatal conductance related limitations of photosynthesis due to soil water stress**

Evidence for any reductions in photosynthetic capacity under soil moisture stress was assessed by comparing rates of photosynthesis at different soil moisture contents but over a restricted range of leaf $C_i$ concentrations ($220 < C_i < 300$), radiation (> 250 W m$^{-2}$), and temperature (< 25 °C). Any differences in rates of assimilation under otherwise non-limiting conditions could then be attributed to non-stomatal soil water effects, rather than changes in the rate of supply of CO$_2$ to the intercellular spaces, light limitation, or any temperature effects.

**1.2.1.3 Data selection**

All analysed FLUXNET data was first screened to remove night time values and gaps in the original data. Only daytime values were considered by selecting data corresponding to half-hours with radiation of 200 W m$^{-2}$ or greater, and assimilation rates of 2 μmol CO$_2$ m$^{-2}$ s$^{-1}$. Screening was also performed to remove data points measured during, or close to, precipitation events, and extreme temperatures (below 5 ° or above 35°).
1.2.2 Ecosystem Models

1.2.2.1 GOTILWA+

GOTILWA+ ("Growth Of Trees Is Limited by WAter"), (Gracia et al., 1999; Keenan et al., 2008; www.creaf.uab.es/GOTILWA+), is a process-based forest growth model that has been developed to simulate tree growth and to explore how it is influenced by climate, tree stand structure, management techniques, soil properties, and climate (including CO₂) change. GOTILWA+ simulates carbon and water fluxes within forests in different environments, for different tree species, and under changing environmental conditions, either due to climate or to management regimes.

The model treats monospecific stands which can be even- or uneven-aged. Individual trees are aggregated into 50 DBH (Diameter at Breast Height) classes and all calculations are performed for each class. Ecosystem carbon and water fluxes are estimated using hourly meteorological forcing.

GOTILWA+ includes a two-layer canopy photosynthetic model (Wang and Leuning, 1998), coupled to a carbon allocation and growth model and a soil respiration and hydrology model. The photosynthesis sub-model treats the C₃ photosynthetic pathway. Leaves in each layer are split into sun and shade leaves, with intercepted radiation depending on the time of the day, and the area of leaf exposed to the sun based on leaf angle and the canopy’s ellipsoidal leaf distribution. Assimilation rates are calculated using the approach of von Caemmerer and Farquhar (1981), with dependencies on intercepted direct and diffuse radiation, species-specific photosynthetic capacities, leaf temperature, and the available leaf Ci concentration. Stomatal conductance is calculated using the BBL model.

Each tree cohort is represented by three carbon compartments, leaf, sapwood, and fine roots. Labile carbon is allocated to each, and maintenance respiration is calculated as a function of temperature. Fine litter fall (i.e. leaves), gross litter fall (i.e. bark, branches) and the mortality of fine roots add to the soil organic carbon content. The soil in GOTILWA+ is divided into two layers, an organic layer and a mineral layer, with a rate of transfer of soil organic carbon between them. Soil water content is described as in
Section 2.1.1. Flux calculations are performed hourly, whereas slower processes such as growth and other state variables are calculated daily.

1.2.2 ORCHIDEE

ORCHIDEE is a process-based terrestrial biosphere model that simulates terrestrial vegetation and soil energy, water, and carbon fluxes (Krinner et al., 2005). Changes in vegetation structure and distribution in response to environmental parameters are also simulated.

ORCHIDEE consists of three major components: (a) SECHIBA, which calculates the exchanges of energy and water between the atmosphere and the land surface; (b) STOMATE, which simulates photosynthesis, carbon allocation, litter decomposition, soil carbon dynamics, maintenance and growth respiration and (c) the LPJ dynamic vegetation model (Sitch et al., 2003), which simulates long-term changes in the composition and structure of vegetation resulting from sapling establishment, light competition, and tree mortality. Energy, water, and carbon fluxes resulting from photosynthesis and autotrophic and heterotrophic respiration are calculated on a half-hourly basis, whereas plant growth, phenology, and vegetation structure occur on a daily timestep.

Vegetation in ORCHIDEE is classed into plant functional types with different phenological, physiological and morphological characteristics; temperate needle-leaved evergreen, temperate broadleaved evergreen, temperate broadleaved deciduous, and C₃ herbaceous types are used in this study. In contrast to GOTILWA+, vegetation is represented as an average individual plant, with no accounting for size-distribution effects.

ORCHIDEE simulates photosynthesis for both C₃ (using the method of Farquhar et al., 1980) and C₄ (using the method of Collatz et al., 1992) photosynthetic pathways, with stomatal conductance calculated using the BB model. Soil water content is treated in two layers, with inputs from precipitation less canopy interception loss, and outputs to drainage, runoff, soil evaporation, and transpiration.
1.2.2.3 The effect of soil water stress on coupled photosynthesis-conductance models

The original GOTILWA+ and ORCHIDEE models applied very different strategies to model responses to drought stress. Two common approaches are used to incorporate the effect of drought stress on the coupled photosynthesis-conductance model. The first limits photosynthesis through stomatal limitations by applying a direct reduction of stomatal conductance during drought stress (Sala and Tenhunen, 1996) as in Eq. 1.4 (a modified version of Eq. 1.2), thus limiting the carbon available for photosynthesis:

\[
G_s = G_{s0} + (W_{fac_{stoma}}m)(A_n - R_d)/(C_n - \Gamma^*)(1 + (vpd/D_o)) 
\]  
(Eq. 1.4)

where \(W_{fac_{stoma}}\) is a soil moisture dependent scalar with values between 0 and 1.

This was the approach used in original GOTILWA+ model. In GOTILWA+, the stomata played the dominant role in the control of photosynthetic responses to drought stress, through the application of a linear scalar of soil moisture, as in Eq. 1.4, to reduced conductance in line with reductions in soil moisture.

The second approach used to incorporate the effect of drought stress on coupled photosynthesis-conductance models is that of non-stomatal limitations. Non-stomatal limitations are simulated by applying a reducing function \(W_{fac_{photo}}\) to photosynthetic potential as follows:

\[
V_{c max} = V_{c max}^* W_{fac_{photo}} \\
J_{max} = J_{max}^* W_{fac_{photo}} 
\]  
(Eq. 1.5)

Where \(V_{c max}\), and \(J_{max}\) are the maximum rate of RuBP carboxylation, and the maximum rate of electron transport, respectively.

This was the approach used in the original ORCHIDEE, where soil moisture was assumed to directly influence photosynthetic capacity during drought stress. At soil water values of below 50% maximum soil water content, the potential rates of
carboxylation, $V_{cmax}$, and electron transport, $J_{max}$, were linearly reduced as suggested by McMurtrie et al. (1990).

The form of the reduction function applied varies between studies (McMurtrie et al., 1990; Sala and Tenhunen, 1996; Reichstein et al. 2002; Dufrene et al., 2005). In order to remove variability due to the application of different reduction functions in the two models, we incorporated the same function in both GOTILWA+ and ORCHIDEE. Here we investigate the effectiveness of a simple non-linear reduction function (Eq. 1.6), derived from the flux data for both stomatal and non-stomatal limitations independently:

$$ W_{fac} = \begin{cases} 1, & \text{if } S(t) \geq S_{max} \\ \left( \frac{s(t) - s_{min}}{s_{max} - s_{min}} \right)^q, & \text{if } S(t) < S_{max} \end{cases} \quad (\text{Eq. 1.6}) $$

where $q$ is a measure of the non-linearity of the effects of soil water stress, $s_{max}$ the point at which reductions are first evident, and $s_{min}$ is the minimum soil water content. In order to assure that this accurately represents the response for both stomatal and non-stomatal limitations ($W_{facstoma}$ and $W_{facphoto}$), these parameters were estimated independently for each site. These functions were then tested in each model to identify the most effective approach. We tested 3 hypotheses: 1) applying stomatal limitations ($W_{facstoma}$) sufficiently constrains carbon and water fluxes during drought stressed periods, 2) applying non-stomatal limitations ($W_{facphoto}$) sufficiently constrains carbon and water fluxes during water stressed periods, and 3) the application of both stomatal and non-stomatal limitations is necessary to accurately reproduce the flux data.

1.2.2.4 Modelling protocol and model evaluation

Simulations with each model were run separately at each of the four sites to test the effectiveness of the different approaches to modelling drought stress responses. For each model and site, 4 runs were made with: 1) with the models original water stress response function, 2) the application of stomatal limitations ($W_{facstoma}$), 3) the
application of non-stomatal limitation (Wfac\textsubscript{photo}), and 4) the application of both stomatal and non-stomatal limitations together.

Both models were run using the same half-hourly meteorological variables (temperature, precipitation, vapour pressure deficit, wind speed, global radiation, and atmospheric CO\textsubscript{2} concentration), which have been taken from site observations, and site conditions including soil characteristics and hydrological parameters (Table 1). The conductance parameters, \( m \) and \( G_{s0} \) were calculated from the data (Table 2). In addition to the above variables required by the two models, GOTILWA+ requires descriptions of stand characteristics (including the structure of the canopy and the DBH class distribution), and also some tree physiological parameters (biomass allocations and compartment specific growth and maintenance respiration rates).

GOTILWA+ simulations were initialised by specifying forest structure parameters specific to the first year of simulation at each site, with data obtained from the literature. ORCHIDEE simulations were initialised by prescribing the cover of each plant functional type for each site. ORCHIDEE was spun up to equilibrium values repeating the climate inputs for 100 years. After this spin-up, ORCHIDEE was driven by half-hourly values over the time periods considered at each site.

To allow for the evaluation of the canopy physiological process description independent of potential inaccuracies in the modelled latent or sensible heat fluxes, which would induce errors in simulated soil moisture values, the soil water contents in the models were controlled artificially by fixing hourly values to those given by the reconstruction. This decoupling of the simulation of soil and canopy processes was achieved by removing the water volume equivalent of the observed latent heat flux at each time step instead of the simulated evapotranspiration.

**Statistics**

The models were evaluated using the correlation coefficient \( r^2 \), the Root Mean Squared Error (RMSE), and the statistic Model Efficiency (MEF). The last is a complement to the \( r^2 \) statistic, and can be interpreted as the proportion of variation explained by a fitted line (Byers et al., 1989; Loague and Green, 1991). The upper bound of MEF is one
(perfect match) and the (theoretical) lower bound is negative infinity (Loague and Green, 1991). The MEF statistic is more sensitive than $r^2$ to systematic deviations and is a useful additional tool in the assessment of goodness of fit (Mayer and Butler, 1993).

**Golden Days**

In order to evaluate model performance under optimal conditions, we first consider ‘Golden days’ at each site. Golden days are defined as days with no precipitation, midday radiation of greater than 400 W m$^{-2}$, frictional velocity of greater than 0.15 m s$^{-1}$, and fully developed canopy leaf area index. Days with gaps in any data were excluded. Theoretically, for such days, the model performance should not be confounded by daily variability in environmental conditions, and the analysis of model performance made easier.

1.3 Results

1.3.1 Primary Fluxes

All sites showed typical Mediterranean-type climate conditions during the studied years, including an extended summer drought. Strong seasonal patterns were observed of reduced photosynthesis and transpiration during peak summer periods (Fig. 1) in comparison to higher fluxes during late spring. This was particularly noticeable at Puechabon, Collelongo and Roccarespampmani, and less so at the Blodgett site.
1.3.2 Data Analysis Results

Soil Water Content

Figure 1.2 shows the evolution of soil water content for each simulated site and year, derived by inverting latent heat fluxes as described in Section 1.2.1.1. At each site, the soil water content stays high throughout spring, being recharged by precipitation events to compensate for the loss from evapotranspiration. The effect of the dry Mediterranean-type summer can be seen through strong decreases in the soil water content. Soil water levels generally returned to maximum during autumn, and remained relatively stable through winter. The Blodgett site soil water content shows little inter-annual variability due to the lack of inter-annual variability in its climate during the studied period. In comparison, at Puechabon, soil water varies over a large range, with
levels reaching a prolonged low during 2003 due to the notable drought experienced in Europe in that year. This drought period is also reflected at the Roccarespampani site, with soil water levels in 2003 reaching less than half of those in 2004. These levels correspond well with levels reported in other studies at the Puechabon site (Hoff et al., 2002; Rambal et al., 2003). No similar published data could be found for the other sites.

![Figure 1.2: Reconstructed daily relative soil water content (RSWC) for the simulated periods at each of the studied sites, separated by year.](image)

**Limitations of photosynthesis due to soil water stress**

The estimates of soil water content in Figure 1.2 are used with the observed flux data to determine whether, stomatal limitations, non-stomatal limitations, or both, vary significantly with soil moisture. Changes in the slope \((m, \text{Eq. 1.2})\), and/or intercept (Residual conductance \(-G_{s0}, \text{Eq.1.2}\)) would indicate a stomatal limitation to photosynthesis. Values for the slope and intercept of the BBL conductance model at
high soil water content for each site are given in Table 1.2, and correspond well with reported values (e.g. Ball et al., 1987; Leuning, 1990; Collatz et al., 1991; Schultz and Lebon, 1995). These values were also calculated for the BB model (for use in ORCHIDEE) and were found not to change in dependence of the choice of model.

Table 1.2. Parameters for the calculation of stomatal conductance, and water stress parameters applied to stomatal conductance (Fig. 1.3) and photosynthetic potential (Fig. 1.4) for each site.

<table>
<thead>
<tr>
<th>Site</th>
<th>Stomata - Wf_{stoma}</th>
<th>Photosynthesis - Wf_{photo}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope (mol m^{-2} s^{-1})</td>
<td>Intercept</td>
</tr>
<tr>
<td>Puéchabon</td>
<td>9</td>
<td>0.0017</td>
</tr>
<tr>
<td>Roccarespampani</td>
<td>8.5</td>
<td>0.0015</td>
</tr>
<tr>
<td>Collelongo</td>
<td>10.5</td>
<td>0.000025</td>
</tr>
<tr>
<td>Blodgett</td>
<td>10.5</td>
<td>0.00002</td>
</tr>
</tbody>
</table>

The fitted slope of the relation of stomatal conductance to assimilation in the BBL model, $m$, did not change notably during the slow onset of soil water stress and declined only slightly at very low soil water levels (<0.3 RSWC) (Fig 1.2a). The fitted intercept in Eq. 1.3 did not change with decreases in available soil water in any of the sites (Fig 1.2b). A similar lack of response was found at all sites.
Chapter 1: Drought controls on carbon and water fluxes

Figure 1.3: (a: Left): Changes in the slope parameter, m, of Eq. 1.3, (the empirical species specific factor that specifies the baseline ratio between conductance and net photosynthesis) with relative available water for each site. Regression lines represent the functions \( W_{fac_{stoma}} \) applied in the models; (b: Right): Intercept parameter (Residual of Eq. 1.3), at each site, as a function of soil water content (proportional to maximum soil water content).

Strong non-stomatal limitations were found under conditions of soil moisture stress at all sites except Blodgett. The point at which limitations were first encountered was between 60% and 80% RSWC, though at Blodgett non-stomatal limitations were not detected until 50% RSWC. Although inferred soil water in our reconstruction falls quite low in Blodgett, very little water stress is observed, suggesting either access to ground water, or an underestimation of the maximum soil water content used for the reconstruction. At the other three sites, stress progressed with site dependent intensities. Roccarespampani showed the strongest non-stomatal limitation, followed by Puechabon and then Collelongo. The fitted \( W_{fac_{photo}} \) functions from Eq. 1.5 are shown in Fig. 1.4, with parameters given in Table 1.2.
1.3.2 Diurnal Cycle

*Evaluation of the stomatal vs. non-stomatal limitation hypotheses using process based models*

The effect of the alternative hypotheses is tested by incorporating the observed empirical relationships from Figures 1.3 and 1.4 into the framework of the process-based models. The simulation of the diurnal courses of carbon and water fluxes during periods of high water availability showed to be very accurate (Fig. 1.5, $a_1$, $a_2$, $b_1$, $b_2$). Simulations during high water availability were relatively unaffected by the modelling approach chosen, due to the fact that the approaches only differ in their treatment of responses to water stress. Conversely, during periods of drought, responses to water stress were highly dependent on the chosen response description.
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Figure 1.5: 20 dry golden day diurnal courses for the observed hourly photosynthesis (An, in umol m$^{-2}$ s$^{-1}$) and actual evapotranspiration (Ea, in mm hour$^{-1}$), and average modelled (a: GOTILWA+, b: ORCHIDEE) values for the same golden days, for the Roccarespampani site, using 3 different modelling approaches, with the soil water content prescribed in each: 1) Applying the factors to stomatal conductance only. 2) Applying the factors to Photosynthetic potential only, and 3) Applying the original parameterisations. Wet and dry golden day periods are compared.
The shape of the diurnal cycle during dry periods, shown for the Roccarespampani site (Fig. 1.5), is relatively insensitive to the chosen soil moisture limitation approach for each process-based model system, demonstrated by a comparable $r^2$. The different approaches gave marked differences, however, in the Root Mean Squared Error. Applying the calculated water stress functions presented in Table 1.2, to photosynthetic potential, leads to a reduction of 80% in the RMSE for assimilated carbon using the GOTILWA+ model, and 47% in the case of ORCHIDEE, when compared to the original model parameterisation. Applying the water stress functions solely to stomatal control produces a marked increase in the RMSE (13%, GOTILWA+; 9%, ORCHIDEE, Table 1.3).

The same pattern was reproduced at all sites, with marked improvements in the accuracy of simulations of the diurnal cycle of both net photosynthesis and transpiration when photosynthetic capacity was reduced using Eq. 1.5. No direct effect of soil moisture on stomatal conductance was necessary to successfully reproduce the response of transpiration to water stress periods, i.e. both the standard BBL and BB models were sufficient for this purpose. Conversely, the application of the function only to stomatal conductance led to photosynthesis being overestimated under drought stress (by an average of 47% in Puechabon, 55% in Roccarespampani, 34% in Collelongo, and 8% in Blodgett, of monthly net photosynthesis values during water stressed months) (Table 1.3). Applying a stronger stomatal conductance restriction than that calculated from the data allowed for the effective simulation of stomatal conductance, but the lowered conductance was not sufficient to decrease assimilation rates.
Table 1.3: Statistics for the comparison of each model (GOTILWA+ and ORCHIDEE) and approach (Stomatal Vs. Non-Stomatal restrictions) with FLUXNET data at each site, for assimilation ($A_n$) and actual evapotranspiration ($E_a$) during wet and dry period Golden Days, with soil water in both models prescribed (SL – Applying stomatal limitations only, NSL – Applying Non-stomatal restrictions, Original – Original models).

GOTILWA+:

<table>
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<tr>
<th></th>
<th>$A_n$</th>
<th></th>
<th>$E_a$</th>
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<tbody>
<tr>
<td></td>
<td>SL</td>
<td>NSL</td>
<td>Original</td>
</tr>
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</tr>
<tr>
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<td>-18.6</td>
<td>0.63</td>
</tr>
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<td>0.82</td>
<td>0.85</td>
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<tr>
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<td>2.7</td>
</tr>
<tr>
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<td>RMSE</td>
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<tr>
<td>Roccarespampani</td>
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<tr>
<td>Model Efficiency</td>
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<td>-5.2</td>
<td>0.76</td>
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### Blodgett

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<tr>
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<td>2.9 1.61</td>
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### ORCHIDEE:

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<thead>
<tr>
<th></th>
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<tr>
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### Collelongo

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<tr>
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### Roccarespampani

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<tr>
<td>R²</td>
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<td>0.82 0.83</td>
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### Chapter 1: Drought controls on carbon and water fluxes

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<td>0.27</td>
<td>0.18</td>
<td>0.27</td>
<td>0.18</td>
</tr>
</tbody>
</table>

- 59 -
We also tested the effects of applying the calibrated functions to both conductance and assimilation (Eq. 1.4 and Eq. 1.5), thus applying stomatal and non-stomatal limitations together. This gave no improvement in the modelled carbon and water fluxes when compared to simulations applying just non-stomatal limitations to photosynthesis.

**Comparing GOTILWA+ and ORCHIDEE**

To evaluate the capacity of the two models to reproduce the observed fluxes simply by introducing the best fitting empirical model of soil moisture effects (i.e. non-stomatal limitations only), we now relax the constraint on soil water content by observation (i.e. the models simulate their own water balance based on modelled latent heat fluxes and runoff) and repeat the simulations for each site (Fig. 1.6).

In well-watered conditions at Puechabon, both models accurately reproduced the shape of the diurnal time courses of carbon and water fluxes, with ORCHIDEE performing slightly better. Under dry conditions at Puechabon, both models responded accurately to the effect of drought.
Figure 1.6: Diurnal cycles of observed photosynthesis ($A_n$, in $\mu$mol m$^{-2}$ s$^{-1}$) and actual evapotranspiration ($E_a$, in mm h$^{-1}$), and average modelled values for the same golden days for both GOTILWA+ and ORCHIDEE at all sites for both wet and dry Golden day periods (with dynamic soil water).

Carbon and water fluxes were accurately modelled by both models in Collelongo. Both models were capable of calculating net photosynthesis to a very high degree of accuracy in both wet and dry conditions. Water fluxes proved more difficult, with both models encountering the same problems, underestimating actual evapotranspiration during wet periods and overestimating in dry periods, possibly due to inaccuracies in model parameterisation.

At Roccarespampani, (Fig. 1.6) both models accurately reproduced carbon assimilation and actual evapotranspiration with an average model $r^2$ of 0.89 and a Mean Squared Error of 0.025 (Table 1.4).
Table 1.4: Statistics for the comparison of GOTILWA+ (GOT+) and ORCHIDEE (ORCH) diurnal cycles of assimilation (An) and actual evapotranspiration (Ea) with FLUXNET data at each site, for wet and dry period Golden Days, with free simulated soil water content, based on hourly data.

<table>
<thead>
<tr>
<th></th>
<th>Wet An</th>
<th>Dry An</th>
</tr>
</thead>
<tbody>
<tr>
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<td>ORCH</td>
</tr>
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<td><strong>Roccarespampani</strong></td>
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<tr>
<td>Model Efficiency</td>
<td>0.81</td>
<td>0.87</td>
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<td>$R^2$</td>
<td>0.89</td>
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</tr>
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<td>RMSE</td>
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<td><strong>Blodgett</strong></td>
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<table>
<thead>
<tr>
<th></th>
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<tr>
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<tr>
<td>RMSE</td>
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<td>0.04</td>
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<tr>
<td><strong>Collelongo</strong></td>
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<td></td>
</tr>
<tr>
<td>Model Efficiency</td>
<td>0.79</td>
<td>0.78</td>
</tr>
</tbody>
</table>
Drought events at the Blodgett site are less severe than at the other chosen sites. Although the seasonal drought does not lead to a drop in assimilation rates and conductance it does prevent both from reaching their potential seasonal maximum. This was well captured by both models. ORCHIDEE correctly calculated the rate of photosynthesis and conductance in wet conditions, as did GOTILWA+. In dry conditions GOTILWA+ accurately modelled assimilation rates, but both models had difficulties in calculating the quantity of water transpired, giving much lower transpiration rates than those observed.

It is worth noting that, statistically (Table 1.4), both models simulate the diurnal cycle of assimilated carbon equally well in water stressed conditions as they do in well watered conditions when including the new water stress functions.

### 1.3.3 Seasonal cycle and inter-annual variations

Finally, ORCHIDEE and GOTILWA+ simulations for each site and the entire time series are compared to the observations in Fig. 1.7. The eleven site-years cover a wide range of inter-annual and inter-site variability in climatic variables, and thus varying levels of drought, with a particularly strong drought experienced in 2003 at the European sites. The effect of drought on photosynthesis, which is characterised by a
sharp decline in assimilation rates in otherwise favourable conditions, is most obvious at Puechabon, at Collelongo in 1998, and at Roccarespampani in 2003.

For Puechabon, both models were capable of accurately predicting photosynthesis in non water stressed conditions. The timing of the onset of stress was also well captured in each year. The magnitude of stress encountered was accurately simulated in 2002, but overestimated in 2003 and 2004 when simulating with a dynamic soil water content. This was due to an overestimation of evapotranspiration in the period of high production before water stress was encountered, producing a lower soil water content during the water stressed period and a greater reduction in photosynthesis than observed. GOTILWA+ outperformed ORCHIDEE at Puechabon, giving a better correlation to the EUROFLUX data and a lower standard error. GOTILWA+ also performed better at the Blodgett site when simulating with a dynamic soil water content, where ORCHIDEE accurately captured carbon and water flux dynamics during spring, but largely overestimated the effect of drought on assimilation and conductance during summer periods.
Figure 1.7: Measured (black solid line) and modelled (both GOTILWA+ (red long dash) and ORCHIDEE (blue short dash)) seasonal cycles of daily assimilation rates ($A_n$) and evaportranspiration ($E_a$) at all sites (data shown has been smoothed using a ten-day running mean).
Simulations at the two deciduous sites were complicated by an active understorey and problems in the prediction of phenological events. In Roccarespampani, both models produced an equal match to the data, but were prone to higher levels of standard error. Roccarespampani had significant vegetative growth beneath the canopy, which was not taken into account in GOTILWA+, but was simulated by ORCHIDEE. Relatively high latent heat fluxes were observed outside of the growing season of the deciduous oak, *Quercus cerris*. These were not reproduced by the models, which led to difficulties when simulating the evolution of available soil water at the Roccarespampani site. Phenology also proved difficult to model, with neither model capable of accurately predicting the timing of budburst or leaf-fall, particularly in 2003. In Collelongo, ORCHIDEE preformed better than GOTILWA+, which accurately reproduced the strength and duration of the drought in 1998 and the effect on photosynthesis, but due to inaccuracies in the prediction of budburst and leaf-fall dates, total photosynthesis over the growing season was overestimated.
Table 1.5: Assimilation and actual evapotranspiration statistics for the comparison of GOTILWA+ and ORCHIDEE with FLUXNET data at each site, for seasonal daily simulation values. For Collelongo and Roccarespampani, which are deciduous sites, data from outside the growing period was omitted. New and original model formulations are compared.

<table>
<thead>
<tr>
<th>Location</th>
<th>GOTILWA+</th>
<th>ORCHIDEE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>An</td>
<td>Ea</td>
</tr>
<tr>
<td></td>
<td>New</td>
<td>Original</td>
</tr>
<tr>
<td>Puechabon</td>
<td>0.76</td>
<td>-0.09</td>
</tr>
<tr>
<td></td>
<td>0.90</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>1.23</td>
<td>1.96</td>
</tr>
<tr>
<td>Collelongo</td>
<td>0.82</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>0.93</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>1.68</td>
<td>1.78</td>
</tr>
<tr>
<td>Roccarespampani</td>
<td>0.68</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>0.88</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>2.36</td>
<td>3.11</td>
</tr>
<tr>
<td>Blodgett</td>
<td>0.69</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>0.89</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>1.47</td>
<td>1.74</td>
</tr>
</tbody>
</table>
With the inclusion of the water stress response calculated in Section 3.1, both models accurately capture the observed fluxes. GOTILWA+ give an average site $r^2$ of 0.9 for $A_n$ and 0.82 for $E_a$, and an average MEF of 0.74 for $A_n$ and 0.41 for $E_a$, over all sites. ORCHIDEE gave an average site $r^2$ of 0.84 for $A_n$ and 0.59 for $E_a$, and an average MEF of 0.61 for $A_n$ and 0.34 for $E_a$ (Table 1.5). These values represent a marked increase in model accuracy when compared against the original models. For GOTILWA+ the new implementation lead to an average decrease of 22% in the RMSE over all sites for simulated $A_n$ and a decrease of 9% in the RMSE of simulated $E_a$ over all sites when compared against the original model formulation. For Orchidee the RMSE for $A_n$ was relatively unchanged (though the $r^2$ increased by 15%) and the RMSE for $E_a$ decreased by an average of 21% over all sites. This also represents a big improvement when compared against the last published model comparisons including water stressed sites (Morales et al., 2005) (This study also included ORCHIDEE and GOTILWA+).

