Doctoral Thesis

Root growth, soil nutrient availability and water flow in model ecosystems of young oaks subjected to air-warming and drought

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Root Growth, Soil Nutrient Availability and Water Flow in Model Ecosystems of Young Oaks Subjected to Air-warming and Drought

A dissertation submitted to

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for the degree of

DOCTOR OF SCIENCES

presented by

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Summary

Global climate change is expected to increase annual temperatures and decrease summer precipitation in Central Europe. Being tolerant to heat and drought, oaks are considered promising trees to replace beech and spruce on forest sites that will become too warm and dry for these species. Oak provenances from dry sites are expected to be especially drought tolerant. However, little is known on how the oak species growing in Switzerland (*Quercus robur*, *Q. petraea* and *Q. pubescens*) will respond to the changing climate and how these responses depend on soil properties. Apart from direct drought and heat stress effects on biochemical and physiological processes, trees like other plants will also have to cope with indirect effects such as changes in soil nutrient availability. For example, nitrogen (N) availability depends on soil microbial processes, which are sensitive to soil temperature and moisture. We expected warming to increase and drought to decrease soil N availability and thus N supply to the trees.

In a 3-year factorial model ecosystem experiment, we investigated the responses of young mixed oak stands with *Quercus robur*, *Q. petraea* and *Q. pubescens*, growing on either acidic or calcareous soil, to air-warming (+1 to 2 °C) and drought treatments (-43% to -60% precipitation), corresponding to the IPCC climate change Scenario A2. The air-warming and drought treatments were applied separately as well as in combination on both soils.

The mild air-warming treatment had no effect on soil water availability, evapotranspiration or stand biomass, neither above nor below ground. Water limitation, which was severe during drought periods as shown in low leaf water potentials, significantly reduced the stand foliage, stem + twigs and root biomass. However, no tree died during the
The drought treatment led to higher root:shoot mass ratios and only little reduction in fine root biomass compared to total root mass. Allocating relatively more biomass into structures for water and nutrient uptake than for above ground assimilation can be seen as a response to balance impeded soil water extraction with reduced transpirational water loss. Moreover, evapotranspiration increased within a few days after re-watering to pre-drought levels, indicating a fast recovery potential of the oaks photosynthetic activity. Decreased evapotranspiration from the drought-exposed stands led to significantly higher air and soil temperatures, which were attributed to reduced transpirational cooling. As a major finding of our results, we point out that additional air warming did not enhance the drought effects on evapotranspiration and biomass, although more negative leaf water potentials were observed.

In an additional neutron radiography experiment, it has been shown for the first time that root-shoot growth patterns of oak seedlings are not altered by either water availability or soil properties. Independent from soil type and drought treatment, peaks and dips of shoot and root growth rates occurred at the same time. We conclude therefore that during the first year after germination, environmental conditions were overruled by endogenous growth patterns. Moreover, it has been shown for the first time that neutron radiography is a well-suited, non-destructive method to track root growth patterns of young oak trees.

Stands not subjected to the drought treatment produced significantly less biomass on the calcareous than on the acidic soil. This was probably due to phosphor (P) or manganese (Mn) limitation. In the acidic soil, root mass was maximal in the nutrient-rich topsoil, whereas in the calcareous soil maximum root mass occurred between 0.25 and 0.5 m depth. There was no difference in biomass and water regime between the two soils under drought conditions. As another highlight of our results, we therefore emphasise that nutrient availability was governed by water availability under these conditions.
While total amounts, vertical distribution and availability of soil N showed substantial differences between the two soils, neither warming nor drought had a significant effect on N availability in either of them, in contrast to our expectations. Air-warming also had no effect on N accumulation in the biomass of the trees, whereas the drought treatment increased or tended to increase N accumulation, indicating that growth was not limited by N. $^{15}$N-labelling revealed that this increase was associated with a higher fraction of tracer-N accumulation deriving from $^{15}$N applied in the form of nitrate to the soil surface, while the fraction of $^{15}$N accumulated in the plants decreased when it had been applied as ammonium. As a further highlight of our study, we therefore conclude that climate change is not expected to have major effects on the N dynamics of young oak stands in central Europe.

The provenances of *Quercus robur* showed more vigorous growth in all treatments than those of the other two species, irrespective of soil type, air-warming and drought treatments. However, drought-tolerance in relation to root:shoot mass ratio, leaf stress injuries, photosynthesis and wood anatomy was higher in *Q. petraea* and *Q. pubescens* than in *Q. robur*. Therefore, *Q. robur* appeared to be the most promising species when drought periods are only short, whereas long and severe drought periods would favour *Q. petraea* and *Q. pubescens*. Within each species, provenances originating from drier sites produced more root mass than those from more humid sites. However, given that the provenances from drier sites reacted more sensitive to water limiting conditions than those from more humid sites, it remains questionable whether these provenances are better suited for a future climate. Also other aspects such as frost tolerance and wood quality must be considered in selecting provenances for future forestry. In any case, we demonstrated for the first time under controlled experimental conditions that independent from soil properties young saplings of all three central European oak species can successfully cope with severe drought periods and
increased air temperatures, and thus are suited from this point of view for future forestry in central Europe under conditions of climate change.
Zusammenfassung


In einem 3-jährigen Lysimeterexperiment wurden die Auswirkungen von Lufterwärmung (+1 bis 2 °C) und Trockenstress (-43% bis -60% Niederschlag) auf Eichenbestände mit jungen Stiel-, Trauben- und Flaumeichen auf einem sauren Boden und auf einem Kalkboden untersucht. Die Behandlungen Lufterwärmung und Trockenstress wurden entweder voneinander getrennt oder in Kombination angewendet und orientierten sich
Zusammenfassung

dabei am IPCC Szenario A2, welches auf einem hohen Populationswachstum, einer langsamer Wirtschaftsentwicklung und einem geringen Technologiewandel basiert.

Die Lufterwärmung hatte keinen Effekt auf die Verfügbarkeit des Bodenwassers, die Evapotranspiration oder die Biomasse der Eichenbestände. Im Gegensatz dazu reduzierte Trockenstress die Wurzel-, Stamm- und Blattbiomasse deutlich. Diese Trockenstressbehandlung erwies sich als ziemlich extrem, was gut sichtbar an den sehr tiefen Blattwasserpotentialen war. Trotz dieser starken Trockenstressbehandlung starb während des ganzen Experiments keine einzige Eiche. Darüber hinaus stieg die Evapotranspiration innerhalb weniger Tage nach Beendigung der Trockenperioden auf das Niveau der Kontrolle an, was auf ein hohes Regenerationspotential der Eichen hindeutet. Unter Trockenstress entwickelten die Eichen ein höheres Wurzel:Spross Verhältnis. Ausserdem waren die Effekte der Wasserlimitation auf die Feinwurzelbiomasse geringer als auf die Gesamtwurzelbiomasse. Diese Anpassungen ermöglichten es den Eichen auch unter Trockenheit effizient Wasser und Nährstoffe aufzunehmen und gleichzeitig Wasserverluste durch Transpiration zu reduzieren. Die Evapotranspiration von trockengestressten Eichenbeständen war zeitweise sehr gering, was durch eine geringe Transpirationskühlung zu höheren Luft- und Bodentemperaturen führte. Als wichtige Erkenntnis dieser Studie möchten wir hervorheben, dass die zusätzliche Lufterwärmung in der kombinierten Behandlung jedoch zu keiner Verstärkung der Trockenstressbehandlung auf die Evapotranspiration oder Biomasse der Eichen führte obwohl tiefere Blattwasserpotentiale gemessen wurden.

In einem zusätzlichen Neutronenradiographieexperiment wurde zum ersten Mal gezeigt, dass periodisches Wurzel- und Sprosswachstum von Eichenkeimlingen weder durch Wasserverfügbarkeit noch durch Bodeneigenschaften verändert wird. Unabhängig von Trockenstressbehandlung und Bodentyp traten die höchsten wie auch die tiefsten Wachstumsraten von Spross und Wurzeln immer zur gleichen Zeit auf. Wir folgern daher,...


Im Gegensatz zu den aufgestellten Hypothesen beeinflussten weder Lufterwärmung noch Trockenstress die Stickstoffverfügbarkeit in diesen beiden Böden. Dagegen gab es deutliche Unterschiede zwischen den beiden Böden in den totalen und verfügbaren N-Gehalten. Während die Lufterwärmung keinen Einfluss auf die Akkumulation von N in der Biomasse hatte, war diese in trockengestressten Bäumen höher als in der Kontrolle. Dies deutet darauf hin, dass das Wachstum der trockengestressten Eichen nicht durch die Verfügbarkeit von N limitiert war. Ein Markierungsversuch mit $^{15}$N ergab, dass diese Erhöhung vor allem auf eine vermehrte Aufnahme in Form von Nitrat zurückzuführen war, währenddem die Akkumulation von N in Form von Ammonium tiefer war. Wir sehen es
daher als wichtiges Ergebnis dieser Studie, dass der Klimawandel wohl keine grösseren Effekte auf die Stickstoffdynamik in mitteleuropäischen Eichenökosystemen haben wird.