1.4 Discussion

A number of recent studies have brought into question the ability of process-based models to accurately simulate seasonal changes in carbon and water fluxes in Mediterranean ecosystems (Krinner et al., 2005; Morales et al., 2005; Jung et al., 2007). The use of algorithms in which the photosynthetic response to drought solely depends on stomatal control over $C_i$ concentration fails to capture both the timing and extent of the response of the coupled photosynthesis-conductance system to water stress. From our analysis of half-hourly FLUXNET data at four Mediterranean sites, this appears not to be due to a fundamental inaccuracy in the description of stomatal conductance using the BB-type approach, but rather an incomplete description of the mechanisms controlling the seasonal response of the photosynthetic capacity of Mediterranean trees to high levels of naturally occurring water stress.

Detailed studies on the effect of water stress on the slope parameter of BB-type conductance models are lacking, and reported results are inconclusive. Several studies assert that the slope decreases as soil water stress increases (Harley and Tenhunen, 1991; Sala and Tenhunen, 1996), whilst others maintain that it remains constant (Sellers
et al., 1996; Colello et al., 1998). We found that the relationship between $G_s$ and $A_n$ changed only slightly under soil water stress, giving an almost constant slope parameter. This suggests that stomatal conductance changes in parallel with photosynthesis, as originally reported by Wong et al. (1979). Our finding that the residual conductance does not change under soil water stress is contrary to results previously reported at the Blodgett site (Misson et al., 2004).

It is well known that the stomata react to changes in soil water availability. The water reserves in leaves are very small when compared to the amount of water transpired by a leaf, and thus could be quickly dehydrated in the absence of fast mechanisms, such as stomatal closure, to limit water loss (Slatyer, 1967). The effect of such stomatal closure is most commonly observed in the mid-day decline in stomatal conductance due to decreasing leaf water potential. This limits photosynthetic activity through reducing the availability of leaf $C_i$. Such short-term responses are essential to conserving the leaf hydraulic balance. However, with the slow onset of water stress, as experienced seasonally by all Mediterranean ecosystems, the picture becomes less clear. Other, slow acting processes have been identified (e.g. Loreto and Centritto, 2008), and can be divided into two categories: mesophyll conductance responses and metabolic adjustments. Changes in mesophyll conductance can reduce the concentration of $CO_2$ in the chloroplast with reference to leaf $C_i$. Whilst stomatal conductance rapidly changes to maintain the leaf hydraulic status, mesophyll conductance has been related to anatomical features and thus subject to slower responses, with large changes reported during the onset of slowly developing drought (e.g. Flexas et al., 2008). Metabolic adjustments can take many forms, and include reduced nitrate reductase activity (Smirnoff and Stewart, 1985), the reduction of enzymes necessary for RuBP regeneration and activity (Maroco et al., 2002), and the reduction of sucrose phosphate synthase (Vassey and Sharkey, 1989). It has been suggested that that all of these possibilities play some role in the control of photosynthesis (Loreto and Centritto, 2008), depending on the degree of water stress encountered and the relevant time scales involved.

The non-stomatal limitations in this study vary between sites. The two Quercus species, at Puechabon and Roccarespampani, showed similar responses, perhaps reflecting similarities in their leaf structure. Quercus ilex is an evergreen sclerophyllous species,
commonly found in Mediterranean regions, and well adapted to drought stress with tough well structured leaves. *Quercus cerris*, although deciduous, also has a high sclerophyll index (Kutbay and Kilinc, 1994). Such structures have been reported to have high mesophyll conductance limitations (Loreto et al., 1992; Syvertsen et al., 1995). The *Pinus ponderosa* species studied at Boldgett encountered very little water stress, with only a minimal reduction in photosynthetic activity during periods of low soil water content. This could be explained by access to ground water and mild air temperatures during summer due to high altitude. *Fagus sylvatica*, found at Collelongo, is not a typical Mediterranean species, and is therefore not moisture-stress adapted. The low water stress encountered at this site could also be explained by the possibility of ground water access at this site as suggested by Hickler et al. (2006). The large role of non-stomatal limitations found at each site suggest that the observed reduction in canopy conductance during slowly progressing natural water-stressed periods is, to a large extent, driven by a decrease in demand, and not by a change in the relationship between canopy conductance and photosynthesis. That is to say that there is little evidence that BB-type models do not hold under natural water-stressed conditions.

The work presented here makes several assumptions regarding possible influencing factors which could not be quantified from the available data. It was first necessary to assume that stomatal patchiness does not have a significant effect when making calculations which average over the whole canopy. It has been reported, that, in leaf-level experiments, stomatal patchiness may invalidate leaf C\textsubscript{i} calculations, in particular in drought conditions (Buckley et al., 1997; Mott and Buckley, 2000). However, more recent studies have shown that the influence of stomatal patchiness on calculations of leaf C\textsubscript{i} is less than once thought (Lawlor and Cornic, 2002). Also, it has been reported that the effect of stomatal patchiness is not as large in the field as it is in laboratory experiments, due to the slow time-scale of the onset of drought (Gunasekera and Berkowitz, 1992; Kubiske and Abrams, 1993). In any case, the nature of the data analysed, gathered at the canopy scale under field conditions, leads to large variability in some of the variables necessary to construct the stomatal and non-stomatal response functions. For example, using observations only from mid-day periods or obtained for the Golden Days studied had minor effects on the calculated parameters of the soil water response functions.
Chapter 1: Drought controls on carbon and water fluxes

The representation of carbon flux responses to drought can vary between process-based models as a function of soil hydrology (with different approaches: single bucket soil module, two layer or multi-layer model), soil structure, and biogeochemistry approaches. Differences in the modelled soil structure and hydrology can lead to significant differences in evapotranspiration calculations and thus sensitivity of land surface fluxes to drought (Vetter et al., 2008). Furthermore, global model parameterisation, notably the assumed maximum soil water content, contributes significantly to the reported model-data difference (Jung et al., 2007). In this study, the inversion of latent heat flux measurements using a soil water flux model, and utilisation of observed maximum soil water storage capacity, has allowed the photosynthetic component in the models to be isolated, and the sensitivity of photosynthesis to soil water stress carefully examined.

Inaccuracies in the simulation of the seasonal cycle of carbon and water fluxes were attributed to difficulties in modelling phenological events, the existence of active understory vegetation (which is not taken into account by GOTILWA+), and possibly different soil hydrology representations. Both models predict phenological events using a running mean of temperature, but were not capable of accurately predicting bud burst at either of the two deciduous sites. Leaf fall estimation was slightly better but was still not consistently accurate. There are various methods for the prediction of phenological events, but as yet none have proven fully reliable across a range of species and climatic conditions (Stockli et al., 2008). This has been shown to have important implications for modelling terrestrial CO2 uptake (Piao et al., 2007).

The role of non stomatal limitations in modelling carbon and water fluxes, shown in this study, should apply to all C3 plant functional types, and will be of particular importance for performing regional scale modelling. Preliminary regional simulations with ORCHIDEE suggest that the new implementation leads, on average, to an 8% reduction in the predicted summer photosynthesis across the Mediterranean area. Any regional model applying only stomatal limitations will, therefore, be expected to overestimate assimilation from Mediterranean-type vegetation by an even greater amount. The applicability of the derived water stress parameterisations across different plant functional types is, however, as yet unclear, given the differences observed in the studied species. More research is needed to identify the range of water stress responses...
and suggest different plant functional types. This, coupled with difficulties in accurately modelling phenological events, which were inaccurately modelled at the two deciduous sites, will be the main challenges for regional modelling efforts in the Mediterranean, particularly for those looking at future vegetation dynamics in the region.

1.5 Conclusions

We have shown that canopy conductance and photosynthesis vary with soil moisture in a consistent manner using observations from four sites. Stomatal conductance was found to vary in parallel with photosynthesis, and with only small changes in the slope or the intercept, of the conductance-assimilation relationship. Changes in photosynthetic capacity not related to stomatal closure under water stressed conditions were found to be more important at each of the sites studied. Incorporating this knowledge into process-based models suggests that accounting for soil-water mediated reductions of photosynthetic capacity alone is sufficient to adequately model carbon and water fluxes during dry and wet periods. Pure stomatal regulation of photosynthesis during seasonal drought episodes is not sufficient to reduce modelled photosynthesis to observed level during drought periods. Accounting for the empirically-derived soil moisture responses of photosynthetic capacity with the derived parameters for each site improved substantially the performance of both models, and allowed for the simulation of carbon and water fluxes with a similar accuracy during wet conditions as under drought conditions. Both models compare well against the FLUXNET data, although GOTILWA+ performed slightly better on average.

This study contributes to improving our ability to model and predict carbon and water fluxes in Mediterranean forest ecosystems, and thereby to reducing uncertainty in future European terrestrial carbon and water fluxes. These results are of particular importance for any study of the effects of climatic changes on Mediterranean ecosystem functioning.
Acknowledgments

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Bibliography


Chapter 1: Drought controls on carbon and water fluxes


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Chapter 1: Drought controls on carbon and water fluxes


Chapter 2

Mesophyll conductance in regulating photosynthetic drought stress responses
The importance of mesophyll conductance in regulating biosphere productivity during drought periods.

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Abstract

Water availability is the most limiting factor to global plant productivity, yet photosynthetic responses to seasonal drought cycles are poorly understood, with conflicting reports on which limiting process is the most important during water stressed periods. We address the problem using a model-data synthesis approach to look at canopy level fluxes, integrating twenty years of half hour data gathered by the FLUXNET network across six Mediterranean sites. The measured canopy level, water and carbon fluxes were used, together with an inverse canopy ecophysiological model, to estimate the bulk canopy conductance, bulk mesophyll conductance, and the canopy scale carbon pools in both the intercellular spaces and at the site of carboxylation in the chloroplasts. Thus the roles of stomatal and mesophyll conductance in the regulation of internal carbon pools and photosynthesis could be separated. A quantitative limitation analysis allowed for the relative seasonal responses of stomatal, mesophyll and biochemical limitations to be gauged. The concentration of carbon in the chloroplast was shown to be a more reliable estimator of assimilation rates than the inter-cellular carbon concentration. Both stomatal conductance limitations and mesophyll conductance limitations were observed to regulate the response of photosynthesis to water stress in each of the six species studied. The results suggest that mesophyll conductance could bridge the gap between conflicting reports on plant responses to soil water stress, and
that the inclusion of mesophyll conductance in biosphere-atmosphere transfer models may improve their performance, in particular their ability to accurately capture the response of terrestrial vegetation to drought.
2.1 Introduction

Our understanding of the potential of terrestrial net primary productivity is not complete without a clear understanding of the main limitations on leaf photosynthesis (Kosugi, Shibata & Kobashi 2003). Water availability is known to be the main limiting factor to global plant photosynthesis (Boyer, 1982), in particular in arid or semi-arid ecosystems within Mediterranean climate regions (Nemani et al. 2003). Projections of climate change suggest that higher temperatures, and increased potential evapotranspiration, as well as changes in seasonal precipitation patterns (IPCC, 2007), will aggravate the seasonal drought stress characteristic to Mediterranean ecosystems (Giorgi et al. 2004; Wang, 2005; Giorgi 2006; Beniston et al. 2007). Our understanding of the impacts of changing climate on these ecosystems is poor due to a lack of understanding concerning ecophysiological responses to soil moisture stress and consequent effects on the cycling of carbon and water (Loreto and Centritto, 2008).

The effect of water stress on plant photosynthesis and stomatal conductance has been widely studied (Wilson, Baldocchi & Hanson 2000; Chaves et al. 2002). It has long been accepted that stomatal control is the main driver behind photosynthetic response to water stress (e.g. Cornic, 2000; Loreto and Cornic, 2002), limiting available leaf inter-cellular carbon, thus slowing down photosynthesis and conserving water. The involvement of non-stomatal limitations has also been suggested which takes the form of an additional metabolic or biochemical constraint (e.g., Smirnoff and Stewart, 1985; Vassey and Sharkey, 1989; Maroco et al., 2002; Reichstein et al., 2002; Keenan et al., 2009). Other studies have shown changes in mesophyll conductance to be one of the possible non-stomatal physiological reactions to stress which can play a part in the limitation of photosynthesis (e.g. Niinemets 2005; Grassi & Magnani 2005; Warren, 2006). Much debate still remains as to the dynamic between stomatal and non-stomatal limitations at different levels of water stress (Lawlor & Cornic 2002; Flexas & Medrano 2002; Loreto and Centritto, 2008) and there is large uncertainty as to the role played by stress related changes in mesophyll conductance.

Mesophyll conductance ($g_{mc}$) has historically been assumed to be infinite, with stomatal control given the sole role in the control of photosynthesis in water stressed conditions through the control of the leaf inter-cellular $CO_2$. However, there is increasing evidence
that \( g_m \) is indeed finite (Evans & Loreto 2000; Flexas et al. 2002; Ethier & Livingston 2004; Flexas et al. 2008), and it has been demonstrated to change during leaf development (Miyazawa & Terashima 2001), with nutrient availability (Warren 2004), with available radiation (Niinemets et al. 2006), leaf temperature (Bernacchi et al. 2002), salinity (Loreto, Centritto & Chartzoulakis 2003), ambient CO\(_2\) concentrations (Flexas et al. 2007) and to be related to soil water availability (Flexas et al. 2002; Flexas et al. 2004; Grassi & Magnani 2005; Warren et al. 2004; Warren 2008a). Finite \( g_m \) would lead to a lower concentration of CO\(_2\) reaching the chloroplast and any changes in \( g_m \) during periods of low soil water availability could potentially play an important role in controlling photosynthetic responses to water stress (Jones 1973; Flexas et al. 2008; Niinemets et al., 2009). This could also account for the differences observed in WUE estimated from FLUXNET data in a conventional way (i.e. not accounting for \( g_m \)) and WUE estimated by sap flow measurements (Rambal et al. 2003).

The logistic complications of making canopy measurements of water stress affects on leaf photosynthesis and \( g_m \) over long periods makes it difficult to scale up results to trees in woodland conditions over entire seasons and years. On top of this, only a few studies have used quantitative analysis techniques to separate the different limitations to photosynthesis (Ellsworth 2000; Wilson et al. 2000). These two problems are being approached by recent advances in inverse modelling techniques which allow for the estimation of leaf ecophysiological parameters from eddy-covariance flux data (e.g. Reichstein et al., 2003; Carvalhais et al., 2008; Lasslop et al., 2008) and recently the consideration of possible limitations of photosynthesis due to a variable \( g_m \) (Grassi & Magnani 2005).

In this chapter, we use data from multiple FLUXNET (http://daac.ornl.gov/FLUXNET/) sites to quantify the role of both stomatal conductance and mesophyll conductance in regulating forest ecosystem productivity during drought periods. Both bulk canopy stomatal and mesophyll conductance were calculated from the FLUXNET data, using an inversion of the McNaughton and Black equation (McNaughton & Black, 1973), the Harley variable \( J \) method (Harley et al. 1992), and an ecophysiological process based photosynthesis-conductance model coupling (Farquhar et al. 1980; Leuning et al., 1995).
together with a canopy distribution model (Campbell, 1986; Dai et al., 2004; Keenan et al., 2009a), thus describing foliar photosynthesis and conductance’s as distributed through the canopy. The resulting responses of the bulk canopy leaf intercellular and chloroplast carbon pool concentrations to changes in soil water availability were thus assessed. A quantitative analysis (Grassi & Magnani, 2005) allowed for the relative soil water related responses of stomatal limitations, mesophyll conductance limitations and biochemical limitations to productivity to be gauged.

2.2 Materials and Methods

**FLUXNET Site Data and Data Manipulation**

The sites chosen cover 6 dominant species, over a wide range of Mediterranean environmental conditions, and include Quercus ilex, Quercus cerris, Fagus sylvatica, Pinus halepensis, Pinus ponderosa, and Quercus douglasii, respectively. Three of the studied sites are situated in Europe (Puéchabon, France; Roccarespampani, Italy; Collelongo, Italy), under the CarboEurope-IP project, one site in Israel (Yatir) and two sites in the United States (Blodgett & Tonzi, California), under the AMERIFLUX project (Table 1.). The most common Mediterranean environments are covered, from a savannah type ecosystem (Tonzi, California) to semi-arid (Yatir, Israel), to mountainous (e.g. Collelongo, Italy; Blodgett, California), across the three continents. FLUXNET provides continuous measurement of carbon dioxide (broken down into net assimilation, and ecosystem respiration) and water fluxes on a seasonal basis with half-hourly discrimination (Wofsy et al., 1993). New flux separation techniques now give the improved level 4 data set (Reichstein et al., 2005), used in this study.
Table 2.1. Characteristics of the FLUXNET sites chosen.

<table>
<thead>
<tr>
<th>Species</th>
<th>Site Location</th>
<th>Period</th>
<th>Longitude</th>
<th>Latitude</th>
<th>Altitude</th>
<th>Annual Precip. (mm)</th>
<th>Soil Water Maximum (Kg m(^{-2}))</th>
<th>Mean Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Quercus ilex</em></td>
<td>Puéchabon, France (1)</td>
<td>2001-2004</td>
<td>3° 35'</td>
<td>43° 44'</td>
<td>270</td>
<td>1028</td>
<td>170</td>
<td>13.7</td>
</tr>
<tr>
<td><em>Quercus cerris</em></td>
<td>Rocarespampa, Italy (2)</td>
<td>2002-2004</td>
<td>11° 55'</td>
<td>42° 23'</td>
<td>223</td>
<td>862</td>
<td>480</td>
<td>14.9</td>
</tr>
<tr>
<td><em>Fagus sylvatica</em></td>
<td>Collelongo, Italy (3)</td>
<td>1998-1999</td>
<td>13° 35'</td>
<td>41° 50'</td>
<td>1560</td>
<td>1181</td>
<td>287</td>
<td>7.6</td>
</tr>
<tr>
<td><em>Pinus ponderosa</em></td>
<td>Blodgett Forest, California (4)</td>
<td>2001-2004</td>
<td>-120° 37'</td>
<td>38° 53'</td>
<td>1315</td>
<td>1402</td>
<td>582</td>
<td>11.9</td>
</tr>
<tr>
<td><em>Quercus douglasii</em></td>
<td>Tonzi, California (5)</td>
<td>2002-2006</td>
<td>-120° 58'</td>
<td>38° 26'</td>
<td>177</td>
<td>601</td>
<td>270</td>
<td>16.3</td>
</tr>
<tr>
<td><em>Pinus halepensis</em></td>
<td>Yatir Forest, Israel (6)</td>
<td>2001-2003</td>
<td>35° 30'</td>
<td>31° 20'</td>
<td>650</td>
<td>279</td>
<td>215</td>
<td>18.7</td>
</tr>
</tbody>
</table>


**Fractional soil water storage**

Understanding the response of observed carbon and water fluxes to changes in soil moisture requires the seasonal evolution of soil water content to be known. In the absence of such measurements over the entire rooting profile at each site, daily soil moisture content at each site was reconstructed using a simple water balance model through inverting the evapotranspiration rate inferred from the measured latent heat flux, and determining the balance with inputs from precipitation and outputs to run-off and below-ground drainage (as in Keenan et al., 2009). Run-off is calculated as a percentage of precipitation, and depends on the soil hydraulic gradient and porosity of the soil upper layer. Drainage is calculated to be inversely proportional to fractional soil water content.
(calculated as in Gracia et al., 1999 and Honeysett and Ratkowsky, 1989; see Keenan et al., 2009 for more details).

A wide range of inter-annual and inter-site variability in climatic variables is encountered over the six chosen sites, leading to varying levels of drought, with a particularly strong drought experienced in 2003 at the European sites. Both inter-site and inter-annual soil water variability is large, giving a broad range of water stress responses in each of the six species. The Blodgett site soil water content shows little inter-annual variability due to the lack of inter-annual variability in its climate during the studied period, and small levels of water stress were experienced. In comparison, at Puéchabon, soil water varies over a large range, with levels reaching a prolonged low during 2003. This drought period is also reflected at the Roccarespampani site, with soil water levels in 2003 reaching half those of 2004. Yatir is a ‘man-made’ forest in a very arid region of Israel and suffers annual periods of strong drought stress. Each site experiences different degrees of water stress related decreases in assimilation rates and observed evapotranspiration. Reconstructed seasonal soil water cycle details are presented in Keenan et al. (2009).

**Calculation of bulk canopy conductance and bulk leaf inter-cellular carbon dioxide concentrations**

Canopy conductance is the bulk leaf-surface conductance to water vapour, expressed on a ground-area basis. It can be estimated from the observed latent heat flux under dry canopy conditions and when soil evaporation is negligible. We inverted the McNaughton and Black equation for canopy latent heat flux (McNaughton and Black, 1973) to estimate bulk canopy conductance, \( G_c \):  

\[
G_c = \frac{LH \cdot \varepsilon \cdot \gamma}{(\rho \cdot Cp \cdot vpd)} \quad \text{(Eq. 2.1)}
\]

where \( LH \) is the observed latent heat flux (W m\(^{-2}\)), \( \varepsilon \) is the coefficient for the conversion of latent heat to its water equivalent (giving actual evapotranspiration \((E_a)\)), \( \lambda \) is the latent heat of vaporisation of water (2.27 MJ kg\(^{-1}\)), \( \gamma \) is the psychrometric
constant (0.66 kPa °K⁻¹), ρ is the density of air (kg m⁻³), Cp is the heat capacity of air (1012 J kg⁻¹ K⁻¹), and vpd is the observed vapour pressure deficit (Pa).

Using the estimated bulk canopy conductance to carbon \( G_{c_{\text{co2}}} = G_c / 1.6 \), observed rates of net photosynthesis from the eddy-covariance measurements, and atmospheric CO₂ concentrations, canopy bulk leaf intercellular CO₂ concentration (\( C_i \)) can be calculated using the simple supply and demand algorithm:

\[
C_i = C_a - \left( A_n / G_{c_{\text{co2}}} \right),
\]

(eq. 2.2)

where \( C_a \) (μmol mol⁻¹) is the atmospheric carbon concentration. As all measurements used in the calculation of bulk conductance have reference above the canopy, boundary layer effects are assumed to be minimal for the calculation of bulk \( C_i \).

**Calculating bulk mesophyll conductance and carbon dioxide concentrations in the chloroplast**

The bulk canopy mesophyll conductance, \( G_m \), from the sub-stomatal cavities to chloroplasts was estimated according to the variable electron transport rate method of Harley et al. (1992):

\[
G_m = A_n / (C_i - \Gamma^* \left( J + 8 \left( A_n + R_d \right) \right) - 4 \left( A_n + R_d \right))
\]

(eq. 2.3)

where \( C_i \) is the bulk canopy leaf intercellular carbon concentration and \( A_n \) is the net photosynthetic assimilation rate taken from the FLUXNET data. The CO₂ compensation point, \( \Gamma^* \), and the mitochondrial respiration rate, \( R_d \), are calculated on a leaf temperature basis using an Arrhenius type equation (Bernacchi et al., 2002). The canopy average electron transport rate, \( J \), was estimated for each site through the calibration of a two-leaf (sun-shade) canopy photosynthetic model (Wang and Leuning, 1998; Farquhar et al. 1980; Dai et al., 2004; but see Keenan et al., 2009), coupled to a canopy distribution model which divides the canopy into sunlit and shaded leaves, with the amount of intercepted diffuse and direct radiation depending on the time of the day, season, and the area of leaf exposed to the sun (Campbell, 1986; 1990). \( J \) depends on the maximal
electron transport rate ($J_{\text{max}}$) and the PAR absorbed by photosystem II (PAR$_{PS2}$), (De Pury & Farquhar 1997), as the minimal solution to:

$$\partial J^2 - (\text{PAR}_{PS2} + J_{\text{max}})J + \text{PAR}_{PS2}J_{\text{max}} = 0 \quad \text{(Eq. 2.4)}$$

where $\theta$ is a curvature parameter. $J_{\text{max}}$ is calculated independently for each site, using a maximal reference value $J_{\text{max25}}$ estimated through the calibrating of the Farquhar et al. (1980) photosynthesis model to the hourly flux data at each site (see Keenan et al., 2009), and an Arrhenius type response based on leaf temperature (Medlyn et al., 2002). Leaf temperature is dependent on the energy balance of the leaf. It was estimated for both sunlit and shaded leaves for each site using the two-leaf canopy photosynthetic model (Keenan et al., 2009) which calculates leaf temperature by using numerical iteration to close the energy balance equations (coupling climate variables, assimilation, and conductance). No direct temperature effect was applied to the Harley approach, as the effect of temperature on mesophyll conductance is unresolved, with both positive and negative responses reported in the literature (Bernacchi et al., 2002; Pons & Welschen 2003; Niinemets et al., 2009b).

Bulk canopy $C_c$ is then a function of the net photosynthesis assimilation rate, stomatal conductance, $G_s$, and $G_m$:

$$C_c = C_a - \left(\frac{A_n}{Gc_{CO2}}\right) - \left(\frac{A_n}{Gm}\right). \quad \text{(Eq. 2.5)}$$

**Data Selection**

The analysed FLUXNET data was filtered to consider only daytime values by selecting data corresponding to half hours with photosynthetically active radiation (PAR) of 400 $\mu$mol m$^{-2}$ s$^{-1}$ or greater, and assimilation rates of greater than 2 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$. Only data corresponding to a leaf inter-cellular carbon concentration of between 100 and 300 $\mu$mol mol$^{-1}$ were used, as data outside of this range was deemed to give unreliable results in the calculation of mesophyll conductance (Harley et al. 1992). Screening was also
performed to remove data relating to precipitation events, and extreme temperatures (below 5° or above 35°). Gap filled data was not considered.

**Quantitative limitation analysis**

The filtered data was used to separate three different limitations on maximum photosynthesis, following Grassi & Magnani’s (2005) elaboration of Jones (1985). Using quantitative limitation analysis, it is possible to calculate the limitations imposed on photosynthesis by stomatal conductance ($SC_L$), mesophyll conductance ($MC_L$), or biochemical processes ($B_L$) at any time of the year, assuming a reference maximum is available. Thus the response of each limitation to changes in soil water availability could be observed. For more details on the methodology and equations used see Grassi & Magnani (2005). Grassi & Magnani’s method of calculating the relative limitations requires knowledge on the photosynthetic Rubisco capacity, $Vc_{\text{max}}$. This was estimated in a similar manner to the estimation of $J_{\text{max}}$, using a maximal reference value $Vc_{\text{max}25}$ (with an Arrhenius type response based on leaf temperature (Bernacchi et al., 2001; Medlyn et al. 2002)), and a two-leaf canopy photosynthetic model calibrated to hourly flux data at each site (Farquhar et al. 1980; Campbell et al., 1986,1990; Wang and Leuning, 1998; Dai et al., 2004; but see Keenan et al., 2009). No response of $Vc_{\text{max}}$ to soil water stress was considered.

**2.3 Results**

**Conductance, assimilation and carbon concentrations**

The rate of photosynthesis, the concentration of carbon in the chloroplast, and mesophyll conductance, were observed to be intricately linked. Mesophyll conductance was found to have a strong role in the control of carbon available for assimilation in the chloroplast. The imperfect scaling with stomatal conductance followed a logarithmic relationship. Changes in high conductance values had little effect on the concentration of carbon in the chloroplast, whilst changes at low rates of conductance lead to big differences in the available carbon at the site of carboxylation (Fig 2.1a). This means that photosynthesis, and the internal carbon dynamics of the leaf are not largely affected by changes in mesophyll conductance when conductance is high, with other processes playing a
stronger role in the control of the concentration of carbon in the chloroplast. A species dependent threshold was observed, between 0.1 and 0.2 mol m\(^{-2}\) s\(^{-1}\), below which mesophyll conductance begins to increasingly limit the concentration of carbon available for assimilation.

Both the *Quercus ilex* species at Puechabon and the *Quercus douglasii* species at Tonzi reached high differences between the carbon pools at low conductance levels, whilst maximum mesophyll conductance was comparatively low (Fig. 1a). Although *Quercus ilex* is a broadleaf evergreen species, and *Quercus douglasii* deciduous, both have very coarse, well structured drought tolerant leaves. The range of C\(_i\)-C\(_c\) reached for the two deciduous species at Collelongo and Roccarespampani was comparatively low, reaching a maximal value of 200 ppm at low levels of mesophyll conductance. Both demonstrated relatively high levels of mesophyll conductance, reaching 0.4 mol m\(^{-2}\) s\(^{-1}\). Blodgett, by far the wettest site included in the study, showed levels of mesophyll conductance comparable to those observed at Puechabon and Tonzi, but the difference between the two carbon pools was small. At Yatir, the driest site in the study, mesophyll conductance levels were very low, and a strong response of drawdown was observed at low levels of conductance.

The deciduous species at Collelongo and Roccarespampani showed high rates of assimilation and conductance compared to the other sites. The other deciduous species at Tonzi, in comparison, showed much lower rates of conductance and assimilation. At Blodgett, the wettest site, both assimilation and conductance were observed within a narrower range than at the other sites. The Yatir site showed very low rates of assimilation and conductance. Assimilation levels corresponded closely to the rate of mesophyll conductance at each site, with increased dispersion observed with increasing conductance levels (Fig 2.1b).
Figure 2.1. **a**) Bulk canopy mesophyll conductance \((G_m, \text{ mol m}^{-2} \text{ s}^{-1})\) against the draw-down \((C_i - C_c, \mu\text{mol mol}^{-1})\) of CO$_2$ from the intercellular spaces to the chloroplast. **b**) Bulk canopy mesophyll conductance \((G_m, \text{ mol m}^{-2} \text{ s}^{-1})\) against net photosynthesis \((A_n, \mu\text{mol m}^{-2} \text{ s}^{-1})\), for each of the studied sites. Fitted lines represent the regression \(y = a_0 + a_1 \log_{10}(x)\) for \(n\) data points, where \(n, a_0,\) and \(a_1\) are: Puechabon **a**) 1465, -10.91, -119.68, **b**) 1465, 13.21, 11.55; Collelongo **a**) 185, 3.93, 114.55, **b**) 185, 9.21, 13.03; Roccarespampiani **a**) 214, 18.9, -97.89, **b**) 214, 15.46, 16.84; Blodgett **a**) 195, -25.52, -132.22, **b**) 195, 10.63, 10.95; Tonzi **a**) 546, -32.74, -151.05, **b**) 546, 15.11, 14.14; Yatir **a**) 78, -18.12, -98.39, **b**) 78, 12.89, 9.94.
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$A_n - C_i$ curves are used extensively to extract parameters for calculating photosynthesis. Figure 2 shows that such curves calculated on the basis of $C_c$ are much less susceptible to variation, i.e. $C_c$ is statistically subject to less variation with respect to $A_n$ and demonstrates a stronger response to changes in $A_n$. Thus, the concentration of carbon in the chloroplast proved to be a more reliable estimator of assimilation rates than the concentration of carbon in the intercellular spaces in all species. This is equally true to conditions of high water availability as to dry conditions.

The data presented here does not give the full range of carbon concentrations normally encountered in leaf measurements due to filtering imposed to assure the reliability of the calculated variables, and thus the regressions are not intended as model $A_n - C$ curves. However, it is clear that carbon in the chloroplast exerts a greater control over photosynthesis than carbon in the intercellular spaces. This is due to the fact that it is the carbon in the chloroplast which is used for assimilation purposes, and thus variance in the relation between carbon concentration and assimilation rates is reduced by using the concentration of carbon in the chloroplast instead of inter-cellular carbon concentrations. Hence, much of the error associated with the use of $A_n - C_i$ curves can be explained by variations in mesophyll conductance.
Figure 2.2. Rate of bulk canopy assimilated carbon ($A_n$) against CO\textsubscript{2} concentrations in the leaf inter-cellular space ($\alpha$: C\textsubscript{i}, \(\mu\text{mol mol}^{-1}\)), and the chloroplast ($\oplus$: C\textsubscript{c}, \(\mu\text{mol mol}^{-1}\)), at each of the studied sites. Fitted lines represent the polynomial regression $y = a_0 + a_1 (x) + a_2(x^2)$ for n data points, where $n = 1465$ (Puechabon), 185 (Collelongo), 195 (Blodgett), 214 (Roccarespampani), 546 (Tonzi), 78 (Yatir).

The C\textsubscript{c} values reported here for optimal assimilation conditions (high $A_n$) for *Quercus ilex* and *Fagus sylvatica* fall within values published for these species in a recent literature review (Warren & Adams 2006; Warren et al. 2007). No published values could be found for the other species included in the study.
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Responses to changes in soil water

Soil water availability induced changes in both mesophyll and stomatal conductance. The magnitude of these changes depended on the species studied and the amount of soil water stress experienced. The resulting effect of soil water availability on the concentration of carbon in the two different pools can be seen in Fig. 2.3. The concentration of carbon in the chloroplast was more responsive to changes in soil water content than the inter-cellular carbon pool at each of the studied sites at low soil water levels, thus in part explaining the reduced variance associated with using the carbon concentration in the chloroplast as an estimator for the rate of assimilation. As soil water stress set in, the carbon pool in the chloroplast diminished in parallel with the carbon concentration in the intercellular spaces, driven by stomatal closure. As the stress progressed in severity, the role of mesophyll conductance increased, reducing the concentration of carbon in the chloroplast at a faster rate than the reductions of the carbon concentration in the inter-cellular spaces. i.e. The stomata responded first, causing a reduction in both carbon pools, and mesophyll conductance decreased with increasing water stress, further limiting the carbon pool in the chloroplast. The large amount of scatter and the low correlation values observed here are explained by the fact that we are looking at the effect of a large scale slow changing process (soil water content), on a fine scale process with a very fast dynamic (carbon pools).
Figure 2.3. The response of concentrations of CO$_2$ in the leaf intercellular spaces ($o$: $C_i$, $\mu$mol mol$^{-1}$) and in the chloroplast ($+$: $C_c$, $\mu$mol mol$^{-1}$) to changes in the available relative soil water content (RSWC) for each site. Fitted lines represent the polynomial regression $y = a_0 + a_1 (x) + a_2(x^2)$ for $n$ midday data points, where $n = 235$ (Puechabon), 120 (Collelongo), 130 (Blodgett), 54 (Roccarespampani), 267 (Tonzi), 64 (Yatir).

The difference between the carbon pools remained steadily constant until a site specific soil water level is met, at which point mesophyll conductance decreased, limiting the transfer of carbon from the intercellular spaces to the site of carboxylation in the chloroplast (Fig. 2.4). This is directly related to changes in soil water content and varied between sites and species. That said, a general response was found among all species studied. As the stress progressed in severity, the proportional role of the stomata decreased, whilst the role of mesophyll conductance became increasingly important in the limiting of assimilation rates. The observed responses were statistically significant at all sites except Yatir. The range of values, reported in Table 2.2, for drawdown and
mesophyll conductance is larger than normally encountered with leaf level measurements due to the large scale nature of the data used for the analysis.

Table 2.2. Drawdown from the intercellular spaces to the sites of carboxylation, and bulk canopy mesophyll conductance in the species studied under different soil water availabilities. High soil water: > 0.65; Low soil water: lowest 10% reached

<table>
<thead>
<tr>
<th>Species</th>
<th>$C_i - C_c$ range (µmol mol$^{-1}$)</th>
<th>$G_m$ range (mol m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High Soil Water</td>
<td>Low Soil Water</td>
</tr>
<tr>
<td><em>Quercus ilex</em></td>
<td>57 – 101</td>
<td>112 – 218</td>
</tr>
<tr>
<td><em>Quercus douglasii</em></td>
<td>69 – 94</td>
<td>98 – 271</td>
</tr>
<tr>
<td><em>Quercus cerris</em></td>
<td>42 – 104</td>
<td>74 – 164</td>
</tr>
<tr>
<td><em>Pinus halepensis</em></td>
<td>56 – 119</td>
<td>61 – 211</td>
</tr>
<tr>
<td><em>Pinus ponderosa</em></td>
<td>53 – 77</td>
<td>56 – 148</td>
</tr>
<tr>
<td><em>Fagus sylvatica</em></td>
<td>34 – 54</td>
<td>52 – 168</td>
</tr>
</tbody>
</table>
Chapter 2: Conductance limitations to photosynthesis

Figure 2.4. The CO₂ concentration gradient between the leaf inter-cellular spaces and the chloroplast (Cᵢ - Cₑ, μmol mol⁻¹) against changes in the relative soil water content (RSWC) at each site. Fitted lines represent the polynomial regression y = a₀ + a₁(x) + a₂(x²) for n midday data points, where n = 235 (Puechabon), 120 (Collelongo), 130 (Blodgett), 54 (Roccarespampani), 267 (Tonzi), 64 (Yatir).

Quantitative limitation analysis

The three different limitations considered in the quantitative limitation analysis are presented in Fig. 2.5. The effect of changes in soil water content on the response of the relative role of each limitation can be seen to vary between sites. Significant relationships between soil water and each limitation were found for all sites, except for biological limitations at the Blodgett site (Table 2.3). The parabolic nature of the responses reflects the fact that other factors such as phenology and leaf development
could not be separated from the soil water effect. This response however, compares well to that reported in Grassi & Magnani (2005, Fig. 2.6), although presented here in a different format.

![Figure 2.5](image_url)

**Figure 2.5.** Regressions of results from the analysis of independent limitations of photosynthesis at each site, over an available soil water gradient (data not shown), relative to their minimum limitation (with non-growth periods excluded). Limitations are: 1. Stomatal limitation (solid line, $S_L$), 2. Mesophyll conductance limitation (dashed line, $MCL$), 3. Biochemical limitation (dotted line, $BL$).

The balance between stomatal and mesophyll conductance limitations ($SC_L$ & $MCL$) was site dependent, but an increasing role of $MCL$ with increased soil water stress was generally observed, with the response of $MCL$ reaching or surpassing that of $SC_L$ at all sites except the Collelongo site. The species at Collelongo is the deciduous *Fagus*
sylvatica, which is the only non-typical Mediterranean species included in the study. Of the remaining species the response of MC\textsubscript{L} was strongest in three (Q. ilex, Q. cerris & P. halepensis). The response of SC\textsubscript{L} was of a similar magnitude to that of MC\textsubscript{L} for two species (P. ponderosa, Q. douglasii). The role of the Biochemical limitation (B\textsubscript{L}) decreases steadily during the season at each site.

Table 2.3. Statistics associated with the regressions of Figure 2.5.

<table>
<thead>
<tr>
<th></th>
<th>Puechabon</th>
<th>Collelongo</th>
<th>Rocca</th>
<th>Blodgett</th>
<th>Tonzi</th>
<th>Yatir</th>
</tr>
</thead>
<tbody>
<tr>
<td>y = a(_0) + a(_1) x + a(_2) x^2</td>
<td>r(^2)</td>
<td>p</td>
<td>r(^2)</td>
<td>p</td>
<td>r(^2)</td>
<td>p</td>
</tr>
<tr>
<td>S(_L)</td>
<td>0.62</td>
<td>&lt;0.01</td>
<td>0.57</td>
<td>&lt;0.01</td>
<td>0.11</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>MC(_L)</td>
<td>0.37</td>
<td>&lt;0.01</td>
<td>0.22</td>
<td>&lt;0.01</td>
<td>0.50</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B(_L)</td>
<td>0.67</td>
<td>&lt;0.01</td>
<td>0.66</td>
<td>&lt;0.01</td>
<td>0.36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>n</td>
<td>1465</td>
<td>185</td>
<td>214</td>
<td>195</td>
<td>546</td>
<td>78</td>
</tr>
</tbody>
</table>

2.4 Discussion

Conductance & Internal carbon dynamics

The finite internal conductance observed in all species results in a significant decrease in CO\(_2\) concentrations from C\(_i\) to C\(_c\), imposing a large limitation on photosynthesis. This supports suggestions that mesophyll conductance plays a big role in the control of photosynthesis, and may decrease rapidly after the onset of water stress (e.g. Centritto et al. 2003; Flexas et al. 2008b; Niinemets et al. 2009b). Measurements in the literature are scarce, but the values reported here compare well against available data.

As mesophyll conductance controls the concentration of carbon in the chloroplast, which in turn limits the rate of photosynthesis, this response can also be seen in the relation between mesophyll conductance and assimilation rates (Fig 2.1b), with little difference resulting in changes of mesophyll conductance above 0.2-0.3 mol m\(^{-2}\) s\(^{-1}\), and large changes below. The higher threshold observed here highlights the sensitivity of the rate of photosynthesis to the concentration of carbon available for assimilation. The tight
coupling between the rate of photosynthesis, the concentration of carbon in the chloroplast and mesophyll conductance shows the importance of mesophyll conductance in the control of photosynthesis.

Similar responses of drawdown to changes in mesophyll conductance were observed in the deciduous species at Collelongo and Roccarespampani, with each showing large gradual responses to changes in mesophyll conductance (Fig. 2.1a), possibly reflecting structural characteristics of deciduous leaves. This was not observed at the deciduous species, *Quercus douglasii* at Tonzi however, though Tonzi receives almost half the rainfall than Collelongo and 50% less than falls in Roccarespampani, leading to high levels of water stress and lower conductance. *Quercus Douglasii* leaves have a low metabolic rate, are very coarse and well structured, thus possibly limiting internal diffusion. The response observed for *Quercus Douglasii* was similar to that of the broadleaf evergreen species *Quercus ilex* at Puechabon. This response was less noticeable for the needle leaf evergreen species *Pinus ponderosa* at Blodgett, possibly due to the large quantity of rainfall and high soil water availability. The real determinants of differences in mesophyll conductance are, however, as yet unknown, though it has been suggested that leaf anatomy, morphology and biochemical factors are all involved (Warren 2008b; Niinemets *et al.*, 2009a).

Finite internal conductance affects estimates of the maximum carboxylation velocity, $V_{c_{\text{max}}}$, and their interpretation. Most published estimates of $V_{c_{\text{max}}}$ will therefore underestimate the true $V_{c_{\text{max}}}$ by not considering the draw-down from $C_i$ to $C_c$. This is of equal importance when considering photosynthesis in well watered conditions as in water stressed conditions, given that the concentration of carbon in the chloroplast proved to be a better estimator of assimilation rates in both.

The finding that $C_i-C_c$ varies between species and as a function of available soil water goes against some other previous studies (von Caemmerer & Evans 1991; Loreto *et al.* 1992; Evans 1999; Evans & Loreto 2000). These studies solely considering response curves of assimilation against mesophyll conductance, with few limited data sets. The analysis here included the response of the concentration of CO$_2$ in the chloroplast, and with an extremely large data set, highlights the imperfect scaling of assimilation with
mesophyll conductance and demonstrates that different magnitudes of species or site dependent responses are possible.

**Limitations**

The debate over the relative roles of stomatal conductance, mesophyll conductance and biochemical processes in the limitation of photosynthesis continues, with several studies favouring one or the other control mechanism (Stomatal: Sharkey 1990; Cornic et al. 1989. Mesophyll: Warren & Adams 2006; Galmes et al. 2007. Biochemical: Wilson et al. 2000; Xu & Baldocchi 2003). Here we have demonstrated that mesophyll conductance reacts strongly to water stress as in previous studies (Flexas et al. 2002; Warren et al. 2004). Both the loss of turgor (Cornic et al. 1989; Renou et al. 1990), and the activity of aquaporins (Terashima & Ono 2002; Miyazawa et al. 2008; Kaldenhoff et al. 2008) have been highlighted as possible mechanisms behind this decline in mesophyll conductance. The magnitude of this reaction to water stress was shown to vary between sites, with no clear signal of a general tendency of either stomatal or mesophyll conductance dominance over photosynthetic reactions to water stress. This study was not capable of ending the debate, but showed that a variety of responses is possible, with \( SC_L \) or \( MC_L \) both capable of responding strongly to water stress in different species under the same analytical methodology. In Puéchabon, Roccarespampani and Yatir, the response of mesophyll conductance to water stress is stronger than that of stomatal conductance at low soil water levels. The contribution of \( SC_L \) at low soil water availability was strongest at Collelongo, Blodgett and Tonzi. This may be due to the amount of soil water stress experienced, with Puéchabon, Roccarespampani and Yatir characteristically encountering more water stress, thus invoking stronger \( MC_L \).

There is no clear understanding of the processes responsible for \( B_L \), with many different possibilities highlighted in the literature. Particular attention has been paid to stress induced leaf senescence (Kramer 1983), inhibition due to high temperatures (Haldimann & Feller 2004), denitrogenisation (Grassi & Magnani 2005), enzyme deactivation (Tezara et al. 1999, Lawlor 2002), activity of aquaporins (Flexas et al. 2004; Kaldenhoff et al. 2008; Miyazawa et al. 2008) among many other possible mechanisms (Kaiser 1987; Chaves et al. 2002; Lawlor & Cornic 2002; Bota, Medrano & Flexas 2004; Flexas et al. 2004). By assuming no stress affect on \( Vc_{\text{max}} \) we explicitly omit the possibility of
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$B_L$ due to water stress changes in carboxylation capacity in our analysis, and focus on the balance between the two conductance limitations. Limited data is available on the response of $V_{C_{\text{max}}}$ to water stress, and that which is available has not taken into account the effect of a variable mesophyll conductance. The inclusion of a stress induced reduction in $V_{C_{\text{max}}}$ proportionally reduces the role of both $SC_L$ and $MC_L$, and has been demonstrated to be sufficient for modelling the stress response of carbon and water fluxes (Reichstein et al., 2003; Keenan et al., 2009). With higher $B_L$, $SC_L$ and $MC_L$ would decrease in parallel (Grassi & Magnani, 2005), but the $SC_L:MC_L$ ratio reported here would not change. More field measurements of $V_{C_{\text{max}}}$, together with measurements of mesophyll conductance, are needed to fully understand the processes involved and their role under increasing stress levels.

The presented results suggest that mesophyll conductance may be more limiting for photosynthesis than stomatal conductance in many Mediterranean forest species, regardless of their growth form and leaf anatomy. This is consistent with a predominant role of metabolic rather than structural determinants of mesophyll conductance, such as aquaporins (Flexas et al. 2006b). The relative limitations reported here are similar to those reported for other tree species (e.g. Epron et al. 1995; Warren et al. 2003a) and underline the fact that internal conductance constitutes a large limitation on CO$_2$ fixation and thus its inclusion in process based models of carbon exchange may improve our ability to model carbon and water fluxes, particularly in water stressed environments (Ethier & Livingston 2004; Niinemets et al., 2009b).

**Methodological considerations**

The presented work makes several assumptions regarding possible influencing factors which could not be quantified from the available data. It was first necessary to assume neither stomatal patchiness nor cuticular conductance have a significant effect when making calculations which average over the whole canopy. It has been reported that, in leaf level experiments, stomatal patchiness may invalidate leaf internal carbon calculations, in particular in drought conditions (Buckley, Farquhar & Mott 1997; Mott & Buckley 2000). Other more recent studies, however, have shown that the influence of stomatal patchiness over calculations of internal carbon do not carry so much weight as had been earlier reported (Lawlor & Cornic 2002). Also, it has been reported that the
effect of stomatal patchiness is not as large in the field as it is in laboratory experiments, due to the slow time scale of the onset of drought (Gunasekera & Berkowitz 1992; Kubiske & Abrams 1993).

The procedure used to estimate the electron transport rate through the closure of the canopy energy budget by numerical iteration could generate inaccuracies. In particular, it has been suggested that the electron transport rate is negatively affected by water stress (Sharkey et al. 1982, Flexas et al. 1999), though the results were not transferable to field studies and were only significant at extremely low soil water potentials. It has been shown that even a large overestimation by 50% in the electron transport rate results in at most a 4–8% underestimation of $G_m$, (Niinemets et al. 2006) and the degree of underestimation was larger for higher values of internal diffusion conductance, that is, for a situation where a large difference in internal diffusion has a small effect on the concentration of carbon in the chloroplast. This agrees with previous evidence demonstrating that the sensitivity of $G_m$ to minor errors in gas-exchange and fluorescence measurements increases with increasing $G_m$ (Harley et al. 1992; Ethier & Livingston 2004). The larger scatter observable between in the values of response variables at higher $G_m$ values in our study further supports this. Therefore, the determination of $G_m$ for species with inherently low internal conductance such as the Mediterranean species studied here is concluded to be robust against small deviations in the estimation of the electron transport system (Harley et al. 1992; Niinemets et al. 2006). We tested this robustness by reducing $J_{\text{max}}$ in proportion to soil water levels and comparing the resulting mesophyll conductance levels to those reported above. This led, on average, to an 11% reduction in the estimated mesophyll conductance during periods of high water stress, but did not change the qualitative nature of the responses shown. The presence of alternative electron sinks may underestimate the rate of internal conductance. However, a lack of alternative electron sinks has been demonstrated over a large temperature gradient (Badger et al., 2000).

2.5 Conclusions

The results of this study highlight the importance of mesophyll conductance in the control of photosynthetic rates, both in well watered conditions and in response to soil
water stress. The drawdown of carbon from the leaf inter-cellular spaces to the chloroplast was shown to scale imperfectly with mesophyll conductance, implying a strong role of mesophyll conductance, in particular at low internal carbon concentrations in the regulation of photosynthesis. The concentration of carbon in the chloroplast was shown to be a more reliable estimator of assimilation rates than the inter-cellular carbon concentration. This is of importance when modelling photosynthetic rates, both in well watered and dry conditions. A strong response of mesophyll conductance to soil water stress was observed at all sites, further limiting the concentration of carbon available for assimilation. A complex dynamic was observed at each site between the responses of stomatal and mesophyll conductance limitations. These results have important implications for canopy-level photosynthesis modelling, and may explain the some of the difficulties ecosystem models encounter when modelling carbon and water fluxes in Mediterranean conditions as, to date, no published model fully incorporates mesophyll conductance. This could be of particular importance in the estimation of Mediterranean primary production, both in current day conditions and when modelling responses to future climate change scenarios.

Acknowledgments

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Chapter 3

Modelling Water Stress Effects on Photosynthesis with Mesophyll Conductance
Mesophyll conductance, soil water stress and coupled photosynthesis-conductance models: Bridging the gap between conflicting reports on the relative roles of stomatal, mesophyll conductance and biochemical limitations to photosynthesis.

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2 Department of Ecology, University of Barcelona (UB).

Abstract

Process based terrestrial vegetation models perform poorly in water stressed environments. Various plant responses to water stress have been reported, but conflicting reports as to which limiting process is the most important and ecophysiological relevant during water stressed periods make it difficult to confidently model carbon and water flux responses to water stress. It has become increasingly accepted that mesophyll conductance could play a role in regulating photosynthesis during periods of water stress. We adapt the Farquhar BB-type canopy photosynthesis-conductance model coupling to incorporate mesophyll conductance, embed it in an ecophysiological forest model and use it to simulate photosynthesis and the effects of seasonal soil water stress on canopy carbon and water fluxes at a Quercus ilex forest in southern France. The tests of the various hypotheses regarding the relative roles of stomatal conductance limitations ($SC_L$), mesophyll conductance limitations ($MC_L$) and biological limitations ($B_L$) confirm that during water stressed periods, applying only $B_L$ allows for the accurate simulation of carbon and water fluxes. Neither $SC_L$ nor $MC_L$ alone could accurately reproduce the observed carbon and water fluxes. However, a
combination of both $MC_L$ and $SC_L$ was successful at reproducing water stress induced reductions in carbon and water fluxes, suggesting that previous calls for a revision of the current hypothesis on the role of stomata in the regulation of plant responses to water stress may be premature and that mesophyll conductance could bridge the gap between conflicting reports on the processes behind responses to water stress in the field.
3.1 Introduction

Process-based models that incorporate the relevant ecosystem processes to successfully simulate the sensitivity of ecosystem functioning to drought stress at all relevant time scales are indispensable tools. Such models of natural vegetation can assess the influence of stresses on the photosynthetic capacity of plants (Centritto et al., 2003; Loreto et al., 2003; Loreto & Centritto, 2008) and predict the effects of climate change on photosynthesis (Rogers & Humphries, 2000), thus helping to assess the vulnerability of ecosystems. Moreover, they are the only available tool for scaling carbon assimilation up from the leaf to whole plant and/or ecosystem (Harley & Baldocchi, 1995; Niinemets & Anten, 2009). Previous modelling studies, however, have highlighted the lack of model skill, at site, regional and global scales, to successfully reproduce the effects of seasonal stress due to low soil water availability, in particular in Mediterranean ecosystems (Morales et al., 2005, Jung et al., 2007).

Projections of climate change (IPCC, 2007) suggest that higher temperatures, and increased potential evapotranspiration, as well as changes in seasonal precipitation patterns, will aggravate the seasonal drought stress characteristic to Mediterranean ecosystems (Giorgi et al., 2004; Giorgi, 2006; Beniston et al., 2007). Soil water availability is already the main limiting factor to global plant photosynthesis (Nemani et al., 2003), in particular in arid or semi-arid ecosystems within Mediterranean climate regions (Boyer, 1982). An increase in anomaly events such as the 2003 European drought could also increase the amount of stress experienced in many non-Mediterranean forest ecosystems in Europe. However, despite recent advances (e.g. Grassi & Magnani, 2005), the effect of soil water stress on photosynthesis, and the vulnerability of Mediterranean vegetation to climate change, will not be fully understood until we untangle the ecophysiological responses and the main limitations imposed by water stress on leaf photosynthesis (Loreto & Centritto, 2008).

Many different approaches have been developed over the last two decades to simulate the carbon and water fluxes of a forest ecosystem. These biogeochemical models assess mass and energy exchange between the canopy and the atmosphere by coupling the fluxes of carbon dioxide and water vapour (Wang & Jarvis, 1990; Aber & Federer,
1992; Amthor, 1994; Baldocchi & Harley, 1995; De Pury & Farquhar, 1997). The leaf
physiologically based photosynthesis model by Farquhar et al. (1980), linked with a
stomatal conductance model (Jarvis, 1976; Ball et al., 1987; Collatz et al., 1991;
Leuning et al., 1995) provides a theoretical framework for spatially scaling up fluxes
from leaf to canopy level. This process-based model coupling has proven hugely
popular, and accurately integrates the canopy functioning over time scales from seconds
to years (e.g., Caldwell et al., 1986; Leuning et al., 1995; Williams et al., 1997; Wang
& Leuning, 1998; Keenan et al., 2009). Although such models are capable of giving
very accurate results, they are not efficient at dealing with all conditions, in particular
with water stress (Kramer et al. 2002; Morales et al. 2005).