Zusammenfassend wir haben in dieser Studie zum ersten Mal unter kontrollierten Bedingungen gezeigt, dass junge Eichen unabhängig vom Bodentyp heisse und trockene Sommer gut überstehen können. Somit sollten alle drei mitteleuropäischen Eichenarten in der zukünftigen Forstwirtschaft Verwendung finden und die heute dominierenden Buchen und Fichten ersetzen können.
1. Introduction

1.1. Background

Swiss forests are usually cultivated under near-natural conditions. This means that foresters primarily grow trees that would occur also under natural conditions on a site, by exploiting spontaneous propagation, where possible, and by controlling species composition and tree density through selective thinning. They are not allowed to apply fertilisers and pesticides. Consequently, the prevalences of foreign tree species (1%) and monocultures (19%) are quite low (Brändli 2010). While most Swiss forests are mixed stands, they are dominated by only a few species. The main species are Norway spruce (*Picea abies*, dominating on 40% of the forested area), beech (*Fagus sylvatica*, 18%), silver fir (*Abies alba*, 10%) and larch (*Larix decidua*, 7%) according to the last forest inventory 2004 – 2006 (Brändli 2010). Oaks dominate on only 2% of the forested area of Switzerland. The three most abundant oak species in Switzerland today are *Quercus robur* (0.5% of all trees by number), *Q. petraea* (1.4%) and *Q. pubescens* (0.3%). In addition, there are a few *Q. cerris* and *Q. rubra* stands.

In the past, oaks were much more abundant, because oak timber was an important product of traditional forestry for construction purposes and furniture, while acorns were used as a nutritious feedstuff in pig husbandry. Over the last centuries, the demand for oaks declined (Bonfils *et al.* 2005) and oaks were replaced by beech and spruce, which produce more biomass and need less care than oaks. This trend continued until very recently. From 1995 to 2006, the number of *Q. robur* trees declined by 13% and that of *Q. petraea* by 8%, while the standing biomass of oaks increased due to aging and lack of rejuvenation (Brändli
Fig. 1.1: Distribution of *Quercus robur*, *Q. petraea* and *Q. pubescens* in Europe. Picture taken from Professur für Waldbau & Professur für Forstschutz & Dendrologie (ETHZ) (1995).

Only the abundance of *Q. pubescens* increased (+36%) during this time, mainly in the Valais, which is the driest region of Switzerland.

Oaks require a temperate climate with a low number of frost days (Bonfils et al. 2005; Landolt & Bäumler 2010). Therefore they are confined to relatively warm, low-lying, colline regions without extended cold periods (Fig. 1.1). *Quercus pubescens* occurs naturally only in southern Europe. In contrast to the narrow restrictions in temperature, oaks are able to grow under a wide range of humidity and soil chemical conditions, *i.e.* on dry to wet as well as on acidic to alkaline soils (Fig. 1.2A, Ellenberg & Klötzli 1972; Ellenberg et al. 2010; Landolt & Bäumler 2010). Beech (*Fagus sylvatica*) occupies a similar physiological optimum as oaks, but grows faster and is more competitive than oaks under moist conditions and on not too nutrient-poor soils. Without forest management oaks dominate only on sites that are too dry for beech and most other tree species (Fig. 1.2B). Beside low soil water availability, also alluvial forests, where the root zone is affected by frequently changing water levels, provide favourable conditions for oaks. Norway spruce (*Picea abies*) is the other important tree species now dominating in forests that were once occupied by oaks. In contrast to the displacement by beech, this is entirely a forestry artefact. As a mountainous tree in Switzerland, spruce would not be much in competition with oaks under natural conditions.
Recently, cultivation of oaks has gained renewed interest in forestry. Oak stands provide a unique habitat for many plants and animals that would disappear with them; and having played an important role in human culture and land use in the past, continued maintenance of oaks is also considered a preservation of national heritage (http://www.foersterschule.ch/proquercus/). With increasing demand for oak reforestation, a better understanding of the ecophysiology of oaks and how different oak species and provenances will perform under climate change conditions is needed in future forestry.

Climate change scenarios generally predict increasing temperatures and more extreme precipitation patterns in central Europe (IPCC 2007). According to IPCC Scenario A2, for example, summer temperatures are expected to increase by 3.2 - 4.8 °C during the 21st century in central Europe (CH2011 2011; Schär et al. 2004). With a reduction in summer precipitation by 21 to 28%, severe drought periods as well as extreme rainfall events will become more
frequent at the same time according to this scenario (IPCC 2007). Oaks are known to be
tolerant to drought and heat. Their long taproots allow them to take up water from deep soil
layers; the xeromorphic structure of their leaves provides efficient reduction of water loss;
and their ability to resume photosynthesis within a few days after re-wetting shows that their
physiology can recover quickly from drought stress (Arend et al. 2012; Galle et al. 2007;
Günthardt-Goerg et al. 2012; Kubiske & Abrams 1993). Therefore, with warmer and drier
summers the frequency of niches matching the ecological optimum of oaks is predicted to
increase. Oaks are expected to outcompete beech under water limiting conditions and
therefore to become more prevalent again in the future (Friedrichs et al. 2009; Leuschner et
al. 2001a; Scharnweber et al. 2011; Thomas 2000a; van Hees 1997). Also Norway spruce is
known as drought-intolerant species due to its shallow root system, as discussed by Zang et
al. (2012). Therefore, the frequency of spruce planted at low altitudes is expected to decrease
as well. In agreement with these hypotheses, modelling studies predict an increase in oak
forests, whereas beech and spruce forests are expected to decline at low altitudes
(http://www.wsl.ch/land/products/klimaanimation; Kienast et al. 1996; Hlasny et al. 2011). In
the Swiss Rhone valley the abundance of *Q. pubescens* has already increased by a factor of
1.8 from 1995 to 2006, whereas the abundance of Scots pine (*Pinus sylvestris*) decreased by
12% in the same period, and it is assumed that these changes are due to a trend in reduced soil
water availability (Brändli 2010; Wohlgemuth 2006).

Generally, higher temperatures are expected to favour tree growth by extending
growing seasons and accelerating plant metabolism (Saxe et al. 2001; Way & Oren 2010).
However, as temperatures approach optimum conditions, variations in temperature loose in
importance relative to other factors such as water, light and nutrients (Pregitzer et al. 2000;
Teskey & Hinckley 1981). In general, temperatures optima for deciduous tree growth range
between 15 and 25 °C (Lütte et al. 2010). Lyr & Garbe (1995) and Lyr (1996) found an
optimum soil temperature of 25 °C for oak root growth. With mean monthly daytime temperatures around 19 °C at low altitudes in Switzerland during the growing season (May to September, 8-18 h, UTC+1), temperatures are neither very close nor far from the optimum of oak trees. Thus, it is not clear if an increase of around 2 - 4 °C as predicted in medium IPCC scenarios will have a significant influence on oak growth or not.

In contrast to the often positive effect of increased air temperatures on plant growth, lack of water generally has a negative effect on trees, including oaks (Bruschi 2010; Jensen & Hansen 2010; Thomas 2000a; Thomas et al. 2002; Thomas & Gausling 2000). However, there are large differences in drought sensitivity among species. Beech and spruce are known to be more drought sensitive than oaks (Friedrichs et al. 2009; Leuschner et al. 2001a); and oak provenances originating from drier sites are known to be more drought tolerant than provenances from mesic sites (Bruschi 2010; Jensen & Hansen 2010). Increasing transpirational water demand, air warming is expected to exacerbate drought stress.

Apart from direct drought and heat stress effects on metabolism processes, trees including oaks may also have to cope with indirect effects of climate change, e.g. on nutrient availability. Whereas in the absence of fertilisation slow weathering processes are a major net source of nutrients such as phosphor (P) and potassium (K) in forest soils, nitrogen (N) availability depends mainly on the microbial processes of organic matter decomposition, ammonification and nitrification. Microbial activity is highly dependent on soil temperature and moisture and shows substantial short-term fluctuations with the variation of these factors during a growing season. Generally, higher air temperatures increase litter decomposition, N mineralisation and nitrification rates (Rennenberg et al. 2009; Saxe et al. 2001), whereas droughts reduce the supply of inorganic N to plant roots (Chen et al. 2011; Sardans et al. 2008b). Decreased soil N concentrations in combination with reduced soil water uptake could lead to N limiting conditions during drought periods. As nitrification depends on ammonium
as substrate, we expect stronger effects of climate change on nitrate (NO$_3^-$) than on ammonium (NH$_4^+$) availability. A shift in the relative availability of soil nitrate and ammonium may influence the growth of oak trees, as they seem to take up N preferably in form of nitrate when the inorganic N forms are supplied at equal rates (Berger & Glatzel 2001; Schulz et al. 2011).

1.2. Objectives

This dissertation was part of the multidisciplinary “Querco” project, which was conducted in the model ecosystem facility of WSL, Birmensdorf, Switzerland (other manuscripts of the “Querco” project see Appendix). The main goal of “Querco” was to determine potential climate change effects on young oaks, and their dependence on soil conditions. For this purpose, *Quercus robur*, *Q. petraea* and *Q. pubescens* saplings were grown on acidic and calcareous soil (Table 2.1) and subjected over three consecutive growing seasons to different air-warming and drought treatments. Each species was represented by four provenances, originating from different sites in Switzerland and in one case of *Q. pubescens* also from a site in Italy (Table 3.1), in order to evaluate the role of site adaptation in the seed trees on the physiological responses of their offspring to the treatments.

The PhD project focused on the soil water and temperature regime, water and nutrient uptake by the oaks, and root growth the two soils in response to the treatments. Addressing these topics, the dissertation is structured into the following four main chapters:

(i) *Water regime and growth of young oak stands subjected to air-warming and drought on two different forest soils in a model ecosystem experiment (chapter 2)*:

Soil temperatures, water availability and nutrient concentrations were measured at different depths to characterise the two soil types and the treatment effects on the
microclimate. Furthermore, it was tested whether increased temperature would enhance the severity of drought effects on oak growth and evapotranspiration. In addition, it was evaluated how fast the water regime of young oak stands can recover from such stresses.