It has recently been shown that part of the model deficiency is due to the assumption of
the sole control of stomatal conductance limitations (SC_L) over photosynthesis during
water stressed periods (Keenan et al., 2009). The inclusion of empirical ‘non-stomatal’
limitations has been shown necessary to successfully model carbon and water fluxes in
water stressed environments (e.g. Colello et al., 1998; Reichstien et al., 2002; Rambal et
al., 2003; Xu & Baldocchi, 2003), with both carbon and water fluxes being accurately
modelled during water stressed periods through the reduction of photosynthetic
potential (B_L) in line with reductions in soil water availability (Keenan et al., 2009).
These model results suggest that stomatal aperture is driven by photosynthetic demand,
and that the stomata do not play an active role in the long term regulation of
photosynthesis during water stressed periods. This is controversial, as stomata have
been widely reported to play a strong role in regulating photosynthesis during water
stress (see Lawlor & Cornic, 2002, for a review). The conclusions from most studies, in
particular model studies, were reached without taking into account the possible effects
of a finite and changeable mesophyll conductance (g_m).

Carbon which enters the inter-cellular spaces of the leaf through the stomata diffuses
through the mesophyll to the site of carboxylation in the chloroplast. It is becoming
increasingly accepted that g_m is finite and variable (Warren, 2008b), and that the
concentration of carbon in the inter-cellular spaces (C_i) differs from the concentration of
carbon in the chloroplast (C_c) in a manner that is highly variable within species and
affected by a range of environmental conditions (Flexas et al., 2007; Warren et al.,
2008b). Limitations of photosynthesis due to changes in g_m (MC_L) could play a role in
plant responses to water stress (Grassi & Magnani, 2005; Galmes et al., 2007; Keenan et al., (in review)). This strengthens the need to incorporate a term that considers $g_m$ in current photosynthesis models, such as that by Farquhar et al. (1980), thus calculating photosynthetic rates based on $C_c$ instead of on $C_i$.

In the current analysis, different hypothesis are tested, challenging current assumptions regarding the relative roles of $SC_L$, $MC_L$ and $B_L$ to photosynthesis and conductance, and how to model them, during seasonal water stressed periods in the field. We modified the Farquhar et al. (1980) model to incorporate mesophyll conductance and thus calculated photosynthesis on a $C_c$ basis. This was coupled to the widely used Leuning et al. (1995) version of the Ball-Berry conductance model (Ball et al., 1987), and embedded in the biogeochemical forest growth model GOTILWA+ (Gracia et al., 1999; Keenan et al., 2008; Keenan et al., 2009). We could therefore test the effect of the inclusion of a water stress responsive stomatal conductance, mesophyll conductance or photosynthetic potential on modelled carbon and water fluxes. Carbon and water eddy-covariance flux data from the five year period from 2001 to 2005 at the Puechabon Mediterranean Quercus ilex forest site in France are used to test model performance during water stressed periods.

3.2 Materials and Methods

*Fluxnet Site Data*

Data and simulations refer to Puechabon State Forest study site, located 35 km NW of Montpellier (southern France) (3°35’45”E, 43°44’29”N, elevation 270 m). Vegetation is largely dominated by a dense over-storey of Quercus ilex trees (Allard et al., 2008). Due to its typical Mediterranean-type climate (with warm and wet winters, and hot and dry summers), the water content in summer falls regularly below the value at which water stress limitations to photosynthesis are expected (Rambal et al., 2003), leading to extended seasonal periods of drought stress. The timing and extent of drought conditions varies from year to year, depending on temperature and precipitation. The mean annual temperature is 13.5 °C with mean annual precipitation of 903 mm (Allard et al., 2008). Rooting depth is 4.5 meters, with a maximum soil water holding capacity
of 210mm (Serge Rambal, personal communication). Soil texture is homogeneous down to 0.5 m depth and can be denoted as silty clay loam (referring to the textural triangle, United States Department of Agriculture), with a limestone rock base. For more details on the site see http://www.cefe.cnrs.fr/fe/puechabon/.

Climate, and eddy-covariance carbon and water (latent heat flux equivalent) flux Measurements from the period 2001 to 2005 are used, which had an average annual air temperature of 14.5 °C and average annual precipitation of 965 mm. The selected focus year 2002 was wetter (total precipitation 1166 mm) than the long-term average but was also warmer than average, (average temperature for 2002 of 14.7 °C). The high soil water availability during spring, and the relatively high temperatures, led to high production in spring 2002. The corresponding high levels of production related transpiration, and a relatively dry summer which was uninterrupted by strong rainfall events led to a sharp and extended drought period during 2002. This contrast between high spring production and a prolonged uninterrupted summer drought makes 2002 at Puechabon a good example year to test different approaches to modelling soil water stress responses.

**Modified photosynthesis model with mesophyll conductance**

The photosynthesis model of Farquhar, von Caemmerer & Berry (1980) was modified to explicitly calculate mesophyll conductance and therefore accurately calculate the quantity of carbon available for assimilation in the chloroplast, through a set of coupled equations. The Farquhar *et al.* (1980) model states that the rate of net photosynthesis ($A_n$) is the minimum of the carboxylation rate limited by the amount, activation state, and kinetic properties of Rubisco (RuBP limited $A_c$); and the carboxylation rate limited by the rate of RuBP regeneration (Electron transport limited $A_j$):

$$A_n = \min \{A_c, A_j\} \quad (3.1)$$

For RuBP limited photosynthesis the net CO₂ assimilation rate is limited by $A_c$:

$$A_c = Vc_{\text{max}} \ast (C_c - \Gamma^*) / (C_c + (K_c \ast (1 + (O_i / K_o)))) \quad (3.2)$$
where $C_c$ is the concentration of CO$_2$ in the chloroplast, $\Gamma^*$ is the CO$_2$ compensation point in the absence of mitochondrial respiration, $V_{C_{\text{max}}}$ is the maximum rate of Rubisco carboxylation, $O_i$ is the O$_2$ concentration, and $K_c$ and $K_o$ are the Michaelis–Menten constants describing carboxylation and oxygenation. For electron transport limited photosynthesis (Harley & Tenhunen, 1991):

$$A_j = ((J / 4) * (C_c - \Gamma^*) / (C_c + (2 * \Gamma^*)))$$  \hspace{1cm} (3.3)

where $J$ is the rate of electron transport, dependent on irradiance, leaf temperature, and the capacity of electron transport ($J_{\text{max}}$) (De Pury & Farquhar 1997).

These calculations of photosynthesis require the concentration of carbon in the chloroplast, which is a function of $A_n$, stomatal conductance ($g_s$) and mesophyll conductance ($g_m$). Traditional modelling approaches estimate stomatal conductance as a function of irradiance, the balance of the supply and demand of carbon, and vapour pressure deficit. Ball et al. (1987) proposed a semi-empirical stomatal model (BB model) in which stomatal conductance was expressed by the leaf photosynthetic rate, relative humidity over a leaf surface, and the ambient CO$_2$ concentration, under conditions of ample water supply (Ball et al., 1987). This model was later developed by Leuning (1995) (BBL model) to include the effects of vapour pressure deficit and the photorespiratory compensation point (Leuning, 1995; Leuning et al., 1995):

$$g_s = g_{s0} + m(A_n - R_d)/(C_a - \Gamma^*).(1 + (vpd / D_0)))$$  \hspace{1cm} (3.4)

where $g_s$ (mol H$_2$O m$^{-2}$ s$^{-1}$) is stomatal conductance to moisture, $g_{s0}$ is the value of $g_s$ at the light compensation point (mol m$^{-2}$ s$^{-1}$), $A_n$ is the rate of net photosynthesis (µmol m$^{-2}$ s$^{-1}$), $R_d$ is the rate of dark respiration (µmol m$^{-2}$ s$^{-1}$), $C_a$ is the atmospheric concentration of CO$_2$ at the canopy surface (µmol mol$^{-1}$), $\Gamma^*$ is the photorespiratory compensation point (µmol mol$^{-1}$), $D_0$ (unitless) is an empirical coefficient that describes the sensitivity of conductance to $vpd$ (vapour pressure deficit, kPa), and $m$ is an empirical species-specific factor that specifies the baseline ratio between conductance and net photosynthesis (unitless).
The concentration of carbon inside the chloroplast, $C_c$ is then a function of the net photosynthesis (assimilation rate) $A_n$, stomatal conductance to carbon, $g_{sc} (=g_s/1.6)$, and $g_m$:

$$C_c = C_a - (A_n/g_{sc}) - (A_n/g_m)$$  \hspace{2cm} (3.5)

where $C_a$ (μmol mol$^{-1}$) is the atmospheric carbon concentration and $g_m$ is the mesophyll diffusion conductance from the sub-stomatal cavities to the chloroplasts.

This set of equations (Eqn. 3.1 – 3.5) complete the mesophyll conductance based photosynthesis model, and are solved iteratively by closing the leaf energy balance through leaf temperature. Values for $K_c$, $K_o$, $O_i$ and $\Gamma^*$ were taken from Bernacchi et al. (2001). $J_{\text{max}}$ and $V_{c\text{max}}$ were calculated following Farquhar et al. (1980) and de Pury & Farquhar (1997).

The assessment of the inclusion of $g_m$ in the Farquhar BB-type model coupling was conducted using three reference values for $g_m$. A $g_m$ of 0.2 mol m$^{-2}$ s$^{-1}$ corresponds to a relatively high rate of mesophyll conductance encountered in plants adapted to relatively moist growing environments. The values of $g_m$ applied of 0.1 and 0.05 mol m$^{-2}$ s$^{-1}$ correspond to moderately low diffusive conductance, and very low diffusive conductance (see Niinemets et al., 2009, Galmes et al., 2007 for recent reviews of species $g_m$ values). $g_m = \infty$ refers to the original Farquhar et al. (1980) model, which assumes infinite mesophyll conductance. Many other factors have been suggested to affect $g_m$, e.g. leaf age, environmental, ontogenic and genetic modification in leaf structure, salinity stress, water stress, radiation and VPD (see Flexas et al., 2008 for a review). There is as yet no consensus as to how such factors could be implemented when modelling with consideration of $g_m$. No direct temperature effect was applied to $g_m$, as the effect of temperature on $g_m$ is also unresolved, with both positive and negative responses reported in the literature (Bernacchi et al., 2002; Pons & Welschen, 2003).
The effect of soil water stress on coupled photosynthesis-conductance models

Model studies have shown that the application of $B_L$ is sufficient to accurately model water stress affects on carbon and water fluxes (Reichstein et al., 2002; Keenan et al., 2009), whilst it is commonly upheld that the stomata ($S_{CL}$) (e.g., Cornic, 2000), and the mesophyll ($M_{CL}$) (e.g. Warren, 2008), play a strong role in regulating water stress responses (e.g., Grassi and Magnani, 2005, but see Loreto & Centritto, 2008). We test the following four hypotheses for the incorporation of the effect of drought stress on the (Farquhar - BB-type) coupled photosynthesis-conductance model:

1) Directly reducing photosynthesis in relation to reductions in soil water availability sufficiently constrains carbon and water fluxes during water stressed periods (biological limitations, $B_L$). This incorporates all reported non-conductance limitations.

2) Directly reducing stomatal conductance in relation to reductions in soil water availability is sufficient to explain variation in carbon and water fluxes during drought stressed periods (stomatal conductance limitations, $S_{CL}$).

3) Directly reducing mesophyll conductance in relation to reductions in soil water availability is sufficient to explain variation in carbon and water fluxes during drought stressed periods (mesophyll conductance limitations, $M_{CL}$).

4) A combination of conductance limitations ($C_L$) can accurately simulate the observed flux data ($C_L = S_{CL} + M_{CL}$).

We tested the hypotheses by imposing the three limitations, either individually or combined, to: a) photosynthetic potential ($B_L$), b) stomatal conductance ($S_{CL}$), and c) mesophyll conductance ($M_{CL}$).

Biological limitations were imposed by the direct reduction of photosynthetic potential through reducing both $V_{C_{\text{max}}}$ and $J_{\text{max}}$ during water stressed periods, in line with the relative soil water content (as in Chapter 1):

$$V_{C_{\text{max}}} = V_{C_{\text{max}}} * W_{\text{fac}_{\text{photo}}}$$
$$J_{\text{max}} = J_{\text{max}} * W_{\text{fac}_{\text{photo}}},$$

(3.6)
where $V_{c_{\text{max}}}$ and $J_{\text{max}}$ are the maximum rate of RuBP carboxylation, and the maximum rate of electron transport, respectively, and $W_{\text{fac}_{\text{photo}}}$ is a soil moisture-dependent scalar with values between 0 and 1. This $B_L$ function directly reduces photosynthesis under drought stress, thus reducing the demand for carbon and reducing conductance.

Stomatal control of photosynthetic responses to drought stress is modelled through the application of a linear scalar of soil moisture, in a modified version of Eq. 3.4 (as in Chapter 1):

$$g_s = g_{s0} + ((W_{\text{fac}_{\text{stoma}}})(A_n - R_d)) / ((C_a - \Gamma^*)(1 + (vpd / D_o)))$$  \hspace{1cm} (3.7)

where $W_{\text{fac}_{\text{stoma}}}$ is a soil moisture-dependent scalar with values between 0 and 1. This $SCL$ function results in a reduction in conductance with reductions in soil moisture, thus limiting the leaf internal CO$_2$ available for photosynthesis.

Mesophyll conductance limitations were modelled by applying a similar reduction function $W_{\text{fac}_{\text{meso}}}$ to $g_m$ ($g_m = W_{\text{fac}_{\text{meso}}}, g_m$). This $MC_L$ function thus results in a direct reduction in mesophyll conductance with reductions in soil moisture.

The three limitation functions, $W_{\text{fac}_{\text{photo}}}, W_{\text{fac}_{\text{stoma}}}$ and $W_{\text{fac}_{\text{meso}}}$, have the following form:

$$W_{\text{fac}} = \begin{cases} 1, & \text{if} \ S(t) \geq S_{\text{max}} \\ \left[ \frac{s(t) - s_{\text{min}}}{s_{\text{max}} - s_{\text{min}}} \right]^q, & \text{if} \ S(t) < S_{\text{max}} \end{cases}$$ \hspace{1cm} (3.8)

where $q$ is a measure of the non-linearity of the effects of soil water stress on physiological processes, $s_{\text{max}}$ the relative soil water content at which reductions are first evident, and $s_{\text{min}}$ is the wilting point. For the sake of comparative simplicity, we have assumed that increases or decreases in limitation strength for a particular limitation ($B_L$, $SCL$, $MC_L$)}
$S_L$, $MC_L$) can be represented by changes in the $q$ value. Thus $s_{\text{max}}$ and $s_{\text{min}}$ remain constant between limitations ($s_{\text{max}}=0.8$, $s_{\text{min}}=0.38$ (168, 80 mm)).

**The biosphere model platform**

The modified coupled Farquhar BB-type model was embedded in the ecophysiological model platform GOTILWA+ (Growth of Trees Is Limited by WAter), (Gracia et al., 1999; Chapter 1; www.creaf.uab.es/Gotilwa+). GOTILWA+ is a process based forest growth model, described in Chapter 1, that has been developed to simulate the processes underlying growth and to explore how these processes are influenced by climate, tree stand structure, management techniques, soil properties and climate change. The GOTILWA+ model provides the biogeochemical background, and canopy microclimatic conditions necessary for the simulation of carbon and water fluxes from forests in different environments, for different tree species, and under changing environmental conditions.

See Keenan et al. (2007) and Chapter 1 for model applications at this site.

**Simulation methodology used**

The inclusion of $g_m$ requires the recalibration of photosynthetic parameters (Niinemets et al., 2009 (in press)). Simulations were run with each fixed $g_m$ value (0.2, 0.1, 0.05 mol m$^{-2}$ s$^{-1}$) for the year 2002 at Puechabon, to calibrate the photosynthetic parameters $V_{C_{\text{max}}}$ and $J_{\text{max}}$, and to assess the impact of the inclusion of $g_m$ in the Farquhar-Ball Berry model coupling. Soil water stress was not considered for these simulations (i.e. Soil water content = soil water holding capacity at all times).

Independent simulations were then run with a dynamic soil water content to test the different water stress response hypotheses as outlined above. In each case, the strength of the limitation was incrementally increased (see Eq. 3.8) until the least square fit was satisfied for either the carbon (net assimilation rate) or water (latent heat flux) fluxes, when compared against the FLUXNET data. The different hypotheses which proved valid for 2002 were then applied, without further calibration, in comparative simulations.
for the five year period from 2001 to 2005, and compared against FLUXNET carbon and water flux data.

3.3 Results

The effect of the inclusion of mesophyll conductance on simulated carbon and water fluxes

Simulations using a \( g_m \) of 0.2 or 0.1 mol m\(^{-2}\) s\(^{-1}\) compared well to results from the traditional \( g_m = \infty \) approach, once the photosynthetic parameters (\( V_{\text{c,max}}, J_{\text{max}} \)) were adjusted (Fig. 3.1). No significant differences were observable for daily assimilation rates or rates of transpiration between the \( g_m = \infty \) and the \( g_m = 0.2 \) (\( A_n \) t-stat = 1.02, \( p = 0.9 \); \( E_a \) t-stat = 0.9, \( p = 0.42 \)) or \( g_m = 0.1 \) (\( A_n \) t-stat = 0.09, \( p = 0.92 \); \( E_a \) t-stat = 0.82, \( p = 0.41 \)) simulations. For \( g_m = 0.1 \) simulations, largest differences were observed in the simulated daily assimilation and transpiration rates at high temperatures and high \( vpd \) when compared to the \( g_m = \infty \) simulations. No change in water use efficiency was observed for \( g_m = 0.1 \) simulations compared to \( g_m = \infty \) simulations. Simulating with \( g_m = 0.05 \) led to significant differences in daily assimilation rates (t-stat = -3.2, \( p < 0.01 \)), most notably at high temperatures and high \( vpd \). Large significant differences (t-stat = 4.8, \( p < 0.001 \)) were observed in daily transpiration rates between the \( g_m = \infty \), and \( g_m = 0.05 \) simulations, due to increased water use efficiency (Fig. 3.1). These differences were larger at higher temperatures, radiation and high \( vpd \) values.
Chapter 3: Modelling photosynthesis with mesophyll conductance

Figure 3.1. Modelled non water stressed responses of daily canopy net assimilation (An), transpiration (Ea, mm day$^{-1}$), and water use efficiency (WUE, mmols mol$^{-1}$) to temperature (T, °C), global radiation (MJ m$^{-2}$ day$^{-1}$), and vapour pressure deficit (VPD, kPa) for different values of mesophyll diffusion conductance ($g_m$, mol m$^{-2}$ s$^{-1}$), at the Puechabon Forest in 2002. Simulations were run using the coupled Farquhar BB-type model coupling, embedded in the GOTILWA+ forest model (Keenan et al., 2008, 2009), modified to calculate assimilation based on $C_c$ ($An = f(C_c, V_c max, J_{max, Rd})$ through equations 1-5. Curves for ‘$g_m = infinity$’ represent simulations run with no consideration for mesophyll diffusion conductance ($An = f(C_i, V_c max, J_{max, Rd})$). Error bars represent the standard deviation from the mean. WUE is defined as the molar ratio of assimilation to transpiration. Daily values were assigned to 14 bins ($n = 364$). For each $g_m$ value, the following canopy integrated photosynthetic parameters were used: $g_m = \infty$, $V_c max = 35$, $J_{max} = 70$; $g_m = 0.2$, $V_c max = 42.5$, $J_{max} = 70$; $g_m = 0.1$, $V_c max = 51$, $J_{max} = 70$; $g_m = 0.05$, $V_c max = 85$, $J_{max} = 80$)
Testing of the first three hypotheses showed that the application of restrictions to photosynthetic potential ($B_L$) allowed for the effective reproduction of both carbon and water fluxes (Fig. 3.2) during the water stressed period in 2002 at Puechabon (An $r^2$: 0.92, RMSE: 1.8; LHF $r^2$: 0.66, RMSE: 1.8). Both the assimilation rate and the latent heat flux (equivalent to evapotranspiration) reduced in parallel when simulating with $B_L$ (Eq. 3.6), thus conserving the water use efficiency (Fig. 3.3). The reduction of $g_s$ alone through the application of $Wfac_{stoma}$ ($SC_L$: Eq. 3.7) reduced stomatal conductance (and thus the latent heat flux), but assimilation rates remained high due to increased water use efficiency (Fig. 3.3). This lead to a large overestimation of total assimilated carbon during the water stressed period of 2002 (An $r^2$: 0.7, RMSE: 8.9; LHF $r^2$: 0.7, RMSE: 5.0).

Implying mesophyll conductance limitations ($MC_L$) through reducing $g_m$ proved more effective at modelling the water stress induced drop in assimilation rates than did $SC_L$, but less effective at simulating water fluxes (An $r^2$: 0.75, RMSE: 4.5; LHF $r^2$: 0.53, RMSE: 5.9) (Fig. 3.2). The application of $MC_L$ was most ineffective at reproducing fluxes at mild water stress (between RSW of 0.5 and 0.7), where water fluxes were accurately simulated but slight overestimations in water use efficiency (Fig. 3.3) led to a much higher predicted assimilation rate than that observed (Fig. 3.2).
Figure 3.2. Water stressed simulated and FLUXNET measured responses of daily canopy net assimilation ($A_n$, gC m$^{-2}$ day$^{-1}$), and daily latent heat flux (LHF, W m$^{-2}$) to changes in soil water content at the Puechabon forest during 2002. Simulations use the coupled Farquhar BB-type model, on a C$_3$ basis, embedded in the GOTILWA+ forest model (Keenan et al., 2008, 2009). Results from three simulations are presented, applying independently either: direct biological limitations to photosynthesis during water stress ($B_L$, Eq.6), stomatal limitations to photosynthesis ($SCL$, Eq. 7), or mesophyll conductance limitations to photosynthesis ($MC_L$). Non-stressed mesophyll diffusion conductance ($g_m$) was set at 0.1 mol m$^{-2}$ s$^{-1}$. Figure insets represent linear regressions between the measured and predicted variables ($B_L$: $A_n$ $r^2 = 0.92$, LHF $r^2 = 0.65$; $SCL$: $A_n$ $r^2 = 0.7$, LHF $r^2 = 0.69$; $MC_L$: $A_n$ $r^2 = 0.75$, LHF $r^2 = 0.52$. ($n = 77$)).
Figure 3.3. Water stressed simulated and FLUXNET derived responses of daily canopy water use efficiency (WUE, $\mu$mol mmol$^{-1}$) to changes in soil water content at the Puechabon forest in 2002. Simulations use the coupled Farquhar BB-type model, on a $C_3$ basis, embedded in the GOTILWA+ forest model (Keenan et al., 2008, 2009). Results from three simulations are presented, applying independently either: direct limitations to photosynthesis during water stress ($V_{c_{max}}/J_{max}$ Restrictions), stomatal limitations to photosynthesis ($g_s$ Restrictions), or mesophyll conductance limitations to photosynthesis ($g_m$ Restrictions). $g_m$ was set at 0.1 mol m$^{-2}$ s$^{-1}$. Figure insets represent linear regressions between the FLUXNET estimated and the simulated WUE ($B_L: r^2 = 0.25; SC_L: r^2 = 0.06; MC_L: r^2 = 0.25$. ($n = 77$)).
Our last hypothesis was that a joint combination of diffusive conductance limitations ($C_L$), with no limitation of photosynthetic potential, could match observed carbon and water fluxes during water stressed periods. Setting both conductance limitations to be of equal strength ($SC_L = MC_L$) allowed for an moderately accurate simulation of water (latent heat flux equivalent) fluxes, but carbon fluxes were not as well reproduced (Fig. 3.4a) (An $r^2$: 0.48, RMSE: 6.9; LHF $r^2$: 0.72, RMSE: 5.4). This follows from the results presented in Figures 2 and 3, as $SC_L$ reduces conductance but is not sufficient to reduce photosynthesis even with an additional $MC_L$ of equal strength. Further independent increases in stomatal restrictions ($SC_L > MC_L$) could not reduce assimilation (Fig. 3.4b), due to the increases in water use efficiency (see Fig. 3.3). An even further increase in $SC_L$, leads to underestimated latent heat fluxes, and overestimated assimilation rates (data not shown). Applying stronger $MC_L$ than $SC_L$ allowed for an accurate simulation of both carbon and water fluxes (Fig. 3.4c) (An $r^2$: 0.82, RMSE: 3.0; LHF $r^2$: 0.73, RMSE: 1.7). Carbon fluxes were not as accurately simulated as through the application of $B_L$ (Hypothesis 1, Fig. 3.2, 3.3), but the simulation of water fluxes slightly improved.
Figure 3.4. Water stressed simulated (applying combined conductance limitations: \( C_L \)) and FLUXNET measured responses of daily canopy net assimilation \((A_n, \text{gC m}^{-2} \text{day}^{-1})\), and daily latent heat flux \((\text{LHF}, \text{W m}^{-2})\) to changes in soil water content at the Puechabon forest during 2002. Simulations use the coupled Farquhar BB-type model, on a Cc basis, embedded in the GOTILWA+ forest model (Keenan et al., 2008, 2009). Results from three simulations to test \( C_L \) are presented, applying either: a) equal limitations to stomatal conductance and mesophyll conductance during water stress \((SCL = MCL)\), b) stronger stomatal limitations than mesophyll conductance limitations \((SCL > MCL)\), or c) stronger mesophyll conductance limitations than stomatal conductance limitations \((MCL > SCL)\). Non-stressed mesophyll diffusion conductance \((g_m)\) was set at 0.1 mol m\(^{-2}\) s\(^{-1}\). Figure insets represent linear regressions between the measured and predicted variables \(a: A_n r^2 = 0.47, \text{LHF } r^2 = 0.73; b: A_n r^2 = 0.43, \text{LHF } r^2 = 0.65; c: A_n r^2 = 0.81, \text{LHF } r^2 = 0.73 (n = 77))\).
**Long term comparison of approaches**

We tested the two effective modelling approaches (1: the application of $B_L$, 2: the application of $C_L$ with $MC_L > SC_L$) against five years of flux data for the period from 2001 to 2005 at Puechabon forest (data not used for the calibration of the response functions). Both model approaches proved effective at capturing the seasonal effect of soil water stress on carbon and water fluxes during the five year period (Fig. 3.5). Simulations with the $C_L$ (with $MC_L > SC_L$) approach and the $B_L$ approach explained 78% and 79% of the observed variance in canopy carbon fluxes respectively. This was confirmed by similar values of the Root Mean Squared Error (RMSE) statistic over the period, of 1.54 and 1.49 respectively. This was not true for the simulated latent heat flux. The $B_L$ approach achieved a higher correlation to the data ($r^2 = 0.75$ vs 0.71) but did not perform as well as the $C_L$ (with $MC_L > SC_L$) approach when considering the RMSE (17.1 vs 14.8). The $C_L$ approach performed markedly better than the $B_L$ approach at simulating water fluxes for 2001, 2004 and 2005, which were the years which received the least precipitation (2001: 847 mm; 2004: 705 mm; 2005: 875 mm; 5 year average: 966 mm).
3.4 Discussion

A common approach to modelling stomatal conductance is to assume a linear relationship with leaf- or canopy-level photosynthesis (Wong et al., 1979), the leaf surface concentration of CO₂, and relative humidity or vapour pressure, the so-called “Ball-Berry” (BB) and “Ball-Berry-Leuning” (BBL) parameterisations (Ball et al., 1987).
1987; Leuning et al., 1995). Such assumptions underpin the coupling of water and carbon cycles in many process-based ecosystem models, but water stress induced changes in conductance-photosynthesis relationships are as yet poorly understood.