(ii) \textit{Root growth of different oak provenances in two soils under drought stress and air-warming conditions (chapter 3):}

At the end of the experiment, total and fine root biomass as well as vertical root distribution were measured. They were related to shoot growth to study the oaks ability to shift assimilation allocation from above- to belowground growth. Furthermore, root mass of the different provenances from the experimental study was correlated with climate conditions at the seed origin sites to verify whether provenances originating from xeric sites had a higher stress tolerance than those from mesic sites. In an additional neutron radiography experiment root-shoot growth patterns were measured to study drought- or nutrient-deficiency effects on root-shoot competition for assimilates.

(iii) \textit{Root and shoot growth patterns of Quercus robur seedlings under different environmental conditions (chapter 4):}

In an additional neutron radiography experiment, root-shoot growth patterns of \textit{Quercus robur} seedlings were measured to study drought- or nutrient-deficiency effects on root-shoot competition for assimilates.

(iv) \textit{Nitrogen dynamics in oak model ecosystems subjected to air warming and drought on two different soils (chapter 5):}

Soil respiration, microbial biomass and N concentrations in soil and plants were analysed to test the influences of drought and air warming on microbial activity
and soil N availability in form of nitrate and ammonium. Furthermore, a $^{15}$N labelling experiment was conducted to test whether a drought-induced shift in nitrate and ammonium availability changed the uptake preferences of oaks.
2. Water regime and growth of young oak stands subjected to air-warming and drought on two different forest soils in a model ecosystem experiment

T. M. Kuster, M. Arend, P. Bleuler, M. S. Günthardt-Goerg & R. Schulin

Chapter 2

Summary

Global climate change is expected to increase annual temperatures and decrease summer precipitation in Central Europe. Little is known of how forests respond to the interaction of these climate factors and if their responses depend on soil conditions. In a 3-year lysimeter experiment, we investigated the growth response of young mixed oak stands, on either acidic or calcareous soil, to soil water regime, air-warming and drought treatments corresponding to an intermediate climate change scenario. The air-warming and drought treatments were applied separately as well as in combination. The air-warming treatment had no effect on soil water availability, evapotranspiration or stand biomass. Decreased evapotranspiration from the drought-exposed stands led to significantly higher air and soil temperatures, which were attributed to impaired transpirational cooling. Water limitation significantly reduced the stand foliage, shoot and root biomass as droughts were severe, as shown in low leaf water potentials. Additional air warming did not enhance the drought effects on evapotranspiration and biomass, although more negative leaf water potentials were observed. After re-watering, evapotranspiration increased within a few days to pre-drought levels. Stands not subjected to the drought treatment produced significantly less biomass on the calcareous soil than on the acidic soil, probably due to P or Mn limitation. There was no difference in biomass and water regime between the two soils under drought conditions, indicating that nutrient availability was governed by water availability under these conditions. The results demonstrate that young oak stands can cope with severe drought and therefore can be considered for future forestry.

Keywords: biomass, evapotranspiration, root:shoot ratio, soil temperature, soil-plant interactions, water use efficiency
2.1. Introduction

Based on the IPCC Scenario A2, summer temperatures in Central Europe are expected to increase by 3.2 to 4.8 °C through the 21st century, while the amount of summer precipitation in Central Europe is predicted to decrease by 21 to 28% (CH2011 2011). Moreover, contrasting periods without any precipitation, as well as extreme rainfall events, are expected to become more frequent (Schär et al. 2004; IPCC 2007). How will higher air temperatures and extended drought periods affect forests in Central Europe? Picea abies and Fagus sylvatica, the most common and economically important tree species in Central European forestry, are known to suffer during periods of high temperature and low soil water availability (Leuschner et al. 2001a; Gessler et al. 2007; Friedrichs et al. 2009). Therefore, forest management has to consider a shift towards heat- and drought-tolerant tree species. Oak trees growing in regions with relatively warm and dry climates are considered to be tolerant to heat and drought (Ellenberg et al. 2010; Landolt & Bäumler 2010); their long taproots allow them to take up water from deep soil layers, the xeromorphic structure of their leaves provides efficient reduction of water loss, and their ability to resume photosynthesis within a few days after a drought period shows their flexibility to changing water availability (Epron & Dreyer 1993; Kubiske & Abrams 1993; Canadell et al. 1996; Leuschner et al. 2001b; Galle et al. 2007).

Increased air temperatures were found to favour plant growth by extending the growing season, increasing soil nutrient turnover rates and accelerating plant metabolism (Saxe et al. 2001; Scheffer et al. 2002; Morin et al. 2010; Way & Oren 2010). A positive response of plant growth and function to increasing temperatures may be expected when temperatures are below the optimum, whereas a negative response to elevated temperatures may be expected above the optimum due to a larger increase in respiration than in assimilation (Saxe et al. 2001; Way & Oren 2010). For example, Usami et al. (2001) studied the response of a
Japanese evergreen oak, *Quercus myrsinaefolia*, to air temperature increases of 3 and 5 °C and found that biomass production increased over the entire study period, but that the net assimilation rate was reduced on hot days. Such results raise the question of how close actual summer temperatures (15 - 20 °C; Schär *et al.* 2004) are to the optimum temperatures of Central European oaks. Surprisingly, optimum growth temperatures are only vaguely known for oaks. Lüttge *et al.* (2010) gave a range of 15 - 25 °C for the optima of deciduous tree growth in general. However, it is still uncertain how an increase in air temperature affects the performance of oak.

Although oaks are considered to be more tolerant to water shortage than most other deciduous trees in Central Europe, low water availability limits their growth (Thomas 2000a; Thomas & Gausling 2000; Thomas *et al.* 2002; Bruschi 2010; Jensen & Hansen 2010). Reallocation of new assimilates from shoots to roots is a frequently observed growth response of oaks to water shortage, thus strengthening the capacity for soil water acquisition during periods of drought relative to the capacity for assimilation (Broadmeadow & Jackson 2000; Thomas 2000a; Thomas & Gausling 2000; Bruschi 2010). Previous drought experiments with oaks were performed on soil substrates with pH values above 6.5 (Thomas 2000a; Thomas & Gausling 2000; Gieger & Thomas 2005; Bruschi 2010; Jensen & Hansen 2010); however, oaks growing in temperate forests generally prefer acidic soils (Burger 1931; Gaertig *et al.* 2002; Landolt & Bäumler 2010). Experiments, on how soil properties, in particular soil reaction, influence drought responses of oaks are still outstanding. Furthermore, drought and air-warming effects have been studied separately, but under global climate change conditions they will occur simultaneously, and there is uncertainty about their combined effects on forests, including oak stands.

In this study we investigated how the growth and water regime of young oak forest stands may be affected by the interaction of air-warming and increased drought in dependence
on soil conditions. In particular, we wanted to know whether increased temperatures would enhance the severity of drought effects on oak growth and evapotranspiration, and how fast the water regime of young oak stands can recover from such stresses. Young mixed oak stands, composed of the three most common oak species of Central European forests, *Quercus robur*, *Q. petraea* and *Q. pubescens*, were grown in open-top chambers on two different soils (acidic versus calcareous) and subjected to factorial treatment combinations of air-warming and extended drought periods, corresponding to an intermediate climate change scenario. In this paper we report how evapotranspiration, biomass production and water use efficiency of the oak stands respond to drought and air-warming.

Our hypotheses were that: (i) predicted air-warming alone will only have minor effects on oak stands, as average temperatures are probably close to optimum temperatures and thus there is little sensitivity to comparatively small temperature changes; (ii) air-warming will enhance drought effects due to increased transpiration demand; (iii) oak stands will recover rapidly after drought periods; and (iv) oak stands can withstand drought effects better on acidic soils than on neutral or alkaline soils.

2.2. Material and methods

2.2.1. Study site and experimental design

The study was conducted in the model ecosystem facility of the Swiss Federal Research Institute WSL, Birmensdorf, Switzerland (47° 21’ 48’’ N, 8° 27’23’’ E, 545 m a.s.l.), which has already been successfully used in other experiments (Sonnleitner et al. 2001; Menon et al. 2005). The facility consists of 16 hexagonal open-top chambers (OTC) of 3 m height (Fig. 2.1A). Each OTC contains two 1.5 m deep concrete-walled lysimeter compartments, each with a soil surface of 3 m². Each compartment was filled with 0.5 m drainage packing.
consisting of three layers of pure quartz gravel of decreasing grain size from bottom to top. On top of this drainage layer, one compartment of each OTC was filled in spring 2005 with a single layer of calcareous sandy loam and the other with two layers of acidic loamy sand (Table 2.1), originating from mixed oak forests. One year later, in spring 2006, two mixed oak stands were established on each soil compartment, separated in the soil by a dividing wall. In each stand, 12 two-year-old saplings (*Quercus robur, Q. petraea* and *Q. pubescens*, of four different provenances each) were planted randomly (Fig. 2.1B; Arend et al. 2011). All trees were grown with sufficient water supply and at ambient air temperatures in the first year.

From spring 2007 to October 2009 we applied the following four treatments, each in four replicates arranged in a Latin square, to the OTCs: air-warming, drought, their combination and a control (Fig. 2.1A). Air-warming was achieved by closing the side walls of the OTCs more than those of the control treatment (Fig. 2.1C). Unless drought was imposed, the stands were irrigated by six sprinklers in each OTC compartment, at a height of 1 m, with 10 mm of deionised water enriched with nutrients, simulating the average composition

**Table 2.1:** Physical and chemical properties of the soil types used in the experiment at the end of the growing season in 2009. The concentrations of the extractable cations were measured in 0.1 M BaCl₂ extracts.

<table>
<thead>
<tr>
<th>Depth</th>
<th>Acidic soil (Eiken, haplic Alisol)</th>
<th>Calcareous soil (Brugg, Fluvisol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.15 m</td>
<td>0.85 m</td>
</tr>
<tr>
<td>Texture (%)</td>
<td>85, 10, 5</td>
<td>87, 8, 5</td>
</tr>
<tr>
<td>pH (0.01M CaCl₂)</td>
<td>3.93</td>
<td>4.00</td>
</tr>
<tr>
<td>Cₜot (%)</td>
<td>2.06</td>
<td>0.48</td>
</tr>
<tr>
<td>Nₜot (%)</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td>Pₜot (mg kg⁻¹)</td>
<td>524.46</td>
<td>469.43</td>
</tr>
<tr>
<td>Caₚₑch. (mg kg⁻¹)</td>
<td>364.07</td>
<td>142.21</td>
</tr>
<tr>
<td>Mgₑch. (mg kg⁻¹)</td>
<td>25.40</td>
<td>9.48</td>
</tr>
<tr>
<td>Kₑch. (mg kg⁻¹)</td>
<td>30.69</td>
<td>18.98</td>
</tr>
<tr>
<td>Mnₑch. (mg kg⁻¹)</td>
<td>45.79</td>
<td>18.63</td>
</tr>
<tr>
<td>CEC (mmolc kg⁻¹)</td>
<td>48.33</td>
<td>24.12</td>
</tr>
<tr>
<td>Base saturation (%)</td>
<td>44.65</td>
<td>36.75</td>
</tr>
</tbody>
</table>
Water regime and growth of young oaks subjected to air-warming and drought

Fig. 2.1: Overview of the experimental design used: A) Set up of the treatments and the split-split-plot arrangement in each open top chamber with two soil types, each split into two stands. B) Within chamber arrangement of the trees and measuring devices. C) Side view of the open top chambers (1.1-1.4) on the 21st August 2009 with their movable roofs and the different openings of the side-walls according to the air-warming treatment.

(30-year means) of ambient rainfall (2 mg l⁻¹ N, 0.1 mg l⁻¹ P, 0.3 mg l⁻¹ K, 0.2 mg l⁻¹ Ca, 0.03 mg l⁻¹ Mg, 0.1 mg l⁻¹ Na, 0.01 mg l⁻¹ Zn, 0.6 mg l⁻¹ Cl and 0.3 mg l⁻¹ SO₄²⁻), every 2 - 3 days to ensure sufficient water supply. Compared to the long-term mean of the site (728 mm during the growing season from April to October), the amount of irrigation in the control was 16% lower in 2007, 26% higher in 2008 and 30% higher in 2009 (Fig. 2.2A). Experimental droughts were imposed by stopping irrigation for several consecutive weeks during selected periods in the growing season (Fig. 2.2A). The experimental drought periods were stopped when the soil moisture reached a threshold of 0.05 m³ m⁻³ for several days. Two drought periods were imposed in each growing season, with an interruption to study stand regenera-
Fig. 2.2: A) Yearly accumulated precipitation ($P$, during the non-growing season from November to March) and irrigation ($I$, during the growing season from April to October) from 2007 - 2009 in either control or drought treatment (mm) and long term ambient precipitation 1963 – 2009 at the site. B) Treatment effects on the average monthly daytime (8 a.m. – 6 p.m., UTC+1) air temperature (°C) from 2007 – 2009 at a height of 120 cm. The effects are presented as differences between the respective treatment and the control treatment. Statistical analyses are presented in Table 2.2. Bold lines on the x-axis indicate periods when all chambers were irrigated.

At the end of the drought periods, more intensive irrigation was applied to the drought-treated stands than to the other stands for a few days, simulating heavy rainfall, according to the increasing probability of extreme precipitation due to global warming. The total input of irrigation water to the drought-treated stands was 60% lower than the long-term mean precipitation during the growing season in 2007 and 43% lower in 2008 and 2009. Natural precipitation was excluded from the plots by automatically closing roofs during rainfall (Fig. 2.1C). During the seasons without growth activity, the roofs of all OTCs were
kept open to allow watering from natural precipitation. Rainfall at the experimental site was measured daily with a pluviometer.

The experiment represents a compromise between natural conditions and experimental control of climate and soil factors. For example, the air temperature in the control OTC was no different from that of ambient stands (data not shown) and a potential reduction in wind speed due to the OTC walls from 1.9 to 1.0 m s\(^{-1}\) was equivalent to a decrease in potential evapotranspiration of no more than 5\%, according to estimates using the Penman–Monteith equation (data not shown). While our model ecosystems were not identical to young oak stands growing under natural conditions, this does not invalidate the main conclusions of this study, as the chamber effect was the same in all treatments.

2.2.2. Measurements of soil water, leaf water potential and temperature regime

Soil water regime was monitored by manually recording weekly measurements of soil water concentration using time-domain reflectometry (TDR 100; Campbell Scientific Inc., Logan, Utah, USA) at depths of 0-25 cm, 25-50 cm, 50-75 cm and 75-100 cm, and weekly measurements of soil water potential using tensiometers (self-made, measuring device: DPM-802; Keller, CH) at depths of 6-18 cm, 32-44 cm, 56-68 cm and 82-94 cm, with two replicates per depth and lysimeter compartment (Fig. 2.1B). Also, the volume of discharge water, which was collected in containers at the bottom of the lysimeters, was recorded weekly. Evapotranspiration (ET) was calculated from the water balance as \( ET = I + P - DR \pm DW \), where \( I \) is irrigation, \( P \) precipitation, \( DR \) drainage and \( DW \) the change in soil water storage. Evapotranspiration was not measured in 2007 and spring 2008. Predawn leaf water potentials were measured in selected trees on each soil compartment at the end of both drought periods in June and August 2009 using a Scholander pressure chamber (M 600; Mosler Tech Support, Berlin, Germany) according to Scholander et al. (1965).
Air temperature was automatically measured every hour at a height of 120 cm using shaded EL-USB-2 data loggers (Lascar Electronics Ltd, Whiteparish, UK; Fig. 2.1B) in each OTC. Soil temperatures were measured at hourly intervals in each compartment at depths of 1 cm, 5 cm, 12 cm and 38 cm using iButtons (Maxim Integrated Products Inc., Sunnyvale, California, USA; Fig. 2.1B).

2.2.3. Biomass measurements

At the end of each growing season from 2006 to 2009, all foliage (including leaf litter on the ground) was collected separately for each lysimeter compartment. In September 2009, all aboveground wood biomass was also harvested, and in spring 2010 soils were carefully excavated and roots separated. Dry weights of foliage, aboveground wood (stem and twigs) and roots were determined after drying at 65 °C for several days. The root:shoot biomass ratio was calculated as the ratio between the dry weight of roots of a stand and the respective dry weights of the combined aboveground wood and foliage collected in 2009 at the end of the experiment.

2.2.4. Statistical analysis

All statistical analyses were carried out using R 2.11.1 (R: A language and environment for statistical computing, R Development Core Team, Vienna, AT). Treatment and interaction effects were analysed using a three-way fully factorial (irrigation, air-warming and soil) ANOVA model and tested at a significance level of P < 0.05 (linear mixed-effect model). The split-plot design with two subplots (soil compartment) in each plot (OTC) was considered in the statistical analyses of soil temperature, evapotranspiration, soil water regime, leaf water potential and stand biomass parameters (Fig. 2.1). In the latter, the split-split-plot design with two replicates of the stands on each soil compartment was taken into account. The split-plot design was not considered in analysis of air temperature measurements, as air temperatures
were the same in the two subplots of each OTC. Measurements were transformed by means of Tukey’s first aid transformation before analysis to fulfil the assumptions of normal distribution. Significant differences between treatments and soil types were tested with Tukey HSD.

2.3. Results

2.3.1. Air and soil temperature

The air-warming treatment increased the mean monthly daytime (08:00 - 18:00 h, UTC+1) air temperature in the OTCs during the growing seasons by about 1 - 2 °C compared to the control (Table 2.2, Fig. 2.2B). Comparing whole-day average temperatures (00:00 - 23:00 h, UTC+1) the mean air temperature difference was smaller as there was no air-

<table>
<thead>
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<th></th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>AW</td>
<td>D</td>
<td>Soil</td>
</tr>
<tr>
<td>evapotranspiration</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>air temperature</td>
<td>***</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>+ 120 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soil temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1 cm</td>
<td>**</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>-5 cm</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>-12 cm</td>
<td>(*)</td>
<td>(*)</td>
<td>ns</td>
</tr>
<tr>
<td>-38 cm</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
Fig. 2.3: Monthly average soil temperature (°C, 0 a.m. – 23 p.m., UTC+1) in relation to the control treatment at four different depths (-1 cm, -5 cm, -12 cm, -38 cm) in both acidic (left) and calcareous soils (right). Bold lines on the x-axis indicate periods when all chambers were irrigated. Statistical analyses are presented in Table 2.2. Please note the different scales at each depth.

warming during the night (data not shown), but still up to 1 °C higher than without air-warming during the growing season. Also, the drought treatment resulted in higher air temperatures, which can be attributed to lower evaporative cooling. The development of the canopies from year-to-year (Table 2.4) increased the water consumption of the stands, which led to more intensive drought stress from 2007 to 2009 and to less evapotranspiration during
drought peaks. In 2007, the drought stress was weak and the reduction in evapotranspiration had no significant effect on air temperature. In 2008, the drought effect on air temperature almost reached significance ($P = 0.087$), and in 2009 it was highly significant and of the same magnitude as the air-warming treatment alone. Transient re-watering tended to reduce the monthly temperature difference only slightly between control and drought-treated stands in July 2009, but was not statistically significant. In combination, air-warming and drought treatment led to an increase in temperature of $+3 \, ^\circ C$ compared to the control during the growing season in 2009. Thus, the two treatments had, approximately, an additive effect.