The effect of water stress on plant photosynthesis and stomatal conductance has been widely studied (Wilson et al., 2000; Chaves et al., 2002; Grassi & Magnani, 2005), but there is little consensus as to those processes governing responses over seasonal time-scales (Loreto & Centritto, 2008; Warren, 2008). Recent studies have shown that ‘non-stomatal’ limitations remove the need for stomatal limitations when modelling drought affects on coupled carbon-water photosynthesis models (Rambal et al., 2002; Reichstein et al., 2002; Reichstein et al., 2003; Keenan et al., 2009), due to the linear relation between photosynthetic demand and stomatal conductance, and are supported by reported changes in photosynthetic potential in the field (e.g. Xu and Baldocchi, 2003). Such results are controversial, as they suggest that stomatal conductance responses are not necessary for plant function under long term seasonal water stress events.

It is well known that the stomata react to changes in soil water availability (e.g., Jones, 1973; Cornic, 2000; see Lawlor & Cornic, 2002 for a review). Stomata play a fundamental role in maintaining the water balance of the leaf, as water reserves in leaves and stems are very small when compared to the amount of water transpired, and thus could be quickly dehydrated in the absence of fast mechanisms, such as stomatal closure, to limit water loss (Slatyer, 1967). The effect of such stomatal closure is most commonly observed in the mid-day decline in stomatal conductance due to decreasing leaf water potential. This is suggested to limit photosynthetic activity through reducing $C_i$. Such short-term responses are, in theory, essential to conserving the plant hydraulic balance.

There is evidence that water stress impairs mesophyll metabolism (Chaves, 1991; Lawlor & Cornic, 2002; Flexas et al., 2008b). This has been linked to the hardening of cell walls and the increase in cell wall lignification in response to hydraulic signals (Escudero et al., 1992; Sobrado et al., 1992; Chazen et al., 1995; Chazen & Neumannmm, 1994; Henry et al., 2000), but could also be related to modifications in the permeability of membranes on the diffusion pathway from the intercellular spaces to the sites of carboxylation (Miyazawa et al., 2008). Strong responses have been reported in long
term drought cycles of varying length and severity, with the mesophyll response becoming progressively more important with increasing water stress (e.g. Flexas et al., 2002; Warren & Adams, 2004; Monti et al., 2006; Galmes et al., 2007c; Peeva & Cornic, 2009). Any changes in mesophyll conductance during periods of low soil water availability could potentially play an important role in controlling photosynthetic responses to water stress (Jones, 1973; Flexas et al., 2008).

We have shown that the inclusion of mesophyll conductance could bridge the gap between our current understanding gained from field and model experiments (e.g. Reichstein et al., 2002; Xu & Baldocchi, 2003; Keenan et al., 2009), and our functional understanding of leaf responses to water stress. The hypothesis that biological limitations are sufficient to accurately model carbon and water fluxes (without any conductance limitations) has been confirmed (as in Reichstein et al., 2002; Keenan et al., 2009), and it has been shown that stomatal conductance limitations alone inevitably overestimate water use efficiency, and therefore, can effectively simulate water fluxes during water stressed periods but overestimate carbon fluxes. Stomatal conductance limitations become effective at modelling carbon and water fluxes only when combined with mesophyll conductance limitations, and vice versa, suggesting a close relation between the two (Flexas et al., 2008b), and supporting previous suggestions of a joint role in regulating photosynthesis during periods of water stress (Grassi & Magnani, 2005).

Finite mesophyll conductance affects estimates of the maximum carboxylation velocity, $V_{C_{\text{max}}}$, and their interpretation. Most published estimates of $V_{C_{\text{max}}}$, and its responses to soil water stress, will therefore underestimate the true $V_{C_{\text{max}}}$ by not considering the draw-down from $C_i$ to $C_c$, and may potentially assign declines in assimilation rates due to changes in $g_m$ during water stress to declining photosynthetic potential. To the knowledge of the authors no published study, either in the field or model based, which reported strong declines in photosynthetic potential during drought periods, has taken into account the possible effects of a soil water stress responsive $g_m$. This does not suggest the exclusion of the possibility of biological limitations under stronger water stresses. Metabolic adjustments can take many forms, and may include the reduction of enzymes necessary for RuBP regeneration and activity (Maroco et al., 2002), reduced nitrate reductase activity (as an indicator of nitrate utilisation) (Smirnoff and Stewart,
1985), and the reduction of sucrose phosphate synthase (Vassey & Sharkey, 1989; Vassey et al., 2006). More field campaigns in ecosystems which are subject to seasonal cycles of water stressed periods will be necessary to improve our understand of the relative roles of each limitation.

The representation of carbon flux responses to drought often varies depending on the model applied, as it is a function of soil hydrology (with different approaches: single bucket soil module, two layer or multi-layer model), soil structure, forest structure and biogeochemistry assumptions. For example, the sensitivity to drought has been shown to be higher in models with a two layer soil hydrology model, and differences in soil structure and hydrology lead to differences in evapotranspiration calculations (Vetter et al., 2008). It has been assumed in this study that the eco-physiological state of the forest is well described in the model, as has been validated through previous applications of the model platform at this site (Keenan et al., 2007; Keenan et al., 2008).

\( g_m \) strongly varies both among and within species (Ethier & Livingston, 2004; Flexas et al., 2008; Niinemets et al., 2009; Warren, 2008), and has been shown to be affected by a large range of environmental and physiological factors (see Flexas et al., 2008 for a full review), and demonstrate both fast (Flexas et al., 2007) and slow (Fila et al., 2006) responses. It has been demonstrated to change during leaf development (Miyazawa & Terashima, 2001), with nutrient availability (Warren, 2004), with available radiation (Niinemets et al., 2006), leaf temperature (Bernacchi et al., 2002), salinity (Loreto et al., 2003), ambient CO\(_2\) concentrations (Flexas et al., 2007) and to be related to soil water availability (Flexas et al., 2002; Flexas et al., 2004; Grassi & Magnani, 2005; Warren et al., 2004; Warren, 2008). This complicates the inclusion of a process based description of a dynamically variable and ecophysiological dependent \( g_m \) in current photosynthesis models. As yet, it is not clear as to which factors have the largest effect on \( g_m \) variability, and there is not sufficient physiological information to allow for a reliable estimation of a variable \( g_m \) in process based models.

Few studies have included \( g_m \) in photosynthesis models. Those that have, most often assume a fixed value of mesophyll conductance (e.g. Williams et al., 1996), or varied \( g_m \) based on selected factors such as intercepted radiation, average air temperature, or leaf nitrogen content (e.g. Hoff et al., 2002; Ohsumi et al., 2007). Recent efforts have
focused on reported correlations between $g_m$ and $g_s$ (e.g. Ohsumi et al., 2007; Cai et al., 2008). Such correlations, however, are subject to large variations (Niinemets & Sack, 2006; Warren & Adams, 2006; Flexas et al., 2008), and potentially not physiologically justified. Maximum values of $g_m$ are inherently constrained by leaf structure, e.g. sclerophytic leaves have lower $g_m$, mesophytic leaves have higher (Evans et al., 2009; Flexas et al., 2008; Niinemets et al., 2009; Terashima et al., 2006; Warren, 2008), which may be an effective manner by which to include $g_m$ in photosynthesis models. Large-scale models (e.g. Morales et al., 2005; Jung et al., 2007; Sitch et al., 2008) in particular, due to their characteristic simplification of plant functional type groupings, may benefit from such an approach.

Although there are huge uncertainties regarding the driving factors responsible for variations in $g_m$ we have shown that the simple inclusion of a water stress responsive $g_m$ can help close the gap between conflicting reports on the role of different limitations in controlling carbon and water fluxes from forest canopies during water stressed periods. The inclusion of $g_m$ did not improve our ability to model in ecosystems subject to seasonal cycles of water stress, but much of the remaining variability between measured and modelled carbon and water fluxes may be explained, as new information accumulates in the future, through the inclusion of a dynamically variable $g_m$.

3.5 Conclusions

This work was carried out in response to the recent calls for the inclusion of mesophyll conductance in leaf photosynthesis models (e.g. Bernacchi et al., 2002; Flexas et al., 2002; Long & Bernacchi, 2003; Ethier & Livingston, 2004; Manter & Kerrigan, 2004; Sharkey et al., 2007; Niinemets et al., 2009c), due to an increased number of reported findings on the role of mesophyll conductance in the regulation of photosynthesis. It has allowed for a model based assessment of previous suggestions that biological limitations, stomatal conductance limitations and mesophyll conductance limitations play some role in the regulation of photosynthesis (Grassi & Magnani, 2005; Loreto & Centritto, 2008), depending on the degree of water stress encountered and the relevant time scales involved. This is the first time that each limitation has been tested on a canopy scale in an eco-physiological model, and shows that, with (and only with) the
inclusion of mesophyll conductance, diffusive limitations can explain carbon and water flux responses to seasonal changes in soil water availability. This helps in closing the gap between studies (both model and measurement based) which report dominant roles of biological changes under water stressed conditions and those which maintain stomatal conductance limitations as the main actor.

It is expected that the future development of a dynamic model for mesophyll conductance, and its integration into coupled photosynthesis-conductance models will improve our ability to model carbon and water fluxes from terrestrial ecosystems both in well watered and water stressed periods.

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Chapter 3: Modelling photosynthesis with mesophyll conductance


Chapter 3: Modelling photosynthesis with mesophyll conductance


Niinemets, Ü., A. Cescatti, M. Rodeghiero and T. Tosens, Complex adjustments of photosynthetic potentials and internal diffusion conductance to current and previous light availabilities and leaf age in Mediterranean evergreen species *Quercus ilex*, *Plant Cell Environ.*, 29(6), 1159-1178, 2006.


Chapter 3: Modelling photosynthesis with mesophyll conductance


Xu, L. and D. Baldocchi, Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (Quercus douglasii) under prolonged summer drought and high temperature, Tree Physiol., 23, 865-877, 2009.
Abstract

Large uncertainties exist in our knowledge of regional emissions of non-methane biogenic volatile organic compounds (BVOC). We address these uncertainties through a two-pronged approach by compiling a state of the art database of the emissions potentials for 80 European forest species, and by a model assessment and inter-comparison, both at the local and regional scale, under present and projected future climatic conditions. We coupled three contrasting isoprenoid models with the ecophysiological forest model GOTILWA+ to explore the interactive effects of climate, vegetation distribution, and productivity, on leaf and ecosystem isoprenoid emissions, and to consider model behaviour in present climate and under projected future climate change conditions. Hourly, daily and annual isoprene emissions as simulated by the models were evaluated against flux measurements. The validation highlighted a general model capacity to capture gross fluxes but inefficiencies in capturing short term variability. A regional inventory of isoprenoid emissions for European forests was
created using each of the three modelling approaches. The models agreed on an average European emissions budget of 1.03 TgC a\(^{-1}\) for isoprene and 0.97 TgC a\(^{-1}\) for monoterpenes for the period 1960-1990, which was dominated by a few species with largest aerial coverage. Species contribution to total emissions depended both on species emission potential and geographical distribution. For projected future climate conditions, however, emissions budgets proved highly model dependent, illustrating the current uncertainty associated with isoprenoid emissions responses to potential future conditions.

These results suggest that current model estimates of isoprenoid emissions concur well, but future estimates are highly uncertain. We conclude that development of reliable models is highly urgent, but for the time being, future BVOC emission scenario estimates should consider results from an ensemble of available emission models.
4.1 Introduction

Non-methane biogenic volatile organic compounds (BVOC), emitted by most plant species, is a heterogeneous compound class made up of a wide range of reactive volatile hydrocarbons. European forest species emit large amounts of BVOCs, in particular, volatile isoprenoids: isoprene (C$_5$H$_8$) and monoterpenes (C$_{10}$H$_{16}$) (Arneth et al., 2007; Guenther et al., 1995; Simpson et al., 1999).

For many BVOCs, the function for the emitting plants is not entirely clear (Owen and Peñuelas, 2005; Peñuelas and Llusia, 2004; Sharkey and Singsaas, 1995), although the emissions seem to play multiple roles in plant protection, in particular during episodes of high photosynthetic photon flux density (Sharkey and Singsaas, 1995), high temperatures (Copolovici et al., 2005; Peñuelas et al., 2005; Sharkey, 2005; Sharkey and Yeh, 2001), oxidative stress (Affek and Yakir, 2002; Loreto et al., 2001; Loreto and Velikova, 2001; Velikova and Loreto, 2005), and biotic stress (Miller et al., 2005; van Poecke and Dicke, 2004).

BVOCs play a significant role in atmospheric chemistry (e.g., Fuentes et al., 2000; Gelencser et al., 2007; Helming et al., 2006; Kanakidou et al., 2005; Szidat et al., 2006), in particular in the formation of secondary organic aerosols (Kanakidou et al., 2005) and tropospheric ozone at high light intensities and temperatures, and in the presence of NOX (Fehsenfeld et al., 1992; Monson and Holland, 2001). Emissions from terrestrial ecosystems also cause a decrease in atmospheric hydroxyl radical (OH) concentrations and thereby result in an increase of the lifetime of methane in the troposphere (CH$_4$) (Poisson et al., 2000; Roelofs and Lelieveld, 2000). The emissions of BVOC have therefore far-reaching implications for air quality (e.g., Bell and Ellis, 2004), both globally and locally. The effects of biogenic emissions on methane lifetime and aerosols can further lead to important feedbacks between emissions and climate change (Kulmala et al., 2004; Peñuelas and Llusia, 2003).

Due to their importance in air chemistry and climatic processes, regional-scale emission inventories are needed to predict regional air quality as well as simulate future climatic
conditions (e.g., Collins et al., 2004; Kulmala et al., 2004; Tunved et al., 2006). This requires application of emission models accurately describing the responses of emissions to variation in environmental drivers (Grote and Niinemets, 2008). Temperature and radiation have been found to be the main driving factors for the emission of both isoprene and monoterpenes in broad-leaved species (Dindorf et al., 2006; Hansen and Sharkey, 2001; Monson and Fall, 1989; Loreto and Sharkey, 1990), while only temperature has been suggested to control monoterpane emissions in some conifers (Tingey et al., 1980, but see Staudt et al., 1997). These key findings have driven the development of isoprene and monoterpane emission models from simple empirical models to more process based designs.

Early emission modelling methods took an empirical approach, linking emissions directly with climatic variables (Guenther et al., 1995). These have shown to have high predictive power in some, but not in all situations (e.g., Arneth et al., 2007; Bai et al., 2006; Geron et al., 2002; Harley et al., 2004; Otter et al., 2002, 2003; Wang et al., 2007). With an improved understanding of the biochemical reaction pathways for the formation of various plant volatiles, more mechanistic models have evolved (Bäck et al., 2005; Martin et al., 2000; Niinemets et al., 1999; Niinemets and Reichstein, 2003; Zimmer et al., 2000). In these process based models, responses of key enzymatic activities to environmental variables set the internal biochemical conditions, which finally determines the emission rates. These advanced models, however, still do not describe several key physiological observations (Monson et al., 2007), such as responses to elevated CO₂. These models also not always capture the variability in emission rates due to heat stress, and due to changes in stomatal closure as the result of drought or salinity stress. So far, few attempts have been made to compare the capacity of different emission models to simulate the environmental variability in emissions encountered in the field (Arneth et al., 2007).

Emission models are almost exclusively developed at the leaf level and are then scaled, through spatial and temporal scales, to the canopy, to the stand, and finally to the region level. This requires the coupling of an emission model with a process-based SVAT (soil-vegetation-atmosphere transfer) model. Such models describe leaf scale processes, set the micrometeorological canopy conditions which drive these processes, and
describe the biomass and foliage distribution throughout the canopy (Baldocchi et al., 1999; Grote, 2006; Lamb et al., 1993; Lenz et al., 1997; Schaab et al., 2003). Such an approach also requires reliable information of emission potentials of each individual species.

The BVOC emission potential \( E_S \), the maximum emission rate under standard conditions) of terrestrial vegetation is one of the most important variables in modelling BVOC emissions (Arneth et al., 2008; Grote and Niinemets, 2008). \( E_S \) strongly varies among species with values near zero to greater than 100 \( \mu g \, g^{-1} \, leaf \, h^{-1} \) (Kesselmeier and Staudt, 1999; Wiedinmyer et al., 2004). This high variability also exists between similar taxonomic entities (e.g., Owen et al., 2001) such that the emission potential of species of the same genera may be vastly different (Benjamin et al., 1996; Kesselmeier and Staudt, 1999). For instance, among Quercus species, some species are known to be strong isoprene emitters, some monoterpene emitters, and some species have emissions close to zero (Kesselmeier and Staudt, 1999). While extensive emission potential databases have been collated over recent years (Kesselmeier and Staudt, 1999; Wiedinmyer et al., 2004, http://bai.acd.ucar.edu/Data/BVOC/index.shtml; Prof. Nick Hewitt’s database: http://www.es.lancs.ac.uk/cnhgroup/iso-emissions.pdf), these databases include all the estimates made so far without a critical revision. Even for a single species, a large variability exists in the emission factor estimates which is currently not understood. There is a general tendency of modelling studies to use average values of emission factors proposed by emission factor databases (Guenther et al., 2006; Parra et al., 2004; Simpson et al., 1995). However, as non-standardized methods have been used in the estimation of emission factors in the past, using averages of all estimates results in propagation of errors from study to study. Thus, a critical revision of the emission factors is pertinent to standardize the emission factor estimates as much as possible (Niinemets et al. in preparation).

In this chapter, we first embedded three different isoprenoid emission models into a process based terrestrial biogeochemical model, thus providing a bottom up approach both to quantify the isoprene and monoterpenes emissions being released into the atmosphere under present day and potential future climate change. With these new tools we tested the possible variability due to differences in leaf level models. For this,
isoprene emission flux measurements were used to compare diurnal and seasonal emission predictions at two forest sites with contrasting forest structure and species composition, one in the south of France and the other in Michigan, USA. We further revised the emission potentials for 80 dominant European forest species both occurring naturally and in forest plantations. In all cases, original studies were examined and a new consensus estimate was derived for each species. Using these basal emission potentials we derive an emission inventory for both isoprene and monoterpenes emissions from European forests under current climatic conditions using the three different modelling approaches. Finally, European forests were used as an example to analyse and compare large scale model performances under future climatic conditions.

4.2 Materials and Methods

4.2.1 Leaf Level Emission Algorithms

A recent model comparison study highlighted three isoprenoid emission models as possible candidates for regional or global applications (Arneth et al., 2007). The models take contrasting approaches to modelling emissions, each with different assumptions about the way environmental factors limit the emissions and with different levels of mechanistic detail. Where pertinent, modifications were made in order to ensure consistency between the models (as in Arneth et al., 2007). No direct CO₂ effect on the emissions was applied in the models.

Guenther et al. model:

By far the most widely used models for simulation of natural isoprenoid emissions were developed by Guenther et al. (1991), and Guenther et al. (1993). Their approach was to describe the emission rates by using long-term basal emission factor for isoprene (\(E_I\)) and monoterpenes (\(E_M\)), and adjusting these basal emission potentials by two empirical factors, one describing the response to light intensity and the other to leaf temperature. The correlation between short term fluctuations, light intensity and leaf temperature is
widely studied and much work has gone into validating the Guenther et al. model under different environmental conditions (Monson et al., 1994; Petron et al., 2001).

The emission factors used in the model are emission rates normalized to a leaf temperature (\( T \)) of 30 °C and quantum flux density (\( Q \)) of 1000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (sometimes for monoterpenes normalized only to temperature) (Guenther, 1991; Guenther et al., 1993; Guenther et al., 1995; Guenther et al., 1997). For light and temperature dependent isoprenoid emission, the following algorithm is used:

\[
E = E_S C_L C_T
\]  
(Eq. 4.1)

where \( E_S \) is \( E_I \) for isoprene and \( E_M \) for monoterpenes. \( C_L \) and \( C_T \) are the functions of quantum flux density and leaf temperature, respectively. \( C_L \) is a Michaelis-Menten type saturating function:

\[
C_L = \frac{\alpha C_{L1} Q}{\sqrt{1 + \alpha^2 Q^2}},
\]  
(Eq. 4.2)

where \( \alpha \) and \( C_{L1} \) are empirical coefficients. \( C_T \) is described by an Arrhenius-type equation with an optimum that is commonly used to describe the temperature dependence of biological reactions:

\[
C_T = \frac{E_{\text{opt}} C_{T2} \exp \left( \frac{C_{T1} (T - T_{\text{opt}})}{RT_{\text{opt}}} \right)}{C_{T2} - \left( C_{T1} \left[ 1 - \exp \left( \frac{C_{T2} (T - T_{\text{opt}})}{RT_{\text{opt}}} \right) \right] \right)}
\]  
(Eq. 4.3)

where \( R \), the gas constant, is 8.314 J K\(^{-1}\) mol\(^{-1}\), and \( T_m \) is the temperature optimum, and \( C_{T1} \) the activation energy, and \( C_{T2} \) the deactivation energy. We used values of 95000 J mol\(^{-1}\) for \( C_{T1} \) and 230 000 J mol\(^{-1}\) for \( C_{T2} \) from the original parameterization of Guenther et al. model. \( C_{L1} \), \( \alpha \), \( E_{\text{opt}} \) and \( T_{\text{opt}} \) were determined following the algorithms developed by Guenther et al. (2006), which links these values to short term (24 hours) and long term (10 days) fluctuations in temperatures and light intensity.
For some species, in particular conifers with extensive monoterpenes pools with slow turnover compared with the rate of monoterpenes synthesis, monoterpenes emissions have been related directly to leaf temperature. In such cases, monoterpenes emissions were calculated following Guenther et al. (1993) temperature based monoterpane model, as described by the following relation:

$$E = E_M \exp\left[\beta(T - T_s)\right]$$  \hspace{1cm} (Eq. 4.4)

where $E_M$ is the basal emission rate at standard leaf temperature $T_s$ (303.16 K) and $\beta$ is an empirically determined coefficient. We used a value of 0.09 K$^{-1}$ from the original parameterization of the model.

**Niinemets et al. model:**

The Niinemets et al. model for isoprene and monoterpane emissions takes a process-based approach, linking the emission rates to the activity of the synthase enzyme $S_S$ to predict the capacity of isoprenoid synthesis pathway and to foliar photosynthetic metabolism via the photosynthetic electron transport rate, $J$, to predict substrate availability for isoprenoid synthesis (Niinemets et al., 2002b; Niinemets et al., 1999). Here, the supply of dimethylallyl pyrophosphate (DMAPP) and NADPH, as affected by the rate of photosynthetic electron transport and the competitive strength of the synthase enzyme for electrons, are considered as the main controlling factors for the rate of isoprenoid synthesis.

Emission rates are calculated through the fraction of total electron flow used for the isoprenoid synthesis, the rate of photosynthetic electron transport, and the cost of isoprenoid synthesis in terms of electrons. Thus, the emissions are linked to the photosynthetic activity of the leaf with the use of only one single leaf dependent parameter, $\varepsilon$, the fractional allocation of electron transport to synthase activity. Emission rates are given by the equation (Niinemets et al., 1999; Niinemets et al., 2002a):
where \( J_T \) is the total rate of photosynthetic electron transport, \( C_i \) is the internal \( \text{CO}_2 \) concentration, and \( \Gamma^* \) is the hypothetical \( \text{CO}_2 \) compensation point of photosynthesis that depends on photorespiration \((\text{Farquhar et al.}, 1980)\). \( \varsigma \) is the carbon cost of specific isoprenoid (6 mol mol\(^{-1}\) for isoprene and 12 mol mol\(^{-1}\) for monoterpenes) and \( \vartheta \) is the NADPH cost of specific isoprenoid (mol mol\(^{-1}\)). For isoprene, \( \vartheta = 14 \text{ mol mol}^{-1} \). For monoterpenes, \( \vartheta \) is found as a weighted average of the costs of all terpene species emitted. In practice, \( \vartheta \simeq 28 \text{ mol mol}^{-1} \) as the contribution of oxygenated monoterpenes that may have lower electron cost or reduced monoterpenes that may have higher electron cost is generally small \((\text{Niinemets, 2004; Niinemets et al., 2002a})\). \( \varepsilon \), the fractional allocation of electron transport to isoprenoid synthesis, is given by:

\[
\varepsilon = \frac{F_d S_s}{J_{\text{max}}} \quad \text{(Eq. 4.6)}
\]

where \( S_s \) is the specific activity of isoprenoid synthase (either isoprene or monoterpene synthase) in mol isoprenoid (g isoprenoid synthase\(^{-1}\) s\(^{-1}\) that depends on temperature according to an Arrhenius type equation that has a temperature optimum, and \( J_{\text{max}} \) is the light saturated rate of total electron transport that scales with temperature in a similar manner \((\text{Niinemets and Tenhunen, 1997})\). \( F_d \) (g m\(^{-2}\) mol electrons mol isoprenoids\(^{-1}\)) is a scaling constant that depends on the basal emission rate, the isoprenoid synthase content (g m\(^{-2}\)) and also converts from isoprenoid units to electron transport units (mol isoprenoids mol electrons\(^{-1}\)) \((\text{Niinemets et al., 2002b; Niinemets et al., 1999})\). The isoprene and monoterpene models apply different values of \( \varepsilon \).

**Martin et al. model:**

Martin et al. (2000) developed a process based approach for isoprene emissions using the knowledge of the biochemical pathway of isoprene synthesis, thus providing a highly mechanistic model.
As the rate of isoprene emission will be governed by the rate of the slowest reaction in its biochemical pathway, the emissions can be calculated depending on which partial process is limiting under given environmental conditions. This model formulates the extent different processes in the biochemical pathway of isoprene synthase limit isoprene emission and represents the rate of emission as the minimum of three potentially rate limiting processes:

- The supply of carbon to isoprene synthase via pyruvate;
- The supply of ATP from phosphorylation;
- The temperature dependency of the isoprene synthase reaction rate.

Therefore, the isoprene emissions are given by:

$$E = L_d \min \{W_{iso\text{co}}, W_{isop}, V_{\text{isoMax}} \}$$  

(Eq. 4.7)

where $L_d$ scales the hourly emissions to the basal emission rate. $W_{iso\text{co}}$ is the pyruvate limited rate of synthase activity simulated by the flux of carbon through Rubisco:

$$W_{iso\text{co}} = \eta \cdot \text{FPYR},$$  

(Eq. 4.8)

The parameter $\eta$ describes the fraction of assimilated carbon lost as isoprene, which increases exponentially with temperature, and Fpyr is the rate of pyruvate formation from RUBP carboxylation.