The treatment effects on air temperature were transmitted to the soil, with decreasing amplitudes towards the lower depths (Fig. 2.3, Table 2.2). Air-warming treatment increased the mean soil temperature during the growing season at all depths, except at 12 cm in 2007 and 5 cm in 2008 and 2009. Similar to air temperature, the drought treatment had an increasing effect on soil temperature from 2007 to 2009. In 2007 the drought effect was only significant in the top soil layer, while in 2008 and 2009 drought significantly increased the soil temperature at all depths. The soil type influenced the soil temperature only at a depth of 5 cm in 2009 and 38 cm in 2008 and 2009. In all cases, the soil temperature was higher in the calcareous than in the acidic soil.

2.3.2. Evapotranspiration

The drought treatment reduced evapotranspiration (soil evaporation + tree transpiration) on both soils in 2008 and 2009 to almost zero, whereas air-warming had no effect on evapotranspiration (Table 2.2, Fig. 2.4). After re-watering, evapotranspiration from the drought-treated ecosystems increased within 11 days in August 2008 and within 8 days in July 2009 to the same levels as in regularly irrigated OTCs. In September 2009, the evapotranspiration in the drought treatment did not recover during 1 month to the level of the
control. This could be explained by premature leaf senescence and subsequent leaf shedding in some of the drought-treated oaks (data not shown). Increased dysfunction of the water-conducting xylem due to drought-induced cavitation of vessel cells might additionally have contributed to the incomplete recovery of evapotranspiration (Tyree et al. 1994).

In the absence of drought, evapotranspiration was significantly higher from the acidic than from the calcareous soil in 2009 (control +34%, Table 2.2). In 2008 there was no difference in evapotranspiration from the two soils. Under drought, evapotranspiration decreased to similar rates on both soils.

2.3.3. Soil water regime and leaf water potential

Under the imposed droughts, soil water potentials dropped below the measuring range of the tensiometers (approximately -0.09 MPa) over the entire depth of the rooting zone in both soils (data not shown). In summer 2009, mean predawn leaf water potentials in drought-treated oaks were between -1.5 and -2.9 MPa at the end of the first drought period in June and
Fig. 2.5: Mean weekly soil water concentration in acidic (left) and calcareous (right) soil from 2007 to 2009. Bold lines on the x-axis indicate periods when all chambers were irrigated.
between -2.8 and -3.9 MPa at the end of the second drought period in August, indicating that the applied drought treatment was quite severe (Table 2.3). At the end of the first drought period in June 2009, a significant air-warming effect (P = 0.019) and a significant drought x air-warming interaction (P = 0.007) were observed, indicating that air-warming reinforced the drought effect on leaf water potential. No air-warming effect on leaf water potential was measured at the end of the second drought period in August 2009. At the same time, the soil water concentrations decreased to values between 0.03 and 0.1 m³ m⁻³ (Fig. 2.5). The lowest water concentrations were recorded at the end of the 2008 and 2009 drought periods in the calcareous soil, with < 0.05 m³ m⁻³ throughout the entire soil profile. After re-watering, soil moisture gradually increased again to reach the moisture levels of the control treatments and 2009 than in 2007, showing the higher water demand of the developing vegetation (Table 2.4, Fig. 2.5). During periods of high evapotranspiration in summer 2008 and 2009, soil moisture also decreased in the control and air-warming treatments. This effect was more pronounced in the acidic than in the calcareous soil, which is in line with the higher water consumption of the trees on acidic soil. The only soil moisture effect related to the air-warming treatment was found in the acidic soil at a depth of 50 - 75 cm, where the soil water content was higher in the air-warming treatment than in the control.

Table 2.3: Air-warming (AW) and drought (D) treatment effects on predawn leaf water potential (MPa) measured at the end of the both drought periods in June and August 2009 on two soils (means ± SE). Different letters are meant to indicate significant differences between respective treatment means on the same soil. An asterisk (*) indicates a significant difference between acidic and calcareous soil for the same treatment.
2.3.4. Growth parameters

In 2006, *i.e.* before the experimental treatments started, no significant differences in foliage biomass were recorded between the OTCs, indicating that all stands had the same initial conditions (Table 2.4). From 2006 to 2009, the production of foliage biomass increased by about 6.5 times in the control treatment. The treatment effect on tree growth paralleled that for soil moisture and evapotranspiration (Table 2.4): there was no significant response of foliage and aboveground wood biomass to air-warming under both drought and humid soil conditions, neither on acidic or on calcareous soils. However, root biomass tended to be lower under air-warming conditions (*P* = 0.054).

In contrast, the drought treatment generally reduced biomass production (control and air-warming versus drought and air-warming and drought) in the growing seasons 2008 and 2009 on both soil types (wood biomass 2009: -59% (acidic)/ -41% (calcareous), root biomass 2009: -33% / -25%, foliage 2008: -35% / -31%, 2009: -34% / -34%; all *P* < 0.001; Table 2.4), but none of the trees died. The relative drought-induced biomass reduction was stronger on the acidic than on the calcareous soil. While much more biomass was produced on the acidic than on the calcareous soil in the well-watered treatments (wood biomass 2009: +41%, *P* < 0.001, root biomass 2009: +13%, *P* = 0.043, foliage 2008: +17%, *P* = 0.012, 2009: +25%, *P* = 0.018; Table 2.4), the absolute biomass in the drought treatments was reduced to similar values on the two soils (drought x soil interaction wood biomass 2009: *P* < 0.001, root biomass 2009: *P* = 0.075, foliage 2008: *P* = 0.001, 2009: *ns*). In 2007, the effects of the soil and of the drought treatment on foliage production were smaller as the vegetation was still developing (foliage 2007: -17%, *P* = 0.097 / -13%, *P* = 0.044, soil effect: *ns*; Table 2.4).

The root:shoot biomass ratio was higher on drought-treated than on well-watered soils, indicating a reallocation of resources under water limitation towards root biomass. The plants
Table 2.4: Air-warming (AW) and drought (D) treatment effects on foliage (2006 – 2009), wood (2009) and root (2009) biomass (kg m\(^{-2}\)) and on root:shoot biomass ratio of the mixed oak stands on the two soils (means ± SE, n=8). Different letters are meant to indicate significant differences between respective treatment means on the same soil. An asterisk (*) indicates a significant difference between acidic and calcareous soil for the same treatment.

<table>
<thead>
<tr>
<th></th>
<th>DW foliage 06</th>
<th>DW foliage 07</th>
<th>DW foliage 08</th>
<th>DW foliage 09</th>
<th>DW wood 09</th>
<th>DW root 09</th>
<th>root:shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>acidic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.063(^a) ± 0.005</td>
<td>0.156(^d) ± 0.013</td>
<td>0.394(^a) ± 0.014</td>
<td>0.411(^a) ± 0.035</td>
<td>*2.17(^a) ± 0.10</td>
<td>2.02(^a) ± 0.11</td>
<td>0.79(^a) ± 0.05</td>
</tr>
<tr>
<td>air-warming (AW)</td>
<td>0.067(^a) ± 0.007</td>
<td>0.121(^a) ± 0.009</td>
<td>*0.422(^a) ± 0.023</td>
<td>*0.455(^a) ± 0.025</td>
<td>*2.30(^a) ± 0.17</td>
<td>1.87(^a) ± 0.11</td>
<td>*0.69(^a) ± 0.02</td>
</tr>
<tr>
<td>drought (D)</td>
<td>0.068(^a) ± 0.004</td>
<td>0.110(^b) ± 0.005</td>
<td>0.262(^b) ± 0.010</td>
<td>0.280(^b) ± 0.013</td>
<td>0.93(^b) ± 0.02</td>
<td>1.32(^b) ± 0.03</td>
<td>1.10(^b) ± 0.04</td>
</tr>
<tr>
<td>AW &amp; D</td>
<td>*0.071(^b) ± 0.005</td>
<td>0.119(^b) ± 0.006</td>
<td>0.268(^b) ± 0.012</td>
<td>0.282(^b) ± 0.015</td>
<td>0.88(^b) ± 0.02</td>
<td>1.30(^b) ± 0.03</td>
<td>1.13(^b) ± 0.05</td>
</tr>
<tr>
<td><strong>calcareous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.053(^a) ± 0.002</td>
<td>0.162(^d) ± 0.011</td>
<td>0.351(^a) ± 0.019</td>
<td>0.347(^a) ± 0.014</td>
<td>*1.61(^a) ± 0.07</td>
<td>1.78(^a) ± 0.09</td>
<td>0.91(^a) ± 0.03</td>
</tr>
<tr>
<td>air-warming (AW)</td>
<td>0.054(^a) ± 0.003</td>
<td>0.140(^d) ± 0.004</td>
<td>*0.349(^a) ± 0.008</td>
<td>*0.330(^b) ± 0.008</td>
<td>*1.57(^a) ± 0.04</td>
<td>1.65(^a) ± 0.05</td>
<td>*0.87(^a) ± 0.03</td>
</tr>
<tr>
<td>drought (D)</td>
<td>0.055(^a) ± 0.003</td>
<td>0.141(^d) ± 0.010</td>
<td>0.242(^b) ± 0.005</td>
<td>0.230(^b) ± 0.013</td>
<td>0.95(^b) ± 0.02</td>
<td>1.34(^b) ± 0.03</td>
<td>1.14(^b) ± 0.03</td>
</tr>
<tr>
<td>AW &amp; D</td>
<td>*0.048(^a) ± 0.004</td>
<td>0.126(^b) ± 0.005</td>
<td>0.272(^b) ± 0.007</td>
<td>0.266(^b) ± 0.012</td>
<td>0.93(^b) ± 0.04</td>
<td>1.25(^b) ± 0.02</td>
<td>1.06(^b) ± 0.04</td>
</tr>
</tbody>
</table>
invested more assimilates into water uptake capacity than into photosynthetic capacity. The relative shift was particularly strong on the acidic soil (+50%, \( P < 0.001 \)), but still significant on the calcareous soil (+25%, \( P < 0.001 \)). Well-watered stands of the control growing on the calcareous soil had a higher root:shoot biomass ratio than on the acidic soil (+20%, \( P = 0.002 \)), whereas no differences in the root:shoot ratios biomass were found between the soils in the drought treatments.

2.3.5. Water use efficiency

Figure 2.6 shows a close linear correlation between evapotranspiration and biomass production (\( R^2 = 0.971 \)), independent of soil type. The fact that the regression line describing this relationship had a positive intercept on the biomass axis means that the overall water use efficiency of the investigated oak stands increased with decreasing growth and water consumption rates.

![Graph showing relationship between evapotranspiration and biomass](image)

**Fig. 2.6:** Relationship between evapotranspiration (2008 + 2009) and the sum of above and below ground biomass (\( DW \) foliage 2008 + \( DW \) foliage 2009 + \( DW \) root 2009 + \( DW \) wood 2009) produced on acidic and calcareous soils and mean water use efficiency (± SE).
2.4. Discussion and conclusions

The air temperature treatment did not influence evapotranspiration and biomass production, in agreement with our expectations (hypothesis i). Arend et al. (2011), who studied other growth parameters within the same experiment as described here, found that the growth height of the mixed oak stands was increased by the air-warming treatment but, with respect to stem volume, this effect was counterbalanced by reduced diameter growth. We conclude that the average daytime air temperature, which was 19.7 °C in the control treatment of our study, was apparently already close to the optimum and thus, an increase of 2 °C during daytime had no measurable effect on biomass production. Considering that summer air temperature is expected to increase until 2085 between 3.2 and 4.8 °C in the IPCC Scenario A2 (Schär et al. 2004; IPCC 2007; CH2011 2011), our near-natural treatment was, in comparison, moderate.

The applied drought treatment was on the extreme side of predicted reductions in precipitation (21 - 28%) until 2085 according to the IPCC Scenario A2 (CH2011 2011). Thus, it is hardly surprising that the drought treatment significantly reduced stand biomass of shoots, foliage and roots, in contrast to the comparatively mild air-warming treatment. Similar drought effects have been reported for Quercus robur and Q. petraea by Broadmeadow & Jackson (2000), Thomas (2000a) and Jensen & Hansen (2010). Broadmeadow & Jackson (2000) exposed 2-year-old seedlings of Q. petraea, grown in OTCs, to reduced soil water potentials between -0.02 and -0.2 MPa over two consecutive years and found a reduction in shoot height (-40%), and shoot biomass (-71%) compared to a control treatment with soil water potentials between -0.008 and -0.011 MPa, but no significant effect on shoot diameter or root biomass. Similarly, Jensen & Hansen (2010) exposed seedlings of Q. robur, in their first growing season, to 5 months of constant soil water content equivalent to 38% of field water capacity, compared to 80% in the control. These seedlings were grown in wood
containers filled with calcareous sandy soil in a greenhouse; shoot height (-66%), shoot diameter (-75%), shoot biomass (-42%) and root biomass (-19%) were all significantly lower in the drought treatment. In another experiment, Thomas (2000a) grew 2-year-old seedlings of *Q. petraea* and *Q. pubescens* in big lysimeters filled with calcareous sand. In one treatment, the soil was completely drained over two periods of 10 and 11 weeks to a soil water content below 5%, whereas in the control treatment the soil water content was kept between 20 and 30% above a soil water table at 40-cm depth. Leaf, shoot and fine root biomass was reduced in the drought-treated *Q. petraea* seedlings, whereas none of these parameters responded to the drought treatment in *Q. pubescens*. Similarly, Chiatante *et al.* (2006) found no effects of a 100-day period with no irrigation on leaf and shoot biomass in 1-year-old *Q. pubescens* seedlings grown under a plastic cover in pots filled with a mixture of clayey loam soil and peat, as compared to control trees that were watered every 3 days. However, they found a reduction in the biomass of lateral roots in the drought-stressed seedlings, while the taproot biomass remained unaffected. In another study, Gieger & Thomas (2005) compared the growth responses of 4-year-old *Q. robur* and *Q. petraea* seedlings to drought stress. The trees were grown in big pots filled with calcareous sand. In the drought treatment they were subjected to an 8-week period without irrigation, during which the soil water content decreased to 0.5 - 2.5% for 55 - 60 days. Compared to the adequately watered control treatment where soil water contents were always above 8%, the drought treatment reduced the leaf biomass in *Q. petraea* but not in *Q. robur*, but had no effect on fine root biomass and stem diameter increment. This high diversity in the reactions of growth parameters to drought shows that experimental conditions have to be considered in the evaluation of drought effects on oaks. In our study, with growth conditions somewhere between pot experiments and real forests, the drought effects on growth increased from season to season with stand development – the results would have been different using only one growing season.
According to hypothesis (iii), our experiment shows that oaks recovered after drought relatively quickly. For example, after evapotranspiration dropped to virtually zero within 2 weeks when irrigation was stopped in July 2008 and remained at this low level for another 2 weeks, evapotranspiration rates recovered within only 12 days after re-watering in late August 2008. Also Damesin & Rambal (1995), Thomas & Gausling (2000), Gieger & Thomas (2002) and Galle et al. (2007) observed a rapid recovery of oaks after water availability increased. The fact that none of the trees died during our 3-year experiment is a further demonstration of the high drought tolerance of oak stands.

In contrast to our expectations (hypothesis ii), neither evapotranspiration, soil water content, nor stand biomass differed among drought treatments with or without air-warming, even though the drought effects on leaf water potential were enhanced by additional air-warming at the end of the first drought period in 2009. The data suggest that both drought treatments lowered the availability of soil water to the same critical level for evapotranspiration and growth, regardless of differences in leaf water potential. In an intermediate drought scenario, additional air-warming would probably enhance the drought effects on evapotranspiration, soil water content and stand biomass to a higher extent than in our study.

In the drought treatments, the ratio of growth allocation shifted from above- towards belowground growth, suggesting that drought-stressed trees invested a higher proportion of their assimilates into water and nutrient uptake and less into CO₂ absorption and capturing light. This strategy of optimising the use of assimilates by their investment into the acquisition of resources according to the extent by which they limit growth is in line with the results of other studies on assimilate allocation in plants, including Q. robur and Q. petraea (Guehl et al. 1994; Picon et al. 1996; Broadmeadow & Jackson 2000; Thomas 2000a; Thomas & Gausling 2000; Bruschi 2010). Developing deep root systems is a general strategy
of oaks to access deep layers of soil water, allowing them to sustain a water supply during long periods of drought (Thomas & Gausling 2000; Hanson et al. 2001; Leuzinger et al. 2005). This ability to tap into water resources in deeper soil layers will become more important in the future, as it is expected that precipitation in winter will increase (CH2011 2011), replenishing water reservoirs between the growing seasons that are not accessible to shallow-rooting plants.

The finding that the oak stands of our study consumed more water and produced more biomass on the acidic rather than on the calcareous soil in the absence of drought is in line with reports of other authors that oaks generally grow better on acidic than on calcareous soils, both under natural and experimental conditions (Burger 1931; Gaertig et al. 2002; Landolt & Bäumler 2010). As the water supply was the same and the availability of soil water was continuously high during the growing seasons in both soils, another limiting factor must have been responsible for the lower growth on the calcareous soil in the control treatment. This factor may have been the low availability of a nutrient such as P or Mn in the calcareous soil (Table 2.1). Deficiency in Mn due to high pH has been reported as a growth-limiting factor in oaks in other studies (Messenger 1986; Thomas et al. 1998). The higher average root:shoot mass ratio of the trees growing on the calcareous soil is another hint that a limiting nutrient resource in soil was the responsible factor for lower growth. The fact that the differences in evapotranspiration and growth rates between the two soil types disappeared when drought was imposed indicates that water became the only limiting growth factor. Thus, our results did not support the expectation (hypothesis iv) that oaks cope better with drought on acidic than on calcareous soil. There was no additive effect of drought and the factor responsible for the growth difference between the two soils in the absence of drought. However, the relative growth reduction of the stand on the acidic soil was higher than on the calcareous soil. This finding shows that soil properties, especially pH, must be considered in
climate change scenarios for forests containing oaks, as stronger drought effects can be expected on acidic than alkaline soil.

The results of the present study show that young oak stands can cope with drought periods, even under more severe conditions than predicted. In our study, the effect of drought overrode that of temperature and nutrients, a pattern that was also found in another drought study on pathogen effects on oaks (Desprez-Loustau et al. 2006). However, more attention should be given to the interaction effects of drought with air-warming and soil properties, as these parameters can strongly modify the results.

Our study does not allow us to draw conclusions about growth responses of adult oaks to drought in natural forests, but we can speculate that mature oaks are less vulnerable to drought as they can tap into deeper water reservoirs with their long taproots (Hanson et al. 2001; Leuzinger et al. 2005). Further, at elevated CO$_2$ concentrations, transpiration can be reduced and therefore soil moisture can sustain tree growth for a longer period of time (Bucher-Wallin et al. 2000; Leuzinger & Körner 2007). The findings of this experiment support the view that oaks are a suitable tree species for future forestry in Central Europe, in particular on sites that become too dry for beech (vanHees 1997; Thomas 2000a; Leuschner et al. 2001a).