$W_{isop}$ is the rate of supply of ATP by phosphorylation, that depends on the carboxylation and photorespiration rates, the rate of dark respiration, and internal oxygen and carbon dioxide concentrations:

$$W_{isop} = \eta (V_c + 1.5V_{pr} - R_d) \nu,$$  

(Eq. 4.9)

where $\nu = ((O_i + V_c)/(C_i + V_{pr} + R_d))$, $V_c$, $V_{pr}$ and $R_d$ are the rates of carboxylation, photorespiration and leaf dark respiration, respectively, $C_i$ is the CO$_2$, and $O_i$ the oxygen concentration in the leaf.
$V_{\text{isoMax}}$ is the temperature dependence of the isoprene synthase enzyme as governed by an Arrhenius equation. The version applied here is a slight modification (following Arneth et al., 2007) of the original version used by Martin et al. (2000) in order to assure compatibility with the analogous expression in the model of Niinemets et al.

The rate of isoprene synthesis is thus proposed to be highly dependent on the rate of supply of carbon in the form of phosphoglyceric acid (PGA) or pyruvate (from photosynthesis or photorespiration), the rate of phosphorylation to supply the ATP needed for the conversion of PGA/pyruvate to DMAPP and the amount and in vivo activity of the isoprene synthase enzyme (Lehning et al., 1999; Loreto and Sharkey, 1993; Monson et al., 1992), following the suggestion that Pyruvate is formed from Rubisco carboxylation about 1% of the time (Andrews and Kane, 1991).

Implicit in both the Niinemets et al. and Martin et al. model approaches for the simulation of isoprene emission on the basis of isoprene synthase kinetics (Fall and Wildermuth, 1998) is that DMAPP concentrations must change in response to changes in light availability at any given temperature, i.e. isoprenoid emission rates are substrate-controlled. DMAPP levels have been found to vary within and between days, and varied more in plants which emitted isoprene (Rosenstiel et al., 2002).

*Modifying the leaf-level models to simulate long-term emission responses to environment*

All three leaf-level models provide qualitatively similar responses of isoprenoid emissions to changes in temperature and light over short term (Arneth et al., 2007). However, emission rates also adjust to long term modifications in environmental drivers, implying that it is important to consider such effects as well to simulate long-term emission dynamics.

*Phenology*

Phenology is known to affect isoprenoid emissions and many studies have characterized how emissions respond to phenological events (e.g., Fuentes and Wang, 1999; Monson
et al., 1994; Petron et al., 2001; Kuhn et al., 2004). Leaves begin to photosynthesize soon after budbreak, but isoprenoids are not emitted in substantial quantity for days to weeks after the onset of photosynthesis (e.g., Wiberley et al., 2005). This effect was incorporated into each of the emission models in the same simple manner as phenology affects seasonal photosynthesis, and also empirically implementing a time-lag between the onsets of photosynthesis and isoprenoid emissions. Seasonal photosynthetic capacity follows the Pelkonen and Hari (1980) approach, which introduces a factor K ranging from 0 to 1 depending of the stage of annual development. This factor multiplies the value of the maximum emission potential of fully-developed leaves used by each of the models. In the case of deciduous phenology (Smith et al., 2001) this leads to a decreasing isoprenoid emission capacity in senescing leaves before the leaves die (Goldstein et al., 1998; Geron et al., 2000; Pressley et al., 2005; Arneth et al., 2007).

Soil water availability effects
Isoprenoid emissions are affected by seasonal water stress, though the exact extent and mechanisms behind the emission responses to water stress are yet unclear (Grote and Niinemets, 2008). A strong reduction of emissions is observed in most cases after strong seasonal water stress (Llusia and Peñuelas, 1998; Pegoraro et al., 2004). In the model GOTILWA+, to which the emissions models are coupled, photosynthesis is reduced directly during water stress through a reduction in the rate of electron transport, and the maximum carboxylation capacity (Keenan et al., (in review)). All three emissions models are indirectly influenced by the soil moisture dependence of stomatal conductance which influences the leaf temperature estimated by GOTILWA+. The Guenther et al. model also includes a direct effect of soil water stress. Emissions are reduced during water stress directly in parallel with photosynthesis, by applying the same function which reduces photosynthetic potential (Guenther et al., 2006; Keenan et al., (in review)). Stress affects the Niinemets et al. model emissions indirectly, as the drought-dependent reductions in the rate of electron transport, $J_T$, lead to reduced substrate availability and thereby to reduced emissions. The Martin et al. model was modified in a similar manner as the Guenther et al. model, but here the water stress function was also applied to the supply of emission precursors.
4.2.2 Species-specific emission potentials

Calculated total emissions are highly dependent on the assigned emission potentials $E_I$ and $E_M$ (Eq. 4.1) that are directly applied in the Guenther et al. model, and determine the isoprenoid synthase activities in the Niinemets et al. and Martin et al. models. The emission potentials have recently been concluded to be one of the most important parameters for modelling regional emissions (Arneth et al., 2008; Grote and Niinemets, 2008). In most large-scale modelling studies, the values initially specified by Guenther et al. (1995) are applied. Since then, much more information has become available and been integrated into previous estimates (Guenther et al., 2006). However, for most ecosystems and vegetation types measurements of BVOC emissions are still scarce or absent (Guenther et al., 2006; Grote and Niinemets, 2008). Due to the large variation within families, assigning general emission rates to plant functional types is often done by subjective judgment. It often occurs that very different emission potentials are assigned to a certain functional type or species in different modelling studies, leading to very different results (Arneth et al., 2008 for review). We address this problem through a thorough critical literature review to compile the state of the art knowledge of species-specific emission potentials for 80 species included in the GOTILWA+ forest model (Table A.4.1).

In this analysis, all previous known reports were reassessed with regards to measurement methods and derivation of standardized emission potentials. As there is considerable uncertainty in actual light and temperature environment in whole-tree and whole-branch enclosure studies that can seriously affect standardization of emission rates (Niinemets et al. in preparation), we preferred estimates conducted with single leaf enclosures. As a variety of temperature responses have been used to convert the emission rates to standardized conditions of 30 °C and 1000 μmol m$^{-2}$ s$^{-1}$, whenever actual response curves were absent, we reconverted the data using the Guenther et al. (1993) original model. In all cases, units were homogenized (e.g., μg C g$^{-1}$ hr$^{-1}$ often used in North American studies vs. μg BVOC g$^{-1}$ hr$^{-1}$ often used in European studies). Errors due to the use of inappropriate values of leaf dry mass per unit area, $M_A$, in converting area based relations to mass basis (for instance converting total leaf area based estimates using projected area-based $M_A$ values) were corrected.
Because of the lower sensitivity of analytical techniques in the past, and significant background level of isoprene and monoterpenes in the gas-exchange enclosures either due to isoprenoids in incoming ambient air, or adsorption/desorption processes in the cuvettes, there is considerable uncertainty in detecting minute emission rates with conventional techniques. Due to these uncertainties, emission rates less than 0.1 μg g\(^{-1}\) hr\(^{-1}\) were set to zero in the current compilation. Only the values corresponding to fully-developed leaves in non-stressed conditions were used whenever possible. For several species included in GOTILWA+ model, reliable emission rates were not available. For these species, emission factors of taxonomically closest species were employed (Benjamin et al., 1996 for discussion).

For broad-leaved species not included in Table A.4.1, we used the default values of 10 μg g\(^{-1}\) hr\(^{-1}\) for isoprene and 0.2 μg g\(^{-1}\) hr\(^{-1}\) as suggested by Solmon et al. (2004). For conifers not included in Table 1, the default values were 1 μg g\(^{-1}\) hr\(^{-1}\) for isoprene and 3 μg g\(^{-1}\) hr\(^{-1}\) for monoterpenes (Solmon et al., 2004).

In addition to the emission factors, for the simulation of monoterpane emissions, it is also important to know whether emissions are only temperature dependent or both light and temperature dependent. In several conifers, the emission may be both light and temperature dependent (Staudt et al., 1997; Shao et al., 2001) and information of the share or these two contrasting emission sources are provided in the Appendix Table A.4.1.

### 4.2.3 Scaling from the leaf to the landscape

The three emission models simulate isoprenoid emissions from any given leaf in dependence on climatic conditions. To scale from the leaf to the landscape, the emission models were coupled to the biogeochemical forest model, GOTILWA+. This model described leaf structural and chemical characteristics, and thus foliage physiological potentials. This model also described the microclimatic conditions and forest structure necessary to scale from the leaf to the canopy, and further to region. Using forest
inventories and regional databases of climate on a 10’x10’ scale, simulations were run for each of the forest stands in EU15+2.

**Scaling from the leaf to the stand: The GOTILWA+ model**

Each emission model considered was coupled separately to the photosynthetic submodel of the GOTILWA+ terrestrial biogeochemical model (Gracia et al., 1999; Keenan et al., 2008; www.creaf.uab.es/GOTILWA+). GOTILWA+ is a process-based forest model that has been developed to simulate carbon and water fluxes from forest ecosystems and to explore how the functioning of forests is influenced by climate, tree stand structure, management techniques and soil properties. Carbon and water fluxes of forests are simulated for different environmental conditions, for different tree species, and under changing environmental conditions that result from either climatic modifications or from alterations in management regime.

The GOTILWA+ model includes a two-leaf canopy photosynthetic model (Wang and Leuning, 1998; Dai et al., 2004). The photosynthesis sub-model treats the C3 photosynthetic pathway. The canopy is divided into sunlit and shaded leaves, with the amount of intercepted diffuse and direct radiation depending on the time of the day, season, and the area of leaf exposed to the sun (Campbell, 1986; 1990). Foliage net assimilation rates are calculated using the Farquhar et al. (1980) photosynthesis model, with dependencies on intercepted quantum flux density, species-specific photosynthetic capacities, leaf temperature, and leaf intercellular CO2 concentration (Ci). Stomatal conductance is calculated using the Leuning et al. model (Leuning et al., 1995) that is the advancement of Ball et al. model (Ball et al., 1987). Water stress affects the photosynthesis-conductance coupling by directly reducing the photosynthetic potential through a non-linear relation to soil water content (Keenan et al., (in review)). Canopy isoprene and monoterpenes emission rates were calculated on an hourly basis as the sum of sunlit and shaded leaf fractions using their specific leaf temperature and incident radiation values.

The model treats monospecific stands which can be even- or uneven-aged. Individual trees in a stand are aggregated into 50 DBH (Diameter at Breast Height) classes and all
calculations are performed for each class. Each tree cohort is represented by three carbon compartments, leaf, sapwood, and fine roots. Labile carbon is allocated to each, and maintenance respiration is calculated as a function of temperature. Fine litter fall (i.e. leaves and flowers), gross litter fall (i.e. bark, branches) and the mortality of fine roots add to the soil organic carbon content. The soil in GOTILWA+ is divided into two layers, an organic layer and a mineral layer, with a specific transfer rate of soil organic carbon between these two layers. Flux calculations are performed hourly, whereas slower processes such as growth and other state variables are calculated daily.

**Scaling from the stand to the region:**

To supply the input data required by the model, an extensive database has been built within the framework of the European ATEAM (Advanced Terrestrial Ecosystem Analysis and Modelling) and ALARM (Assessing Large-scale Risks for biodiversity with tested Methods) projects, connecting diverse information sources at a European level and adapting them to fit the same spatial resolution of 10’x10’. The database contains data related to forest species, forest area cover, forest structure, forest function (photosynthesis, respiration rates), soil hydrology, organic matter decomposition rates and management strategies (Schröter et al., 2005). Area explicit estimates of forest cover were made available which specify the eighty dominant forest species in Europe. Simulations were run with GOTILWA+ for each 10’ x 10’ scale forested pixel in Europe (EU15 + 2, pre-enlargement Europe, Norway and Switzerland). For parameterisation of the forest structural components in GOTILWA+, three forest functional types (temperate deciduous, temperate broadleaf evergreen, and temperate needle-leaf evergreen) were considered.

**Scaling through time:**

Simulations were run with each emission model coupled to the GOTILWA+ model for a two hundred year period from 1900 to 2100. From 1900 to 2000, a reconstructed climatic data time series based on the CRU05 (1901-2000) monthly dataset (New, Hulme, and Jones, 1999) was used, with global atmospheric concentrations of CO$_2$ from 1901 to 2000 obtained from the Carbon Cycle Model Linkage Project (McGuire et al.,
The IPCC socioeconomic analysis (IPCC, 2001, 2007) resolved several possible standard scenarios for CO₂ emissions (A1, A2, B2, B1). Different global circulation models use these scenarios to generate future climatic projections. In this work we have applied the climatic projection for period 2001-2100 generated by the HadCM3 global circulation model using the A2 scenario as an indicator for the effect of possible future climate change on the BVOC emissions. This scenario uses an estimated increase in atmospheric CO₂ to 709 ppm by 2080. Using this scenario, HadCM3 predicts an increase in temperature of 2.8 °C by 2080 for the area included in this study (in comparison to the average temperatures for the 1960-1990 period), and regional changes in precipitation.

4.2.4 Stand scale validation

Recent developments in methodologies such as eddy-covariance techniques provide high quality quantitative measurements of isoprenoid emissions (Ciccioli et al., 2003; Spirig et al., 2005). So far, few such measurements are available over forest ecosystems. Here, one relatively short-term series (26-days) of diurnal time-courses of isoprene emissions with a half-hour time-resolution (Arneth et al., 2007) and one long-term series of seasonal time-courses of isoprene fluxes with daily resolution (Pressley et al., 2005; Pressley et al., 2006; Pressley, unpublished data) are used to validate the implementation of the isoprene models at the canopy level. Three simulations were run at each site, one for each model coupled to the GOTILWA+ model, using stand and species parameters gathered from the literature (Arneth et al., 2007; Goldstein et al., 1998; Waring et al., 1998; Curtis et al., 2001, Pressely et al., 2006, Serca, unpublished data). Emissions factors were used from a previous modelling study at these sites (Arneth et al., 2007).

Site 1:
We used measurements from two field campaigns at two Mediterranean Quercus pubescens stands in southern France (43° 39’ N, 6° E) conducted during 2000 and 2001. In these campaigns, isoprene fluxes were measured with the Eddy covariance technique for approximately two weeks in the summer of each year. The fast isoprene sensor (Hills and Zimmerman, 1990) was employed in these measurements, and half hour
values were stored. These data have been used to examine the diurnal course of emissions predicted by each model. Average diurnal courses were constructed by taking the emission value for each hour of each day.

Site 2:
To our knowledge, the only available long-term data set of forest eddy-covariance measurements of isoprene emissions is from the University of Michigan Biological Station (UMBS, 45° 33’N, 84° 43’W), (Pressley et al., 2005; http://www.biosci.ohio-state.edu/~pcurtis/UMBS~Flux). This site supports a mixed forest, dominated by *Populus grandidentata*, *P. tremuloides*, *Quercus rubra*, *Fagus grandifolia*, *Acer rubrum*, and *Pinus strobus* (Curtis et al., 2005; Pressley et al., 2005; Pressley et al., 2006). Three years of eddy covariance measurements, from the growing seasons of 2000, 2001, and 2002, are available to test the model efficiency at capturing seasonal time-courses.

4.3 Results

**Evaluating isoprene model emissions: Diurnal time-courses**

The data from the French site were used to test the capacity of the models to reproduce the diurnal time-courses of isoprene emissions. Each model compared well against the average diurnal time-course (Fig. 4.1). Guenther et al. model failed to reproduce the midday decline in emissions, and thus, overestimated the emissions at this site. Martin et al. model also overestimated the midday isoprene emission rate, but to a smaller extent. Niinemets et al. model accurately reproduced the early morning and late evening emissions, and also gave accurate estimates of the average diurnal midday emissions. When looking at the complete time series, no noticeable differences among model predictions can be discerned, except for the last five days of the measurement campaign in 2001. During this period, a large increase in the emissions was observed, which was accurately reproduced by both the Guenther et al. and the Martin et al. model. Niinemets et al. model, however, proved unresponsive during this period. This increase in the emissions was correlated with an increase in temperature during the last four days.
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Figure 4.1. Hourly measured canopy isoprene emissions (mg C m\(^{-2}\) h\(^{-1}\)), from the French site for two two-week periods in 2000 and 2001 (Arnet h et al., 2007; Serca, unpublished data), compared with the simulation results using GOTILWA+ with the three isoprene emission models (Guenther et al., Niinemets et al., and Martin et al. models). The average diurnal time-course from the measurements is also compared with the simulation results.

**Evaluating isoprene model emissions: Seasonal time-courses**

The seasonal time-course of isoprene emission was accurately simulated by each model (Fig. 4.2). The effect of phenological events during spring and autumn was well captured in 2000 and 2001. In 2002, missing data during spring complicated the comparison. The autumn reduction in isoprene emission was correctly predicted by the models. Day-to-day variability and summer maxima were reproduced with a lesser accuracy than the long-term phenological effects. The actual data exhibited a larger variability than the models in each year, in particular in 2000 and 2001. Both Guenther et al. and the Niinemets et al. model showed non-linear responses in their ability to simulate emissions, accurately capturing low level emissions (~<20mg C m\(^{-2}\) d\(^{-1}\)), but underestimating higher emissions. The Martin et al. model showed a more linear response, with a slight tendency to underestimate emissions in all cases.
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Figure 4.2. Comparison of daily integrated canopy isoprene emission fluxes from the UMBS forest (Pressley et al., 2005) and fluxes simulated by GOTILWA+ with the three emission models compared (Guenther et al., Niinemets et al., and Martin et al. models) over three years (left panels) and the regressions of measurements vs simulations (right panels).

Current Emissions from European forests

Total simulated isoprene emission from pre-enlargement European Union with Norway and Switzerland (EU15 + 2) was 1.03 TgC a\(^{-1}\) for the period between 1960 and 1990. A broad range of canopy emission capacities was observed for the species considered (Fig. 4.3), with order of magnitude differences per unit ground area. However, when considered together with species aerial coverage and climatic distribution, the strongest emitters were not necessarily the largest contributors to the European emission budget.
The Europe-wide isoprene emission was dominated by a few species. \textit{Quercus robur} was by far the highest contributor, with 32\% of total Europe-wide emission. \textit{Quercus pubescens} and \textit{Quercus petraea} contributed 18\% and 16\%. \textit{Eucalyptus} species, \textit{Quercus frainetto}, and \textit{Quercus pyrenaica} each contributed around 6\% to total emissions. Six species contributed between 1 and 2\% (\textit{Quercus faginea}, \textit{Quercus rubra}, \textit{Populus} spp., \textit{Robinia pseudacacia}, \textit{Picea abies}), with the remaining species contributing less than 1\% of the total European emission budget.

Monoterpane emissions showed a similar pattern, with a very broad range of species emissions per unit stand area, but with only a few species dominating the total budget of emissions from European forests when both emission potentials and geographical distribution were taken into account. Total simulated emissions amounted to 0.93 TgC a$^{-1}$. Of this total, 24\% was emitted by forests dominated by \textit{Quercus ilex}, 21.5\% by \textit{Pinus sylvestris}, 16\% by \textit{Fagus sylvatica}, 11\% by \textit{Picea abies} and 7\% by \textit{Quercus suber}. Seven further forest species (Fig. 4.4) contributed between 1 and 2\% to total emissions, with the remaining forest species contributing less than 1\%.
Figure 4.3. Estimated average annual isoprene emissions (log scale) from European forest canopies for the period 1960-1990 (grey bars). Values represent average estimates from the three emissions models (Guenther et al., Niinemets et al., and Martin et al.). Black bars denote the percent contribution of each species to the total European isoprene emissions budget for this period (for contributions of greater than 1%). Species emission potentials are according to Appendix Table A.4.1.
Figure 4.4. Estimated average annual monoterpene emissions (mg C m$^{-2}$ a$^{-1}$) from European forest species for the period 1960-1990 (grey bars). Values represent average estimates from the two emissions models (Guenther et al., and Niinemets et al.). Black bars denote the percent contribution to total European monoterpene emissions budget for this period (for contributions of greater than 1%). Species emission potentials follow Appendix Table A1.
Emissions showed a strong regional pattern, being influenced by the distribution of the species across Europe, and regional weather patterns. For isoprene emission, France showed the highest emissions for the period 1960-1990 (Fig. 4.5), followed by the north-western area of the Iberian Peninsula. The Mediterranean zone, although it is subject to higher temperatures, and radiation, showed lower emissions than central mainland Europe due to lower plant coverage and leaf area index as well as due to suppression of emissions during extensive summer drought periods.

Figure 4.5. Estimated annual isoprene emissions (gC m$^{-2}$ a$^{-1}$) from European forests over the period 1960-1990, using average estimates from the three isoprene emissions models coupled to GOTILWA+. 
Simulated monoterpenic emissions were lower than those of isoprene, with lower interspecific emission rate variability. The emissions of monoterpenes also showed a more uniform spatial distribution. The highest emissions were predicted for the southern Iberian Peninsula, and areas of the Mediterranean zone (Fig. 4.6).

Figure 4.6. Estimated annual monoterpenic emissions (gC m$^{-2}$ a$^{-1}$) from European forest canopies over the period 1960-1990, using average estimates from the two monoterpenic emission models (Guenther et al. and Niinemets et al.) coupled to GOTILWA+.
Model comparisons for the period 1960-1990

The period 1960-1990 is often used as a baseline for comparing regional models in the past and the future (Schröter et al., 2005). For isoprene, no significant difference was observed between the emission model predictions for this period. The three isoprene models agreed on the total emission budget, and gave similar responses to light and temperature ranges across Europe. For monoterpenes, the Guenther et al. model gave slightly higher (4.6%) emissions than the Niinemets et al. model for the simulated period, perhaps due to a slightly stronger temperature response (Arneth et al., 2007).

Projected Future European Emissions

Although the choice of the model had little effect on the estimated budget of isoprenoids during the period 1960-1990, projected future emission estimates were greatly affected by the choice of the emission model. In the case of isoprene, the models showed up to a two-fold difference in predicted emission rates by 2100 (Fig. 4.7). Differences were not fully evident until about 2050, when large disparities began to appear between the models. All models agreed on a general strong increasing trend in both isoprene and monoterpane emissions, though the dynamics of this trend differed between models. For isoprene emission, Martin et al. model showed the strongest response with average per pixel emission of 4400 mgC m\(^{-2}\) a\(^{-1}\) from European forests. This gave a total European budget of 2.36 TgC a\(^{-1}\) for the period 2080-2100, i.e. more than double the average emission predicted for the period 1960-1990 with the same model. Guenther et al. model gave slightly higher emissions than the other models for the early 21st century, but did not respond as strongly as Martin et al. model under conditions of more severe climate change (3720 mgC m\(^{-2}\) a\(^{-1}\) for 2080-2100). Total European isoprene emission simulated with the Guenther et al. model for the period 2080-2100 was 2 TgC a\(^{-1}\). Niinemets et al. model predicted the lowest emission of the three models throughout the century, and did not respond as strongly as the other models to future climate change, giving a total of 1.58 TgC a\(^{-1}\). The electron transport system, on which the Niinemets et al. model is based, showed a response of a similar magnitude. On average, 98% increase
in isoprene emission was predicted for the period 2080-2100 relative to the emission in the period 1960-1990 with the three emission models.

Of the two monoterpene emission models, Guenther et al. model consistently predicted higher emissions than Niinemets et al. model throughout the 21st century, and responded much more strongly to climate change, perhaps reflecting its higher temperature sensitivity as suggested by Arneth et al. (2007). For the period between 2080 and 2100, Guenther et al. model predicted a total European monoterpene budget of 1.27 TgC a⁻¹, which represents a 31% increase in monoterpene emissions with respect to the emissions for the period 1960-1990. Niinemets et al. model predicted a smaller increase in the emissions, giving a total emission from European forests of 1.07 TgC a⁻¹, which represented an 11% increase with respect to the period from 1960 to 1990. As an average of the two models, monoterpene emissions from European forests were predicted to increase by 21% for the period 2080-2100 relative to the period 1960-1990.
Figure 4.7. Average per m² modelled isoprene and monoterpene emissions from European forest canopies from 1900 to 2100. Climate from the CRU (New, Hulme, and Jones, 1999) was used for the period 1900 to 2000. Results from 2001 to 2100 correspond to climate from HadCM3 global circulation model using climate change scenario A2 (IPCC, 2001, 2007). The displayed error bars (in grey) represent the standard error from the mean. The Mean Trend is a quadratic regression ($y = y_0 + ax + bx^2$) of the displayed data.
4.4 Discussion

To our knowledge, this is the first time different BVOC emission modelling approaches have been run in parallel on a regional scale. The results serve to both reduce and highlight uncertainty in the modelling of current emissions of isoprene and monoterpenes from European forest species through a two-pronged approach: the compilation and assessment of species specific emission potentials, addressing the broad variability of values published in the literature, and the comparison of three distinct methodologies for the prediction of regional emissions.

To achieve our goals a revised database of isoprene emission factors was constructed for the key tree species growing in European forests using state-of-the art information of species emission potentials (Table A.4.1). So far, all regional emission inventories in Europe have been based on uncritical use of species emission potentials collected in databases that have not been updated since late 90s (e.g., Prof. Nick Hewitt’s database: http://www.es.lancs.ac.uk/cnhgroup/iso-emissions.pdf) (Parra et al., 2004; Projections, 2007; Simpson et al., 1999; Solmon et al., 2004). However, new information has become available on the emission characteristics of some important species such as important Mediterranean species Quercus suber that has been traditionally considered “non-emitting” species in emission models. Recent data show that this species strongly emits monoterpenes (Pio et al., 2005; Staudt et al., 2004). Analogously, Fagus sylvatica, a dominant component in European temperate deciduous forests has previously been reported to be a minute monoterpane emitter (König et al., 1995; Steinbrecher et al., 1993), while recent data demonstrated that this species is moderate to high monoterpane emitter (Dindorf et al., 2006; Luchetta, 1999; Moukhtar et al., 2005). While for some species, reliable information of emission potentials is still not available, the aerial coverage of these species is generally small and only minor improvement of large-scale emission estimates is expected.

Model comparisons with tower flux measurements at mid-latitude forest sites show that the models do not differ greatly in their ability to reproduce the short-term variations in
isoprene emission, accurately capturing the diurnal time-course of isoprene emission driven by modifications in light and temperature. Guenther et al. model, being solely based on light and temperature, was not effective at capturing the mid-day decline in the emission in drought conditions, leading to a general overestimation of the total emission at the Mediterranean site. The Guenther et al. model is based on two driving variables, and assumes a fixed shape of the response of isoprene emission to these variables. Yet, other factors have been known to affect the emission rates as well (e.g., Affek and Yakir, 2002; Loreto et al., 2001; Loreto and Velikova, 2001; Peñuelas and Llusia, 2001; Sharkey and Singsaas, 1995; Sharkey and Yeh, 2001). Given its simplicity, its performance under present-day conditions is surprisingly comparable with the other two more plastic models.

The Niinemets et al. model performed better for simulation of average diurnal emission time-course, but it was unresponsive to the high temperatures experienced in the last few days at the French site. The response of the Niinemets et al. model to temperature was reported in a previous study (Arneth et al., 2007, Fig. 1), though not discussed in detail. There, it was also shown to be slightly less responsive to temperature than the other two models. Martin et al. model, which takes a more detailed approach to describe the limiting precursors of emissions, better reproduced the diurnal time-series, accurately capturing both the day-to-day variability and the average diurnal time-course. Long-term (daily to seasonal) emissions were moderately well reproduced by the models. The low correlation with the data highlights the fact that a complete understanding of emissions drivers and controls is missing from the models, and we are indeed a long way from fully realistic BVOC emission estimates (Arneth et al., 2008; Monson et al., 2007; Grote and Niinemets, 2008).