**Acknowledgements**

We thank Martin Keller, Matthias Dobbertin, Patrick Schleppi, Terry Menard, the internship students, the research unit Forest Soils and Biogeochemistry, the central laboratory and the garden team at WSL for their support. We express gratitude to the Velux Foundation for funding (project number 489).
3. Root growth of different oak provenances in two soils under drought stress and air-warming conditions

T. M. Kuster, M. Arend, M. S. Günthardt-Goerg & R. Schulin

accepted, to be published in Plant and Soil
Summary

Oaks are considered to be drought- and thermo-tolerant tree species. Nevertheless, species and provenances may differ in their ecological requirements. We hypothesised that (i) provenances from xeric sites are better adapted to drought than those from more humid sites, (ii) oaks direct root growth towards resource-rich layers, and (iii) air-warming promotes root growth. To test different provenances of *Quercus robur*, *Q. petraea* and *Q. pubescens*, we conducted a model ecosystem experiment with young trees, grown on acidic and calcareous soil, subjected to drought, air warming, the combination of warming and drought, and a control. The results were only in partial agreement with the first hypothesis. As expected the provenances originating from drier sites produced more biomass than those from more humid sites under drought conditions. Surprisingly, however, they reacted more sensitive to water limiting conditions, as they produce also substantially more biomass under well-watered conditions. The drought treatment reduced root mass substantially in the upper soil and in agreement with the second hypothesis this led to a shift in the centre of root mass to lower depth, where water was still more available than closer to the soil surface. In contrast to the third hypothesis, the air-warming treatment, which was very mild however compared to climate change scenarios, had no significant effects on root growth. Given that the provenances from drier sites, with overall better growth, showed more biomass loss at water limiting conditions than provenances from more humid sites, it remains questionable whether provenances from drier sites are better suited for a future climate.

*Keywords*: climate change, fine root, root mass distribution, root:shoot ratio, *Quercus robur*, *Quercus petraea* & *Quercus pubescens*
3.1. Introduction

Climate change scenarios of the IPCC predict generally increasing temperatures and changing precipitation patterns all over the world (IPCC 2007). According to IPCC Scenario A2, for example, mean summer temperatures will increase by 3.2 to 4.8 °C until the end of this century in central Europe, while summer precipitation will decrease by 21 to 28% (CH2011 2011). Moreover, periods with extreme weather conditions are expected to become more frequent than today (IPCC 2007; Schär et al. 2004). This implies that drought and heat resistance will become important for tree survival, in particular together with additional stress by competition with other trees for light, water and nutrients.

Oaks are known to be drought-tolerant among European forest trees (Ellenberg et al. 2010; Landolt & Bäumler 2010). They are therefore considered promising candidates to replace beech (Fagus sylvatica), spruce (Picea abies) or even the also drought-tolerant Scots Pine (Pinus sylvestris) on warm and dry sites in future central European forestry (Friedrichs et al. 2009; Leuschner et al. 2001a; Scharnweber et al. 2011; Wohlgemuth 2006). Besides a xeromorphic leaf structure and the ability to rapidly resume assimilation after drought periods (Arend et al. 2012; Galle et al. 2007; Kubiske & Abrams 1993), oaks show root growth patterns that make them particularly well adapted to temporal and spatial variability in soil water and nutrient availability. A common response of oaks to reduced soil water availability is the shift of assimilate allocation from above- to belowground growth, thereby increasing the ability of the root system to exploit water and nutrient resources in the soil and reducing transpirational water losses (Broadmeadow & Jackson 2000; Fotelli et al. 2000; Gieger & Thomas 2002; Joslin et al. 2000; Thomas 2000a; Thomas & Gausling 2000). In many other tree species, including whitebeam (Sorbus aria), ash (Fraxinus excelsior) and beech, the root:shoot or root:leaf ratio was found to respond less to drought conditions compared to oaks (Broadmeadow & Jackson 2000; Thomas 2000a). Furthermore, plant species are able to
respond to uneven vertical distribution of water resources and nutrients by enhancing root proliferation in zones where the limiting resources are most available, as for example shown for oak root growth by Callaway (1990). Again this capacity seems to be particularly pronounced in oaks, in contrast for example to spruce and beech, as shown for nutrient availability by Göransson et al. (2006). Comparing the influence of water and nutrient availability on vertical root growth allocation in Quercus petraea, Thomas (2000b) reported that nutrients, especially N, were more important than water availability.

In comparison to water and nutrient availability, rapid variations in soil temperature are of importance only very near to the soil surface, while in deeper soil layers only seasonal fluctuations are still felt. Generally, root growth requires a minimum soil temperature between 0 and 5 °C (Kozlowski et al. 1991). Above this threshold it increases with soil temperature, until a maximum growth rate is reached at 20 to 25 °C, as for example reported for Q. robur and Q. petraea (Lyr 1996; Lyr & Garbe 1995).

Different oak species and provenances may vary considerably in temperature sensitivity, drought stress tolerance and adaptability of root growth to changing conditions. For example, Q. robur was generally found less drought tolerant than Q. petraea and Q. pubescens (Arend et al. 2012; Günthardt-Goerg et al. 2012), but to be more flexible in adapting to changing soil water conditions (Landolt & Bäumler 2010). Provenances from xeric sites generally appeared to be better adapted to enhanced temperatures and low water availability than provenances from more humid sites (Bruschi 2010; Jensen & Hansen 2010).

Effects of warming, drought and nutrient availability on root growth in oaks have mostly been studied independently of each other, whereas interactions between these factors are less well known. Based on the literature presented above, we hypothesised that (i) oak provenances from xeric sites produce more root mass under drought conditions than those
from more humid sites, (ii) oaks grow roots at higher density in water- and nutrient-rich than in water- and nutrient-poor layers, and (iii) air-warming promotes root growth. To test these hypotheses we conducted a full-factorial model ecosystem experiment in which various provenances of young *Quercus robur*, *Q. petraea* and *Q. pubescens* trees were grown on two different soils and subjected to drought and air-warming treatments.

### 3.2. Material and methods

#### 3.2.1. Study site & experimental design

The experiment was performed in the model ecosystem facility of the Swiss Federal Research Institute WSL, Birmensdorf, Switzerland (47°21’48’’ N, 8°27’23’’ E, 545 m a.s.l.). A detailed description of the experimental design is given in Kuster *et al.* (2012a). Briefly, the facility consists of 16 hexagonal chambers of 3 m height and a surface area of 6 m² each. Using a Latin square design, one of the following four treatments was applied in four replicates from spring 2007 to October 2009: air warming (AW), drought (D), the combination of air warming and drought (AWD), and control (CO). Each chamber is split below ground into two 1.5 m deep concrete-walled lysimeters, filled in spring 2005 with a 0.5 m drainage packing, composed of 3 layers of pure quartz gravel of decreasing grain size (from bottom to top), and a 1 m soil layer on top of this drainage layer. In one lysimeter of each chamber the soil consisted of two layers of acidic loamy sand taken from a Haplic Alisol (pH 4.0, subsoil 0.15 - 1.00 m, topsoil 0 - 0.15 m), while in the other lysimeter it consisted of a single layer of calcareous sandy loam taken from a Calcaric Fluvisol (pH 6.9). Further properties and nutrient concentrations of the two soils, both of which originated from mixed oak forests, are presented in Kuster *et al.* (2012a) and in Kuster *et al.* (2012b). In spring 2006, the same set of two-year-old saplings of *Quercus robur*, *Q. petraea* and *Q. pubescens* was randomly planted on all lysimeters and grown with sufficient water supply at ambient air
temperature during the growing season of 2006 (Arend et al. 2011). For each species, provenances from 4 different oak stands in Switzerland and Italy were selected. Each provenance was represented by two saplings on each lysimeter (statistical unit = oak provenance, n = 8), leading to a total number of 768 trees (4 climatic treatments, 2 soils, 12 provenances nested in 3 species, 8 replications). The sites were different for all 12 provenances. For each species, one site of origin was located in the Swiss Jura, a second in southern Switzerland and a third in a region where the respective species is most abundant in Switzerland. The fourth site was chosen for Q. robur and Q. petraea in the Swiss Plateau and for Q. pubescens in Northern Italy. To indicate soil humidity conditions at these sites during the growing season (May to September), water balances are given in Table 3.1, representing the differences between precipitation and potential evapotranspiration after Primault (1981). The smaller this difference is at a given site, the more limiting is water for growth; a negative value indicates conditions where the water losses are higher than water input through precipitation. For the Italian provenance Arezzo no data was available to calculate a water balance.

The air warming treatment consisted in keeping the side-walls less opened than in the control treatment. As a result, day-time air temperatures were 1 - 2 °C higher than in the control chambers during summer, and topsoil temperatures (at 5 cm depth) increased by 0.5 - 1 °C. In the drought treatment, irrigation was suspended for several consecutive weeks during the growing seasons, while it was continued in the control treatment. Rainfall was excluded from the chambers during the growing season (April – October) in all treatments by automatically closing roofs, while roofs were left open during the dormant seasons to allow for irrigation by natural precipitation. Compared to the long-term (1961 – 2009) mean precipitation from April to October at the experimental site, irrigation was reduced by 60% over the entire growing season of 2007 and by 43% in 2008 and 2009 in the drought treat-
Table 3.1: Location of the origins of the oak provenances, each with the average climate conditions (1981 - 2010) at nearby SwissMeteo WMO weather stations during the growing season (May to September). Water balance data = precipitation - potential evapotranspiration after Primault (1981).