Although there are many strong isoprene and monoterpene emitting tree species in Europe, the regional distribution of these species leads to only a few of these strong emitters being important for the estimation of total European emission budget. These species were not necessarily the strongest emitters, but tended to be species with large coverage in regions with high temperatures and radiation. Total European forest isoprene emissions for the late 20th century were strongly dominated by three Quercus species, making up over two thirds of the total isoprene emission. It is therefore of
considerable importance to focus research efforts on accurately quantifying the emission potentials from these species, and their emission responses to environmental drivers. Monoterpene emissions were similarly dominated by a few species, with five species contributing 80% of the total emission budget. Having more accurate data on these species could greatly improve the reliability of estimates of present day emission budgets.

The resulting inventory of isoprene emission is similar to the value presented by Simpson et al. (1999) (1.4 TgC a\(^{-1}\); Simpson et al., Table 18), and Arneth et al. (2008, 1.2 TgC a\(^{-1}\)) for the same area. Since isoprene emission estimates are highly uncertain, due to their linear dependency on the leaf emission potentials assigned to a species or vegetation type, and due to strong dependence on the kind and quality of the land cover information used (Guenther et al., 2006), the similarity of the calculated annual totals is remarkable. Recently it has been suggested that such agreement between model simulations is due to compensation efforts applied to move model estimates closer to the hypothetical ‘real’ regional or global emission value (Arneth et al., 2008). We made no such effort, and would suggest that the similarity of different modelled isoprene estimates derives from the fact that regional isoprene emissions are dominated by a few highly emitting and well documented species.

Such similarities do not apply for monoterpene emission estimates, with a broad range of global emissions reported in the literature (Adams et al., 2001; Guenther et al., 1995; Kaplan et al., 2006; Lathiere et al., 2006; Levis et al., 1999; Naik et al., 2004; Tao and Jain, 2005; Valdes et al., 2005). Our estimate of total monoterpene emissions from the European forests of 0.97 TgC a\(^{-1}\) is lower than the emission estimate published in Tao and Jain (2005; 1.73 TgC a\(^{-1}\)). However, their simulation is based on a larger part of Europe than ours, and their parameterization also differs from ours by considering six plant functional types only, rather than species-specific parameterization. To our knowledge, no study has simulated the emission of monoterpenes from European forests on a European scale.

Although both the isoprene and monoterpene emission models performed comparably under current climatic conditions, large differences were observed in the emission
estimates for realistic future climatic change scenarios. The differences observed here between model responses for the late 21st century results from the small differences in temperature, radiation and atmospheric CO₂ concentration responses reported in the isoprene model (Arneth et al., 2007, Fig. 1, 2 for review). The isoprene model of Martin et al. is more sensitive to temperature, radiation, and increasing atmospheric CO₂, followed in sensitivity by the Guenther et al. model. The long-term simulations under gradually increasing temperature and atmospheric CO₂ (Fig. 4.7) highlight the implications of such differences in sensitivities between models. Our study demonstrates that the choice of the model used can greatly alter the final result. The non-concurrence of the emission models in simulations of future scenarios calls into question the validity of numerous conclusions regarding future emissions, and the resulting effects on atmospheric chemistry, made so far on the basis of only one emission model.

A recent study hypothesised that we are overconfident about our ability to accurately model BVOC emissions from terrestrial vegetation, and, according to the terminology of Le Quere (2006), we are in the ‘illusion’ phase of model development (Arneth et al., 2008). When we consider the estimates of present day emissions that were obtained by applying the same methodology and with the models coupled to the same terrestrial vegetation model, the comparability of model performance does not seem to support such a conclusion. However, when moving to projected future climatic conditions, it becomes clear that we are far from reaching a clear understanding of the processes governing emission rates (Monson et al., 2007), and their potential responses to future climate change.

Current model development efforts are focused on the improvement of simple empirical algorithms (Guenther et al., 2006) and the development of more process-based emission models (Arneth et al., 2006; Back et al., 2005; Grote et al., 2006; Niinemets et al. 2002a; Martin et al., 2000; Niinemets et al., 1999; Zimmer et al., 2000). Each approach lends itself more easily to different applications, such as the easy implication of simple empirical models in atmospheric chemistry models (e.g., Guenther et al., 2006), or the detailed study allowed for by the more intricate models (e.g., Grote et al., 2006). Despite the big differences in model structures, none of the models included in this
study outperformed the others. In fact, no existing approach has been shown to perform consistently better (Arneth et al., 2007). This is due both to a lack of detailed model inter-comparisons, and a lack of good quality data with which to test the models. Further efforts in both fields, together with the development of new modelling approaches and synergies (Grote and Niinemets, 2008; Monson et al., 2007) will be needed to advance our ability to reliably simulate emissions. Meanwhile, we urge that results from different model approaches should be considered in any simulation project dealing with terrestrial emissions of BVOCs, particularly if considering future climate change scenarios.

4.5 Conclusions

The coupling of the three different model approaches (Guenther et al., Niinemets et al. and Martin et al. models) to an ecophysiological forest model provides a unique opportunity to explore the time-dependent changes in modelled biogenic emissions due to differences in model structure and model responses to changes in climatic and physiological processes. The modelled emissions from present day European forests were shown to be independent of the emission model used, with estimates of 1.03 TgC a⁻¹ for isoprene emission and 0.93 TgC a⁻¹ for monoterpenes, giving a consistent emission inventory for BVOCs from European forests. Relative to previous estimates, the differences are moderate and are driven by use of improved emission factor database for 80 European key forest species, as well as implementation on physiological controls on emissions (water stress and phenology).

Coincidence of model estimates of emissions for current and past climatic conditions in Europe suggest that the present day inventories of BVOC emissions provide realistic estimates. However, model-dependent differences in simulated estimates of future emissions of both isoprene and monoterpenes highlight the fact that we are in the early stages of the path towards a full understanding of the processes governing BVOC emissions. This has important implications for any study seeking to model future BVOC emissions. Many studies involving modelled future BVOC emissions (e.g., potential offsetting of emissions by rising CO₂ concentrations, relative effects of changes in land
use on quantitative emission estimates, effects on future emissions on tropospheric O₃ concentrations and air quality) may need to be revised to take into account the inherent variability introduced by the choice of the emission model used.

Acknowledgments

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Chapter 4: BVOC Emissions from European forests


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## Appendix 4.1

Table A.4.1. Compilation of isoprene ($E_i$) and monoterpene ($E_M$) emission factors and leaf dry mass per unit area ($M_A$) in 80 dominant species of European forests

<table>
<thead>
<tr>
<th>Species</th>
<th>$M_A$ (g m$^{-2}$)</th>
<th>Isoprene</th>
<th>Monoterpene</th>
<th>Light dependence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_i$ (µg g$^{-1}$ h$^{-1}$)</td>
<td>Reference</td>
<td>$E_M$ (µg g$^{-1}$ h$^{-1}$)</td>
<td></td>
<td>Reference</td>
</tr>
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<td>3</td>
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<td>35</td>
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<td>N</td>
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<td>d</td>
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<td>57</td>
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<td>e</td>
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<td>73</td>
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<tr>
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<td>87</td>
<td>48 20, 29</td>
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</table>

**Notes:**
- N: Not detected
- Y: Detected
- i: Interpolated
- m: Missing data
- n: Not applicable
### Chapter 4: BVOC Emissions from European forests

#### Species nomenclature follows ARS/GRIN online database (USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network - (GRIN), National Germplasm Resources Laboratory, Beltsville, Maryland, http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl); \textsuperscript{b} based on the original studies and Niinemets (1999) and Wright et al. (2004) (GLOPNET) databases; \textsuperscript{c} N – the emission is only controlled by temperature, Y – the emission is controlled by both light and temperature, N/Y(xx) part of the emission is controlled by temperature only, part by both temperature and light. The number in parenthesis shows the

<table>
<thead>
<tr>
<th>Species</th>
<th>Value</th>
<th>Value</th>
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\textsuperscript{a} N – the emission is only controlled by temperature, Y – the emission is controlled by both light and temperature, N/Y(xx) part of the emission is controlled by temperature only, part by both temperature and light. The number in parenthesis shows the
percentage of emission controlled by both light and temperature; \( d \) based on default values for \textit{Abies} that was derived from references 21, 23, 26 for isoprene and 21, 23, 26, 85) for monoterpenes; \( e \) based on default values for \textit{Acer} that was derived from references 26, 51, 85, 88, 99 for isoprene and 15, 18, 25, 26, 96, 99 for monoterpenes; \( f \) based on values for \textit{Arbutus unedo}; \( g \) based on values of \textit{Carpinus betulus}; \( h \) based on values for \textit{Fagus sylvatica}; \( i \) based on values for \textit{Fraxinus excelsior}; \( j \) based on values for \textit{Juniperus oxycedrus} and \textit{Juniperus phoenicea}; \( k \) based on values of \textit{Larix decidua}; \( l \) based on values for \textit{Phillyrea angustifolia}; \( m \) based on default values for \textit{Pinus} that were derived from 9, 15, 44, 46, 59, 60, 66, 73, 77, 80, 82, 92, 96 for isoprene and 21, 23, 26, 52, 96 for monoterpenes; \( n \) average values for \textit{Populus alba} and \textit{P. tremula}; \( o \) average values for \textit{Populus deltoides} and \textit{Populus nigra}; \( p \) based on values for \textit{Tilia cordata}.
Table A1 References


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Chapter 5

Seasonality of BVOC Emissions for Regional Modelling
Seasonality of monoterpene emission potentials in *Quercus ilex* and *Pinus pinea*: Implications for regional BVOC emissions modeling

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Santi Sabate 1,3, Carlos Gracia 1,3, and Josep Peñuelas1,4

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2 Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia.

3 Department of Ecology, University of Barcelona (UB)

4 Global Ecology Unit CSIC-CEAB-CREAF, Autonomous University of Barcelona (UAB), 08193 Bellaterra, Spain

*An edited version of this chapter is in review in:*

Abstract

Isoprenoid emissions from terrestrial ecosystems provide one of the principal controls over oxidative photochemistry in the lower atmosphere and the resulting air pollution. Such atmospheric processes have strong seasonal cycles. Although similar seasonal cycles in isoprenoid emissions from terrestrial ecosystems have been reported, regional emissions inventories generally omit the effect of seasonality on emissions. We compiled measurement data on seasonal variations in monoterpene emissions potentials for two evergreen species (*Quercus ilex* and *Pinus pinea*) and used these data to construct two contrasting seasonal response functions for the inclusion in isoprenoid emission models. We included these responses in the Niinemets *et al.* model and compared simulation results to those of the Guenther *et al.* MEGAN model, both with and without its predicted seasonality. The effect of seasonality on regional emissions inventories for European Mediterranean forests dominated by these species was tested for both models, using the GOTILWA+ biosphere model platform.
The consideration of seasonality resulted in a large reduction in estimated emissions using the Niinemets *et al.* model. Total annual emissions were reduced by up to 65% in some regions, with largest reductions at lower latitudes. The MEGAN model demonstrated a much weaker seasonal response, and did not capture between species seasonality differences. Results suggests that species-specific expressions of seasonality may be necessary, and call into question the validity of the application of one response function for all species. The consideration of seasonality is shown to both largely reduce regional annual emissions estimates, and change the expected seasonal distribution of emissions.
5.1 Introduction

Volatile isoprenoids represent a heterogeneous compound class made up of a wide range of reactive hydrocarbons - isoprene, monoterpenes, and sesquiterpenes - emitted by most plant species. Isoprenoid emissions from terrestrial ecosystems provide one of the principal controls over oxidative photochemistry in the lower atmosphere (Fehsenfeld et al., 1992; Crutzen et al., 1999; Monson and Holland, 2001) and have a large impact on local air pollution (e.g., Fuentes et al., 2000; Kanakidou et al., 2005; Helmig et al., 2006; Szidat et al., 2006; Gelencser et al., 2007). The air chemistry and air pollution impacts of isoprenoids depend on the availability of reaction partners, i.e. reactive nitrogen compounds, which have a regionally specific seasonal pattern that is driven by anthropogenic as well as biological activities (e.g., Pierce et al., 1998; Fiore et al., 2005; Tie et al., 2006). Thus, it is not only the overall total emission budget but also the timing of emissions that is important.

Natural seasonal cycles are known to have a strong control over the timing of isoprenoid emissions (e.g., Llusia and Peñuelas, 2000; Hakola et al., 2003; Hakola et al., 2006; Holzinger et al., 2006). Climate change already affects seasonal cycles in terrestrial ecosystems, most notably the timing and duration of phenological events such as the onset of budburst and the rates of foliage development and senescence (Peñuelas and Filella, 2001; Bakkens et al., 2002; Peñuelas et al., 2002; Walther et al., 2002) and is likely to continue to do so in the future (Gitay et al., 2001; Prieto et al., 2009). Compared to other regions, Mediterranean regions in particular are threatened by such changes in the near future due to proportionally higher projected increases in temperature (Giorgi et al., 2004; Giorgi, 2006; Beniston et al., 2007; IPCC, 2007), and the potential for drought-driven phenological shifts in response to changes in precipitation (Peñuelas et al., 2004). Moreover, many dominant ecosystems of the Mediterranean regions contain species that are strong isoprenoid emitters such as the Mediterranean evergreen oak Quercus ilex L. dominated forests, which emit high amounts of highly reactive monoterpenes and exhibit a strong seasonality (Llusia and Peñuelas, 2000). Future changes in environmental conditions are likely to change isoprenoid emissions not only directly through altered temperature effects on the emission rates, but also indirectly due to altered seasonal cycles. Understanding the overall effect of seasonal cycles on emissions (and potential future changes) is thus
necessary to reduce uncertainty in current estimates and future projections of isoprenoid emissions.

Various models exist to describe the emissions of isoprenoids from terrestrial vegetation scales (see Grote and Niinemets, (2008), and Arneth et al., (2008b) for recent reviews). These models are based on the observed short-term response of emissions to temperature and light intensity, and due to a lack of long-term seasonal measurement data, have a bias towards employing only a snapshot of whole season, typically mid-season measurements, for parameterization (for representative emission inventories in mid-season used in major scaling exercises see Geron et al., (2000; 2006), Kesselmeier and Staudt (1999) and Simpson et al. (1999)). Other processes, operating over longer time scales, such as the effect of seasonality, and potential effects of CO₂ fertilization have received little attention. Atmospheric CO₂ concentration changes have been suggested to modify the emission response on the decadal or longer time scale (Possell et al., 2005; Arneth et al. 2007a, 2007b, 2008a). The determination of seasonality effects, however, has not as yet been systematically investigated, and represents a major uncertainty in biogenic emission simulations (Funk et al. 2005; Monson et al., 2007; Arneth et al., 2008b).

Seasonality in emissions has been suggested to affect the species-specific emission factor. This factor describes the potential for emissions under optimal conditions. It is the most important factor for the description of isoprenoid emissions (Arneth et al., 2008b; Grote and Niinemets, 2008) and varies strongly among species from values near zero to greater than 100 μg g⁻¹ leaf h⁻¹ (Kesselmeier and Staudt, 1999; Wiedinmyer et al., 2004). The basal emission factor is known to change considerably during the year (e.g., Llusia and Peñuelas, 2000; Hakola et al., 2006; Holzinger et al., 2006). The mechanisms behind this change are not fully understood, but it has been suggested to be due to the production and destruction of enzymes that are responsible for the formation of isoprenoids (Lehning et al., 1999; Loreto et al., 2001; Mayrhofer et al., 2005). In large-scale simulated emission estimates, the seasonal dynamics generated by such physiological pre-conditioning is almost always neglected. The influence of the omission of seasonal variation in emission potential on regional isoprenoid emission budgets has yet to be quantified.
Few approaches have been developed to simulate this seasonal modification of emissions. Geron et al. (2000) applied a weak seasonality using the integrated temperature of the previous 18 hours instead of instantaneous temperature. Fuentes and Wang (1999) used an empirical function based on cumulated temperature or growing degree days, respectively, and He et al. (2000) varied the emission factor in dependence on the number of monthly sunshine hours. Only one regionally applied model known to the authors, the Guenther et al. (2006) MEGAN model (Müller et al., 2008), explicitly accounts for the seasonal cycle of emissions on the regional scale. This model applies the same empirical adjustment to all plant functional types, based on the light and temperature regime of the past 10 days.

These models have typically resulted in bell-shaped response curves of isoprenoid emissions during the season, reflecting seasonal variation in light and temperature. However, the application of relatively short-term previous integrated climate to describe seasonal variation assumes that seasonal modifications mainly reflect acclimation response of foliage emission potentials. While environmental modifications can trigger the onset of seasonal events such as bud-break or leaf senescence, the control of phenological events by climatic drivers alone is not often strong (Battey, 2000). Seasonal variation in isoprenoid emission is thus often not bell-shaped (Llusia and Peñuelas, 2000), reflecting stronger variation of emissions than predicted by integrated climatic variables, possibly due to direct triggering by phenological events and the seasonal course of enzyme activity (Lehning et al., 2001), as simulated by the detailed SIM-BIM2 model (Grote et al., 2006). The scarcity of whole season emission data has hindered the inclusion of such species controls into the emission models.

The overall uncertainty in our knowledge of the drivers of seasonal dynamics of emissions, and how to model them, is potentially a large source of error when modeling isoprenoid emissions from terrestrial vegetation. In this chapter, we addressed the problem of seasonal dynamics of monoterpene emission potentials by developing seasonal response functions for two key species in Mediterranean forest ecosystems: the broadleaved evergreen sclerophyll Quercus ilex and the evergreen conifer Pinus pinea. The response functions were integrated into the Niinemets et al. (1999, 2002a)
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isoprenoid emission model coupled to the process-based terrestrial biogeochemical model GOTILWA+ (Gracia et al., 1999; Keenan et al., 2008; Keenan et al., 2009 (in press - ACPD)). Simulations were run for these two dominating species over the European Mediterranean region to quantify the effect of the consideration of a seasonally dynamic emissions potential on the total emissions budget for these two species. The simulations were further compared with simulations coupling the commonly used Guenther et al. model development, MEGAN, (Guenther et al., 1995; Guenther et al., 2006; Müller et al., 2008), both with and without its seasonal modifications of emissions, to the GOTILWA+ model.

5.2 Models, Measurements and Methods

5.2.1 Studied species

The Holm oak (Quercus ilex) is an evergreen sclerophyllous tree native to Mediterranean Europe. It is a strong emitter of monoterpenes, and although its distribution is limited to the Mediterranean region, it contributes more than 25% of the total European forest emissions budget (Keenan et al., 2009 (in press - ACPD)). The aerial coverage of Q. ilex is currently increasing in some mesic areas as the result of warmer temperature and reductions in water availability (Peñuelas and Boada, 2003). The Italian stone pine (Pinus pinea) is an evergreen conifer widespread in the European Mediterranean region. It is also one of the strongest monoterpenes emitters of European forest species. In both species, the bulk of monoterpenes are emitted in a temperature- and light-dependent manner (Bertin et al., 1997; Staudt et al., 1997).

5.2.2 Biosphere model platform description

To describe canopy level emissions, and scale the leaf level isoprenoid emission models to the region, we coupled both the Niinemets et al. (1999, 2002b) and the Guenther et al. (1995; 2006) MEGAN model (Müller et al., 2008) isoprenoid emission models to the process based terrestrial biogeochemical model GOTILWA+ (Gracia et al., 1999; Keenan et al., 2008; Keenan et al., 2009 (in press – ACPD)).
The GOTILWA+ model describes leaf structural and chemical characteristics, and thus foliage physiological potentials. The model also describes the forest structure and the microclimatic conditions necessary to scale from the leaf to the canopy, and for correct integration of fluxes distinguishes between sunlit and shaded leaf fractions (Wang and Leuning, 1998; Dai et al., 2004; Niinemets and Anten 2009). The distribution of intercepted diffuse and direct radiation within the canopy depends on the time of the day, season, and the area of leaf exposed to the sun (Campbell, 1986). The photosynthetic module couples the Farquhar et al. (1980) photosynthesis model, with dependencies on intercepted radiation, species-specific photosynthetic capacities, leaf temperature, and leaf intercellular CO₂ concentration (Cᵢ), to the Leuning et al. (1995) stomatal conductance model, that is the advancement of the Ball et al. (1987) model.

To scale from the canopy to the region, an extensive database has been built within the framework of the European ATEAM (Advanced Terrestrial Ecosystem Analysis and Modeling) and ALARM (Assessing Large-scale Risks for biodiversity with tested Methods) projects (Keenan et al., 2009 (in press - ACPD)), connecting diverse information sources at a European level and adapting them to fit the same spatial resolution of 10’ latitude x 10’ longitude (minutes). The database contains data related to forest species, forest coverage, forest structure, forest function (photosynthesis, respiration rates), soil hydrology, organic matter decomposition rates and management strategies (Schröter et al., 2005; Keenan et al., 2009 (in press - ACPD)). The species distribution database was updated using distribution data compiled by members of the EUFORGEN network (www.euforgen.org; Fady et al., 2004) (updated, 2008). The model set up used to scale from the leaf to the region has previously been described in Keenan et al. (2009 (in press – ACPD)).

2.3. Leaf level emissions algorithms
We considered the two leaf level emission models most commonly applied to estimate emissions on the regional scale (Niinemets et al., 2002b; Guenther et al., 2006; but see Arneth et al., 2008a; Müller et al., 2008). Both emissions models take contrasting approaches to modeling emissions, each with different assumptions about the way in which environmental factors limit the emissions and with different levels of mechanistic detail. Where pertinent, modifications were made for consistency between
these models (as in Arneth et al., 2007a). No direct CO$_2$ or water stress effect on the emissions was applied in this modeling exercise.

### 5.2.3.1 The Guenther et al. model

The earliest and, to date, most widely used model for the simulation of natural isoprenoid emissions was developed by Guenther et al. (1991), and Guenther et al. (1993). Its wide use is in large part due to its simplicity, describing emission rates by varying a long-term basal emission factor for isoprene ($E_I$) and monoterpenes ($E_M$) in dependence on light and temperature. These adjustments are applied through two empirical factors, one describing the response to light intensity and the other to leaf temperature, using the following algorithm:

$$E = E_M C_L C_T$$  
(Eq. 5.1)

The emission factor, $E_M$, used in the model is the emission rate normalized to a leaf temperature ($T$) of 30 °C and quantum flux density ($Q$) of 1000 μmol m$^{-2}$ s$^{-1}$ (sometimes for monoterpenes emitted by monoterpene-storing species normalized only to temperature) (Guenther et al., 1991; Guenther et al., 1993; Guenther et al., 1995; Guenther, 1997). $C_L$ and $C_T$ are the functions of quantum flux density and leaf temperature, respectively. $C_L$ follows a Michaelis-Menten type saturating function:

$$C_L = \frac{\alpha C_{L1} Q}{\sqrt{1 + \alpha^2 Q^2}} ,$$  
(Eq. 5.2)

where $\alpha$ and $C_{L1}$ are empirical coefficients. $C_T$ is described by an Arrhenius-type equation with an optimum that is commonly used to describe the temperature ($T$) dependence of biological reactions:

$$C_T = \frac{E_{opt} C_{T2} \exp \left( \frac{C_{T1} (T - T_{opt})}{RTT_{opt}} \right)}{C_{T2} C_{T1} \left[ 1 - \exp \left( \frac{C_{T2} (T - T_{opt})}{RTT_{opt}} \right) \right] } ,$$  
(Eq. 5.3)
where \( R \), the gas constant, is 8.314 J K\(^{-1}\) mol\(^{-1}\), and \( T_{\text{opt}} \) is the temperature optimum, and \( C_{T1} \) the activation energy (60,550 J mol\(^{-1}\)), and \( C_{T2} \) the deactivation energy (252,570 J mol\(^{-1}\)). Values for \( C_{T1} \) and \( C_{T2} \) were chosen to assure consistency between the two models used in the study. In the Guenther et al. model version ‘MEGAN’ (Guenther et al., 2006; Müller et al., 2008), the model was developed to include an empirical measure of seasonality, based on the previous light and temperature regime. In MEGAN, \( C_{L1}, \alpha, E_{\text{opt}} \) and \( T_{\text{opt}} \) are modified following the algorithms developed by Guenther et al. (2006), which links these values to short term (24 hours) and long term (10 days) fluctuations in temperatures \( (T_{24}, T_{240}) \) and light intensity \( (Q_{24}, Q_{240}) \):

\[
\alpha = 0.004 - 0.0005 \cdot \ln(Q_{240}) \quad \text{(Eq. 5.3a)}
\]

\[
C_{L1} = 0.0468 \cdot e^{[0.0005(Q_{24} - Q_{240})]} \cdot Q_{240}^{0.6} \quad \text{(Eq. 5.3b)}
\]

\[
T_{\text{opt}} = T_{\text{opt}} + 0.6 \cdot (T_{240} - 297) \quad \text{(Eq. 5.3c)}
\]

\[
E_{\text{opt}} = \exp(0.05 \cdot (T_{24} - 297)) \cdot \exp(0.05 \cdot (T_{240} - 297)) \quad \text{(Eq. 5.3d)}
\]

\( Q_0 \) varies depending on whether the given leaf is exposed to direct sunlight (\( Q_0 = 200 \) \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) or is in the shade (\( Q_0 = 50 \) \( \mu \text{mol m}^{-2} \text{s}^{-1} \)), when coupled to a biosphere model. Other specifications of the Guenther et al. (2006) MEGAN model, such as the effect of the effect of water stress, have been omitted in this study to ensure comparability with the Niinemets et al. (1999, 2002b) model (MEGAN assumes no leaf age affect for evergreen species). The seasonal cycle of emissions in the Guenther et al. (2006) MEGAN model (based on the previous 10 days light and temperature) has been calibrated using isoprene emission data from five studies (not included in our response function parameterization) (Petron et al., 2001; Monson et al. 1994; Sharkey et al., 2000; Geron et al., 2000; Hanson and Sharkey, 2001), including four different species (Quercus alba, Quercus rubra, Quercus macrocarpa, and Populus tremuloides).

### 2.3.2. The Niinemets et al. model

In the Niinemets et al. (1999, 2002b) model for isoprene and monoterpene emissions the supply of dimethylallyldiphosphate (DMADP) and NADPH, as affected by the rate of photosynthetic electron transport and the competitive strength of the synthase enzyme for electrons, are considered as the main controlling factors for the rate of isoprenoid synthesis. Thus the emission rates are linked to the activity of the isoprene or monoterpene synthase enzyme \( S_S \) to predict the capacity of isoprenoid synthesis...
pathway and to foliar photosynthetic metabolism via the photosynthetic electron transport rate, \( J \), to predict substrate availability for isoprenoid synthesis (Niinemets et al., 1999; Niinemets et al., 2002b).

Emission rates are calculated through the rate of photosynthetic electron transport, the fraction of total electron flow used for the isoprenoid synthesis, and the cost of isoprenoid synthesis in terms of electrons. Emissions can thus be linked to the photosynthetic electron transport activity of the leaf with the use of only one single leaf dependent parameter, \( \varepsilon \), the fractional allocation of electron transport to isoprenoid synthesis. Emission rates are given by the equation (Niinemets et al., 1999; Niinemets et al., 2002a):

\[
E = \varepsilon J_T \frac{C_i - \Gamma^*}{\varsigma(4C_i' + 8\Gamma^*) + 2\{C_i' - \Gamma^\} (\theta - 2\varsigma)},
\]

(Eq. 5.4)

where \( J_T \) is the total rate of photosynthetic electron transport, \( C_i \) is the internal CO\(_2\) concentration, and \( \Gamma^* \) is the hypothetical CO\(_2\) compensation point of photosynthesis that depends on photorespiration (Farquhar et al., 1980). \( \varsigma \) is the carbon cost of specific isoprenoid (6 mol mol\(^{-1}\) for isoprene and 12 mol mol\(^{-1}\) for monoterpenes) and \( \theta \) is the NADPH cost of specific isoprenoid (mol mol\(^{-1}\)). For monoterpenes, \( \theta \) is found as a weighted average of the costs of all terpene species emitted. In practice, \( \theta \simeq 28 \) mol mol\(^{-1}\) as the contribution of oxygenated monoterpenes that may have lower electron cost or reduced monoterpenes that may have higher electron cost is generally small (Niinemets et al., 2002a; Niinemets, 2004). \( \varepsilon \), the fractional allocation of electron transport to isoprenoid synthesis, is given by:

\[
\varepsilon = F_d \frac{S_S}{J_{max}},
\]

(Eq. 5.5)

where \( S_S \) is the specific activity of either isoprene or monoterpene synthase in mol isoprenoid (g isoprenoid synthase\(^{-1}\)) s\(^{-1}\) and \( J_{max} \) is the light saturated rate of total electron transport. Both \( S_S \) and \( J_{max} \) scales with temperature according to an Arrhenius type equation that has a temperature optimum (Niinemets and Tenhunen, 1997). \( F_d \) (g m\(^{-2}\) mol electrons mol isopnenoids\(^{-1}\)) is a scaling constant that depends on the basal
emission rate ($E_M$), the isoprenoid synthase content (g m$^{-2}$) and also converts from isoprenoid units to electron transport units (mol isoprenoids mol electrons$^{-1}$) (Niinemets et al., 1999; Niinemets et al., 2002b).

### 5.2.4 Derivation and implementation of seasonal response functions

An extensive literature search was performed to identify measurements related to the seasonal variation of the basal monoterpene emission factor ($E_M$). Data were compiled from studies explicitly looking at seasonal variation in $E_M$ as well as from studies reporting emission rates for several sampling events during the growing season where the measurement date was reported. In all cases, only measurements from fully sun-exposed branches were included. For *Quercus ilex*, $E_M$ estimates were obtained from (Bertin et al., 1997; Owen et al., 1997; Kesselmeier et al., 1997; Street et al., 1997; Kesselmeier et al., 1998; Llusia and Peñuelas, 2000; Staudt et al., 2004). For *Pinus pinea*, $E_M$ estimates were obtained from (Pio et al., 1993; Kesselmeier et al., 1997; Owen et al., 1997; Staudt et al., 1997; Street et al., 1997; Owen and Hewitt, 2000; Staudt et al., 2000; Owen et al., 2001; Sabillón and Cremades, 2001). The compiled data for both species exhibited a curve with a maximum between the Julian days 200-320 (Fig. 1) and were fitted by different empirical functions. As the seasonal variation in $E_M$ was asymmetric (Fig. 1), fitting the data by simple sinusoidal functions resulted in low degrees of explained variance ($r = 0.51$ for *Q. ilex* and $r = 0.61$ for *P. pinea* for the best sine functions with four parameters). Therefore, we used an asymmetric exponential function that allows for different rates of increase and reduction in $E_M$ during the growing season (June et al., 2004) to describe the seasonal variation in $E_M$:

$$E_M = E_0 + E_{\text{max}} e^{-\left\lfloor \frac{D-D_{\text{max}}}{\varepsilon} \right\rfloor^2},$$  \hspace{1cm} (Eq. 5.6)

where $E_0$ is the minimum and $E_{\text{max}}$ the maximum emission rate during the season, $D$ is the Julian day, $D_{\text{max}}$ is the Julian day at $E_{\text{max}}$, and $\varepsilon$ determines the rate of change of $E_M$ during the season. The data were fitted by Eq. 6 through minimizing the least-squares between the measurements and predictions, resulting in a high degree of correlation between the measured and predicted values ($r = 0.83$ for *Q. ilex* and $r = 0.86$ for *P.
pinea) (see Fig. 1 for the fits). For *Q. ilex*, the model parameters obtained were: $E_0 = 7.49 \, \mu g \, g^{-1} \, h^{-1}$, $E_{\text{max}} = 28.8 \, \mu g \, g^{-1} \, h^{-1}$, $D_{\text{max}} = 222.7$, $\varepsilon = 55.6$, while for *P. pinea* $E_0 = 1.95 \, \mu g \, g^{-1} \, h^{-1}$, $E_{\text{max}} = 7.90 \, \mu g \, g^{-1} \, h^{-1}$, $D_{\text{max}} = 198.1$, $\varepsilon = 42.9$.

![Graph showing seasonal variation in monoterpene emission factor (EM) in Mediterranean evergreen trees.](image)

*Figure 5.1. Seasonal variation in monoterpene emission factor (EM) in the Mediterranean evergreen sclerophyll Quercus ilex and the evergreen conifer Pinus pinea. Data were fitted by Eq. 6. (See Methods section 2.4. for data sources and curve parameters).*

### 5.2.5 Modelling protocol

Simulations were run with each emission model coupled to the GOTILWA+ model for each 10’ longitude x 10’ latitude scale pixel containing *Quercus ilex* or *Pinus pinea* forests in the European Mediterranean region. For parameterization of the forest structural components in GOTILWA+, species-specific parameters for *Quercus ilex* and *Pinus pinea* were applied. Two versions of the Niinemets *et al.* (1999, 2002b) model were considered – one with a fixed species-specific emissions potential (Fig. 1),
and the other varying the emissions potential using the seasonally dynamic response derived in Section 2.4 (Fig. 1). Two versions of the Guenther et al. (1995; 2006) MEGAN model (Müller et al., 2008) were also used – one without the seasonal parameter modification, and the other applying the Guenther et al. (2006) seasonal modification (based on the previous light and temperature regime, Eq. 3a-d). For each species, simulations were performed for years from 1900 to 2000, using the reconstructed climatic time-series based on the CRU05 (1901-2000) monthly dataset (New, Hulme, and Jones, 1999), with atmospheric concentrations of CO₂ from 1901 to 2000 obtained from the Carbon Cycle Model Linkage Project (McGuire et al., 2001). The presented results correspond to the 1960 to 1990 time period.

5.3 Results

The application of seasonal variations in emissions in both models had a large affect on both the total emissions budget and the timing of emissions for the two species (Table 1). For both species the consideration of a seasonally changing emission potential with the Niinemets et al. model reduced the total annual emissions from the European Mediterranean region by roughly 50%, when compared to emission estimates from the Niinemets et al. model with a fixed basal emission factor. The difference between the seasonal and non-seasonal Guenther et al. MEGAN model was not as strong, with an overall difference in annual emissions of 21% over the two species (Table 5.1).
Emissions from winter, early spring and late autumn were most affected. Summer months also showed a large reduction in emissions when considering seasonal driven changes in emissions in both models. In the case of the seasonal Niinemets et al. model, the peak emissions potential was not reached until mid-summer for Pinus pinea (Fig. 5.3) and late-summer for Quercus ilex (Fig. 5.2). For the seasonal Guenther et al. MEGAN model, peak emissions were in late summer for both species. The Guenther et al. MEGAN model predicted only very small reduction in emissions due to seasonality in autumn, due to the fact that it uses the past 10 days light and temperature, which allows high emissions to be sustained after peak summer conditions.
For *Quercus ilex*, the non-seasonal versions of both models gave very similar annual emissions totals. There were large differences within the year however, with the non-seasonal Niinemets *et al.* model giving higher emissions in the early and later parts or the year, and the non-seasonal Guenther *et al.* MEGAN model giving higher peak emissions during summer. The implication of a seasonal variation in emission potentials in the Niinemets *et al.* model explained much of the difference in the shape of the annual emission response when compared to the Guenther *et al.* MEGAN model (Fig. 5.2). The seasonal Guenther *et al.* MEGAN model showed the same shape as the seasonally dynamic Niinemets *et al.* model, with peak emissions around August (Fig. 5.2), but gave higher emissions than those of the seasonal Niinemets *et al.* model at all times during the year (Table 5.1). The total annual emissions budget with the seasonal Guenther *et al.* MEGAN model for *Quercus ilex* over the Mediterranean region was 43% higher than that of the seasonal Niinemets *et al.* model.
Table 5.1. Average emissions (g C m^{-2} month^{-1}) for the Niinemets et al. (1999, 2002b) and MEGAN (Guenther et al., 2006) models for the periods JFM (January, February, March), AMJ (April, May, June), JAS (July, August, September), and OND (October, November, December), for Quercus ilex and Pinus pinea during the years 1960-1990. Emissions for the MEGAN model and Niinemets et al. model are compared both with and without a seasonal adjustment. The percentage reduction refers to the reduction in total emissions from each model due to the implication of a seasonal variation in emissions.

<table>
<thead>
<tr>
<th>Period</th>
<th>Quercus ilex Emissions (g C m^{-2} month^{-1})</th>
<th>Pinus pinea Emissions (g C m^{-2} month^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEGAN model</td>
<td>Niinemets et al. model</td>
</tr>
<tr>
<td></td>
<td>Non-seasonal</td>
<td>Seasonal</td>
</tr>
<tr>
<td>JFM</td>
<td>0.23</td>
<td>0.10</td>
</tr>
<tr>
<td>AMJ</td>
<td>0.73</td>
<td>0.54</td>
</tr>
<tr>
<td>JAS</td>
<td>1.30</td>
<td>1.20</td>
</tr>
<tr>
<td>OND</td>
<td>0.33</td>
<td>0.21</td>
</tr>
<tr>
<td>Totals</td>
<td>2.59</td>
<td>2.05</td>
</tr>
</tbody>
</table>
For *Pinus pinea*, the non-seasonal Niinemets *et al.* model and the non-seasonal Guenther *et al.* MEGAN model again gave similar annual totals (differing by 16%), with a very different distribution of emissions within the year. The distribution of emissions within the year from the seasonally dynamic Niinemets *et al.* model was also more comparable to both the non-seasonal and the seasonal Guenther *et al.* MEGAN models than was the non-seasonal Niinemets *et al.* model (Fig. 5.3). Much of the difference between the original Niinemets *et al.* model and the seasonal Guenther *et al.* MEGAN model was explained by the inclusion of a seasonally dynamic emissions factor in the Niinemets *et al.* model, particularly in spring and early summer (Table 5.1). Marked differences appear in late summer and autumn, with emissions from the seasonal Guenther *et al.* MEGAN model (which applies the same seasonal response to both species) peaking much later than those of the seasonally dynamic Niinemets *et al.* model. This leads to 56% higher emissions in the second half of the year with the seasonal Guenther *et al.* MEGAN model (Table 5.1). The seasonal Guenther *et al.* MEGAN model gave 50% higher total annual emissions than the seasonal Niinemets *et al.* model.
Figure 5.4. Average regional differences (%) in total simulated annual emissions: (a) between the Niinemets et al. seasonal and Niinemets et al. (1999, 2002b) non-seasonal model, and (b) the Guenther et al. (2006) MEGAN model with and without its seasonal response. Both models are run coupled to the GOTILWA+ model (Gracia et al., 1999; Keenan et al., 2009 (in press, ACPD)), for Quercus ilex forests in the Mediterranean Europe region, for the period 1960-1990.

The impact of the consideration of a seasonally dynamic emission potential on the total annual emission budget from the Niinemets et al. model was higher at lower latitudes. The reduction in the total annual emissions from the Niinemets et al. model due to the consideration of seasonal variability in the basal emission factor varied from 25% (in the region of the Pyrenees Mountains), to 65% (in the southern Iberian Peninsula). This trend was reflected in both Quercus ilex (Fig. 5.4a) and in Pinus pinea (Fig. 5.5a). Overall, the impact of seasonal variation in the Niinemets et al. model was higher in Pinus pinea, which shows a stronger seasonal cycle in its emission potential (a 5.2 fold increase over a 62 day period, compared to a 4.8 fold increase over a 98 day period for
Quercus ilex (Fig. 5.1)). Areas subject to warmer winters showed the largest differences.

The difference between the Guenther et al. MEGAN model with and without seasonality was considerably smaller than that observed with the Niinemets et al. model (Fig. 5.4, Fig. 5.5), suggesting that the measure of seasonality in the Guenther et al. MEGAN model is much weaker than that derived from the data in Section 5.2.4. The difference in total annual emissions did not show a strong latitudinal response, due to the fact that the seasonal response in the Guenther et al. MEGAN model is based on temperature and light.

Figure 5.5. Average regional differences (%) in total simulated annual emissions: (a) between the Niinemets et al. seasonal and Niinemets et al. (1999, 2002b) non-seasonal model, and (b) the Guenther et al. (2006) MEGAN model with and without its seasonal response. Both models are run coupled to the GOTILWA+ model (Gracia et al., 1999; Keenan et al., 2009 (in press, ACPD)), for Pinus pinea forests in the Mediterranean Europe region.
5.4 Discussion

Seasonal variation in isoprenoid emissions in Mediterranean plants has been widely reported (e.g., Peñuelas and Llusia, 1999; Seufert, 1997; Staudt et al., 1997; Llusia and Peñuelas, 2000), and has been linked to phenological events, such as leaf development (which involves a hardening of the leaf cuticle and therefore may alter both the diffusive resistance and the isoprenoid vapor pressure). Emissions are also influenced by ontogeny (Grinspoon et al., 1991; Fuentes et al., 1995; Fuentes and Wang, 1999; Kuhn et al., 2004) as well as acclimation to the environment during previous hours to days. Accounting for such seasonal changes in isoprenoid emissions is of great importance for accurately modeling the seasonal dynamics of isoprenoid emissions, in particular for studies which include a strong temporal dependency (e.g. the effect of the timing of emissions on regional air chemistry and pollution). The presented study is the first to demonstrate the importance of considering the seasonal dynamics of emissions on a regional scale, and illustrates the magnitude that the consideration of seasonal variability can have on estimated regional budgets. The scarcity of knowledge on the driving processes behind such seasonal variations leads to large uncertainty when modeling the seasonal cycle of emissions. The use of a fixed seasonality relationship with the time of year, as applied in this study, may not be entirely correct due to the probable dependence of seasonality on past light and temperature regimes (Sharkey and Loreto, 1993; Staudt et al., 2000), phenology, or water availability (Bertin and Staudt, 1996), leading to complex spatiotemporal variations in emission potentials. However, considering such effects in a more detailed manner is not possible until a more process-based knowledge of seasonal variations has been gained.

The Niinemets et al. (1999, 2002b) model has a strong relation to light regime and energy production (see Arneth et al., 2007a), as do the pre-MEGAN versions of the Guenther et al. model algorithms (equivalent to the non-seasonal MEGAN model) (Guenther et al., 1991; Guenther et al., 1993; Guenther et al., 1995; Arneth et al., 2007a), which have been widely used, with a fixed emission factor, in regional and global estimates of biogenic isoprenoid emissions inventories (e.g., Guenther et al., 1995; Levis et al., 1999; Simpson et al., 1999; Wang and Shallcross, 2000; Adams et al., 2001; Naik et al., 2004; Parra et al., 2004; Tao and Jain, 2005; Lathière et al.,
Thus, with a fixed emission factor, emissions can be sustained even in winter because light availability and temperature are still sufficiently high. This is particularly noticeable in the increasing difference in modeled emissions at more southern latitudes (Fig. 5.4, Fig. 5.5), where relatively warm winters lead to large emissions if the seasonal cycle of emissions potentials is not taken into account. Only one large scale regional emissions inventory, known to the authors, has included the effect of seasonality (Guenther et al., 2006; Müller et al., 2008), though the seasonal variation included in that inventory has been shown here to be much weaker than the response derived in this study. This suggests that the previous inventories may need to be revised.

Species differences in the shape of the $E_M$ vs. Julian day dependencies reflect species-specific differences in phenology as frequently observed in Mediterranean species (e.g., Pereira et al., 1987; Flexas et al., 2001; Ogaya and Peñuelas, 2004; Prieto et al., 2009). The large difference (in the magnitude and timing of emissions) between the response function for Pinus pinea and Quercus ilex calls into question the validity of applying one empirical parameterization to all species and functional types. Such differences suggest that the empirical introduction of seasonality by the Guenther et al. (2006) MEGAN model (Müller et al., 2008) (parameterized with data from five studies of four different species) may not be effective in capturing between species/functional type variations. This has proven to be the case for these two studied species, with both exhibiting markedly different seasonal cycles. In addition, the seasonal variation in $E_M$ appears highly asymmetric in both species (Fig. 5.1), while MEGAN assumes a symmetric seasonal variation. These differences in $E_M$ response shapes result in larger differences in predicted emissions among specific months than between annual totals (Table 5.1).

The effect of drought has not been included in this study. Drought occurrences and changes in the distribution of precipitation have been reported to cause changes in phenological cycles (Peñuelas et al., 2004). Drought affects could therefore indirectly lead to complex changes in the timing of emissions. Drought has also been shown to greatly reduce summer emissions from forest canopies in the Mediterranean region (Llusia and Peñuelas, 1998; Grote et al., 2009; Lavoir et al., 2009). Various simple
reduction functions have been used in modeling studies (Guenther et al., 2006; Grote et al., 2009; Keenan et al., 2009 (in press - ACPD)), though there is no clear understanding as to how to model emission responses to drought (Grote and Niinemets, 2008). A drought-induced reduction in emissions during summer would increase the relative importance of the consideration of seasonal variation in the basal emission factor, as spring and autumn emissions (where bigger differences are observed due to seasonality) would have greater weight in the total annual emissions budget. This is important when considering the fact that drought-prone areas have a large aerial coverage of isoprenoid-emitting species due to both natural (Kellomäki et al., 2001) and anthropogenic (Vizuete et al., 2002; Geron et al., 2006; Lathière et al., 2006) reasons (e.g. plantation of crops and fast growing trees to produce bio-energy). Our understanding of the effect of drought on photosynthesis, and how to model it, has recently improved (Keenan et al., 2009 (in press, BGD)) but much work is needed to accurately model phenology and interactions between water availability and the timing of phenological responses.

5.5 Conclusions

We conclude that the consideration of seasonality is necessary for any regional inventory, and a more thorough understanding will be crucial for climate change scenario analyses of isoprenoid emissions for many regions, in particular at lower latitudes. This is particularly true for areas that exhibit drought stress today or in the future and host isoprenoid emitting plants (e.g., Lathière et al., 2005). The empirical approach used by the Guenther et al. (2006) MEGAN model (Müller et al., 2008) compared well in shape to the response derived from the data for Quercus ilex, but was shown to be much weaker than the seasonal response derived in this study. For Pinus pinea, neither the shape nor the magnitude of the response included in the Guenther et al. (2006) MEGAN model (Müller et al., 2008) compared well against the seasonal response derived in this study, suggesting that species specific expressions of seasonality in emissions potentials may be necessary.
The qualitative results of the effect of the inclusion of seasonality, although here specific to the two studied species, are expected to be applicable to any species. This is likely to have large ramifications on regional and global emissions estimates, potentially reducing previous emissions inventories by up to 65% in some areas.

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Chapter 5: Seasonality of BVOC Emissions


Chapter 5: Seasonality of BVOC Emissions


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General conclusions

This PhD thesis investigates the exchange rates of carbon and water fluxes from terrestrial forest ecosystems, with a particular focus on combining data analysis with the modeling of the ecophysiological processes which drive them. We have dealt thoroughly with two areas which lead to large uncertainty when modeling carbon and water fluxes from terrestrial vegetation: The effect of water stress, and intermodel differences in BVOC emission estimates. This has led to the following main conclusions:

1) The response of the coupled photosynthesis-conductance model represents the main uncertainty when modeling CO₂ and water fluxes from Mediterranean forest ecosystems. This has been shown to be due to the assumption of the sole role of stomatal conductance in the regulation of photosynthesis responses to soil water stress. Through the implication of changes in either photosynthetic potential, or (in conjunction with changes in stomatal conductance) mesophyll conductance, carbon and water fluxes can now be modeled to a similar degree of accuracy during periods of soil water stress as during periods of high soil water availability. Whether biological limitations or a combination of conductance limitations to photosynthesis are dominant during water stressed periods remains an open question, and is most likely to be dependent on the species in question and the degree of soil water stress experienced.

2) It has been shown that model predictions of the emissions of BVOCs from European forests agree well under present day conditions but diverge strongly under projected future climatic conditions, due to the different temperature sensitivities of the models. The use of species specific emission potentials and geographical distributions showed that few dominant species contribute the largest portion of the total emissions budget. These will be important focus species for future work, in order to reduce uncertainty in our regional BVOC emissions estimates. Part of this uncertainty derives from lack of knowledge about seasonal variations in the species emissions
potentials. Seasonal variation in emissions potentials was shown to have a very large impact on the regional emissions budget.

Specific conclusions

The specific conclusions of each chapter of this thesis are the following:

Chapter 1. Improved understanding of drought controls on seasonal variation in Mediterranean forest canopy CO2 and water fluxes through combined in situ measurements and ecosystem modelling

1) We have shown that canopy conductance and photosynthesis co-vary with soil moisture in a consistent manner using observations from four sites. Stomatal conductance was found to vary in parallel with photosynthesis, with only small changes in ratio of the conductance-assimilation relationship.

2) Changes in photosynthetic capacity not related to stomatal closure under water stressed conditions were found to be very important at each of the sites studied.

3) Incorporating this knowledge into process-based models suggests that accounting for soil-water mediated reductions of photosynthetic capacity alone is sufficient to adequately model carbon and water fluxes during dry and wet periods. Pure stomatal regulation of photosynthesis during seasonal drought episodes is not sufficient to reduce modelled photosynthesis to observed level during drought periods.

4) This study contributes to improving our ability to model and predict carbon and water fluxes in Mediterranean-type forest ecosystems, and thereby to reducing uncertainty in future European terrestrial carbon and water fluxes. These results are of particular importance for any study of the effects of climatic changes on Mediterranean ecosystem functioning.

Chapter 2: The importance of mesophyll conductance in regulating biosphere productivity during periods of water stress
1) The results of this study highlight the importance of mesophyll conductance in the control of photosynthetic rates, both in well watered conditions and in response to soil water stress.

2) The drawdown of carbon from the leaf inter-cellular spaces to the chloroplast was shown to scale imperfectly with mesophyll conductance, implying a strong role of mesophyll conductance, in particular at low internal carbon concentrations in the regulation of photosynthesis.

3) The concentration of carbon in the chloroplast was shown to be a more reliable estimator of assimilation rates than the inter-cellular carbon concentration. This is of importance when modelling photosynthetic rates, both in well watered and dry conditions.

4) A strong response of mesophyll conductance to soil water stress was observed at all sites, further limiting the concentration of carbon available for assimilation. A complex dynamic was observed at each site between the responses of stomatal and mesophyll conductance limitations.

5) These results have important implications for canopy-level photosynthesis modelling, and may explain some of the difficulties ecosystem models encounter when modelling carbon and water fluxes in Mediterranean conditions as, to date, no published model fully incorporates mesophyll conductance. This could be of particular importance in the estimation of Mediterranean primary production, both in current day conditions and when modelling responses to future climate change scenarios.

Chapter 3: Soil water stress and coupled photosynthesis-conductance models: Bridging the gap between conflicting reports on the relative roles of stomatal, mesophyll conductance and biochemical limitations to photosynthesis

1) This work was carried out in response to the recent calls for the inclusion of mesophyll conductance in leaf photosynthesis models, due to an increased number of reported findings on the role of mesophyll conductance in the regulation of photosynthesis.

2) It has allowed for a model based assessment of previous suggestions that biological limitations, stomatal conductance limitations and mesophyll
conductance limitations play some role in the regulation of photosynthesis, depending on the degree of water stress encountered and the relevant time scales involved.

3) This is the first time that each limitation has been tested on a canopy scale in an eco-physiological model, and shows that, with (and only with) the inclusion of mesophyll conductance, diffusive limitations can explain carbon and water flux responses to seasonal changes in soil water availability.

4) This helps in closing the gap between studies (both model and measurement based) which report dominant roles of biological changes under water stressed conditions and those which maintain stomatal conductance limitations as the main actor.

5) The inclusion of a fixed $g_m$ did not improve our ability to effectively model carbon and water fluxes, but did increase our understanding of the processes involved in photosynthetic limitations during water stress periods. A fixed $g_m$ however is not very realistic, as it has been reported to be highly variable.

6) It is expected that the future development of a dynamic model for mesophyll conductance, and its integration into coupled photosynthesis-conductance models will improve our ability to model carbon and water fluxes from terrestrial ecosystems both in well watered and water stressed periods.

Chapter 4: Process based inventory of isoprenoid emissions from European forests: model comparisons, current knowledge and uncertainties

1) The coupling of the three different model approaches (Guenther et al., Niinemets et al. and Martin et al. models) to an ecophysiological forest model provides a unique opportunity to explore the time-dependent changes in modelled biogenic emissions due to differences in model structure and model responses to changes in climatic and physiological processes.

2) The modelled emissions from present day European forests were shown to be independent of the emission model used, with estimates of 1.03 TgC a-1 for isoprene emission and 0.93 TgC a-1 for monoterpenes, giving a consistent emission inventory for BVOCs from European forests.

3) Coincidence of model estimates of emissions for current and past climatic conditions in Europe suggest that the present day inventories of BVOC emissions provide realistic estimates. However, model-dependent
differences in simulated estimates of future emissions of both isoprene and monoterpenes highlight the fact that we are in the early stages of the path towards a full understanding of the processes governing BVOC emissions.

4) This has important implications for any study seeking to model future BVOC emissions. Many studies involving modelled future BVOC emissions (e.g., potential offsetting of emissions by rising CO2 concentrations, relative effects of changes in land use on quantitative emission estimates, effects on future emissions on tropospheric O3 concentrations and air quality) may need to be revised to take into account the inherent variability introduced by the choice of the emission model used.

Chapter 5: Seasonality of monoterpane emission potentials in *Quercus ilex* and *Pinus pinea*: Implications for regional BVOC emissions modeling

1) We conclude that the consideration of seasonality is necessary for any regional inventory, and a more thorough understanding will be crucial for climate change scenario analyses of isoprenoid emissions for many regions, in particular at lower latitudes. This is particularly true for areas that exhibit drought stress today or in the future and host isoprenoid emitting.

2) The empirical approach used by the Guenther *et al.* MEGAN compared well in shape to the response derived from the data for *Quercus ilex*, but was shown to be much weaker than the seasonal response derived in this study.

3) For *Pinus pinea*, neither the shape nor the magnitude of the response included in the Guenther *et al.* MEGAN model compared well against the seasonal response derived in this study, suggesting that species specific expressions of seasonality in emissions potentials may be necessary.

4) The qualitative results of the effect of the inclusion of seasonality, although here specific to the two studied species, are expected to be applicable to any species. This is likely to have large ramifications on regional and global emissions estimates, potentially reducing previous emissions inventories by up to 65% in some areas.
"Twenty years from now you will be more disappointed by the things you didn't do than by the ones you did. So throw off the bowlines, Sail away from the safe harbor. Catch the trade winds in your sails. Explore. Dream"

Mark Twain