<table>
<thead>
<tr>
<th>Species</th>
<th>Provenance</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude</th>
<th>Weather station</th>
<th>Precipitation</th>
<th>Temp. 3)</th>
<th>WB</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Q. robur</em></td>
<td>Tägerwilen (T)</td>
<td>47° 38’ N</td>
<td>9° 08’ E</td>
<td>510 m</td>
<td>Illhart/ Güttingen</td>
<td>508 mm</td>
<td>15.7 °C</td>
<td>+ 205 mm</td>
</tr>
<tr>
<td></td>
<td>Bonfol (B)</td>
<td>47° 28’ N</td>
<td>7° 09’ E</td>
<td>450 m</td>
<td>Fahy</td>
<td>515 mm</td>
<td>16.2 °C</td>
<td>+ 232 mm</td>
</tr>
<tr>
<td></td>
<td>Hühnenberg (H)</td>
<td>47° 11’ N</td>
<td>8° 25’ E</td>
<td>398 m</td>
<td>Cham/ Luzern</td>
<td>631 mm</td>
<td>17.0 °C</td>
<td>+ 385 mm</td>
</tr>
<tr>
<td></td>
<td>Magadino (Mi)</td>
<td>46° 09’ N</td>
<td>8° 53’ E</td>
<td>199 m</td>
<td>Magadino</td>
<td>975 mm</td>
<td>19.1 °C</td>
<td>+ 686 mm</td>
</tr>
<tr>
<td><em>Q. petraea</em></td>
<td>Corcelles (C)</td>
<td>46° 51’ N</td>
<td>6° 41’ E</td>
<td>550 m</td>
<td>Yverdon/ 2)</td>
<td>436 mm</td>
<td>16.3 °C</td>
<td>+ 94 mm</td>
</tr>
<tr>
<td></td>
<td>Magden (M)</td>
<td>47° 32’ N</td>
<td>7° 48’ E</td>
<td>308 m</td>
<td>Arisdorf/ Basel</td>
<td>514 mm</td>
<td>17.1 °C</td>
<td>+ 251 mm</td>
</tr>
<tr>
<td></td>
<td>Wädenswil (W)</td>
<td>47° 14’ N</td>
<td>8° 38’ E</td>
<td>430 m</td>
<td>Wädenswil</td>
<td>734 mm</td>
<td>16.8 °C</td>
<td>+ 435 mm</td>
</tr>
<tr>
<td></td>
<td>Gordevio (G)</td>
<td>46° 12’ N</td>
<td>8° 44’ E</td>
<td>450 m</td>
<td>Locarno</td>
<td>1023 mm</td>
<td>18.6 °C</td>
<td>+ 615 mm</td>
</tr>
<tr>
<td><em>Q. pubescens</em></td>
<td>Leuk (Lk)</td>
<td>46° 18’ N</td>
<td>7° 38’ E</td>
<td>720 m</td>
<td>Sierre/ Visp</td>
<td>268 mm</td>
<td>16.3 °C</td>
<td>- 282 mm</td>
</tr>
<tr>
<td></td>
<td>Le Landeron (LL)</td>
<td>47° 04’ N</td>
<td>7° 03’ E</td>
<td>700 m</td>
<td>Neuchâtel</td>
<td>456 mm</td>
<td>15.7 °C</td>
<td>+ 7 mm</td>
</tr>
<tr>
<td></td>
<td>Promontogno (P)</td>
<td>46° 20’ N</td>
<td>9° 33’ E</td>
<td>900 m</td>
<td>Soglio/ Poschiavo</td>
<td>794 mm</td>
<td>14.9 °C</td>
<td>+ 457 mm</td>
</tr>
<tr>
<td></td>
<td>Arezzo/Italy (A)</td>
<td>43° 34’ N</td>
<td>12° 04’ E</td>
<td>310 m</td>
<td>Molin Bianco</td>
<td>298 mm</td>
<td>n. avail.</td>
<td>n. avail.</td>
</tr>
</tbody>
</table>

1) Meteorological network for measuring precipitation is denser than for other parameters, therefore different stations were used to describe the climate conditions: 1st station refers to precipitation measurements, 2nd to temperature and parameters needed to calculate potential evapotranspiration (sunshine, relative air humidity). 2) Average climate data of the nearby weather stations Payerne, Neuchâtel and Bullet/La Frétaz. 3) Air temperature was adjusted to the altitude of the provenance by ± 0.65 °C / 100 m.

3.2.2. Biomass sampling

Aboveground biomass (stems, twigs and foliage) was harvested tree by tree at the end of the growing season 2009. Then, 10 soil cores (diameter = 0.08 m, h = 0.22 m) were taken from each lysimeter soil compartment to sample topsoil fine root density (diameter < 2 mm). Thereafter, fine root biomass samples of each lysimeter were pooled for further analysis.
(statistical unit = soil type, n = 4). After thawing in spring 2010, also all rootstocks were carefully excavated and separated from the soil. Dry mass of all plant parts was determined after drying to constant weight at 65 °C. A picture from each rootstock was taken in front of a white background (see example shown in Fig. 3.1). The pictures were converted using ImageJ 1.44h (U.S. National Institutes of Health, Bethesda, Maryland, USA) into black and white images in which black pixels represented root tissue and white pixels the background and analysed for “projected root area” per soil layer using IDL 7.1 (ITT Visual Information Solutions, Boulder, Colorado, USA). These 2D pictures give a representative record of the root distributions over depths. This was confirmed by the finding of a close and approximately linear relationship between “projected root area” and root mass: root area \[\text{[cm}^2]\] = 2.23 x root mass [g] + 135.16 cm² (R² = 0.807). The positive intercept reflects the fact that the ratio between diameter and cross sectional area is larger for fine roots than for coarse roots.

3.2.3. Statistical analysis

For statistical analyses we used R 2.11.1 (R Development Core Team, Vienna, AT). The data were analysed after log-transformation (mass values) or square-root-transformation (vertical root distribution) by multivariate ANOVA using a linear mixed-effect model accounting for the split-plot design of the experiment with two soils in each chamber. The statistical unit oak provenance was nested in species to account for similarity within a given species. Selected differences between treatments, soil and depths were pair-wise tested using contrasts based on t-tests and considered significant at P < 0.05 (Crawley 2009).
3.3. Results

3.3.1. Total and fine root mass

Total and topsoil fine root mass were both higher in the acidic than in the calcareous soil in the treatments without droughts, while there was no difference in total root mass between the two soil types in the drought treatments (Fig. 3.2A/B, Table 3.2). The drought treatment reduced the total root mass by about one-third in the acidic and by one-fourth in the calcareous soil, irrespective of air warming. Fine root mass, in contrast to total root mass, was reduced by the drought treatment only in the acidic soil. However, this reduction was slightly lower compared to total root mass decrease. Air warming had no effect, neither on total nor fine root mass. As expected, the root:shoot (stem, twigs and foliage) mass ratio was increased on both soils by the drought treatment (Fig. 3.2C). The ratio was slightly higher on calcareous than on acidic soils in absence of drought stress, but did not differ between the soils in the drought treatment. The fine root:foliage mass ratio was increased by the drought treatment only in the saplings growing on the calcareous, but not in those on the acidic soil (Fig. 3.2D). In contrast to the total root:shoot mass ratio, the fine root:foliage mass ratio was higher on the acidic than on the calcareous soil in the treatments with no droughts (Fig. 3.2D).
Table 3.2: Main effects and selected interactions of drought (D, continuous vs. discontinuous irrigation), air warming (AW, ambient vs. elevated air temperature), soil type (acidic vs. calcareous), type of species and type of provenance (Prov, nested within species) after three growing seasons on total root mass (n = 8), stem mass (n = 8), on the total root:shoot (stem + foliage) mass ratio (n = 8), on fine root mass in the upper 0.22 m (n = 4), on the fine root mass:foliage mass ratio (n = 4) and the projected root area (n = 8). Bold numbers represent significant main and interaction effects (P < 0.05). Other interactions were not significant.

<table>
<thead>
<tr>
<th></th>
<th>D</th>
<th>AW</th>
<th>Soil</th>
<th>Species</th>
<th>Prov</th>
<th>D x Soil</th>
<th>D x Prov</th>
<th>Soil x Prov</th>
<th>D x Soil x Prov</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total root mass</td>
<td>&lt; 0.001</td>
<td>0.727 &lt; 0.001</td>
<td>0.028 &lt; 0.001</td>
<td>0.025</td>
<td>0.003 &lt; 0.001</td>
<td>0.033</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem mass</td>
<td>&lt; 0.001</td>
<td>0.691 &lt; 0.001</td>
<td>0.052 &lt; 0.001</td>
<td>0.015</td>
<td>0.204 &lt; 0.001</td>
<td>0.228</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total root:shoot mass ratio</td>
<td>&lt; 0.001</td>
<td>0.694 &lt; 0.001</td>
<td>0.359 &lt; 0.001</td>
<td>0.042</td>
<td>0.125 &lt; 0.001</td>
<td>0.262</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine root mass</td>
<td>0.002</td>
<td>0.696 &lt; 0.001</td>
<td>-</td>
<td>-</td>
<td>0.050</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Fine root:foliage mass</td>
<td>0.004</td>
<td>0.769 0.015</td>
<td>-</td>
<td>-</td>
<td>0.114</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total projected root area</td>
<td>&lt; 0.001</td>
<td>0.665</td>
<td>0.451</td>
<td>0.003 &lt; 0.001</td>
<td>0.162</td>
<td>0.003 &lt; 0.001</td>
<td>0.094</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00 - 0.25 m</td>
<td>&lt; 0.001</td>
<td>0.659 &lt; 0.001</td>
<td>0.003 &lt; 0.001</td>
<td>0.001</td>
<td>0.035 &lt; 0.001</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 - 0.50 m</td>
<td>0.001</td>
<td>0.817 0.328</td>
<td>0.006 &lt; 0.001</td>
<td>0.375</td>
<td>0.009 &lt; 0.001</td>
<td>0.348</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.50 - 0.75 m</td>
<td>0.457</td>
<td>0.256 &lt; 0.001</td>
<td>0.007 &lt; 0.001</td>
<td>0.190</td>
<td>0.003 &lt; 0.001</td>
<td>0.116</td>
<td></td>
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</tbody>
</table>

The main and interaction effects on the projected root area in the lowest soil layer (0.75 - 1.00 m) could not be calculated as the model assumptions could not be fulfilled despite square-root-transformations. According to Fig. 3.4 and 3.5, there might be the same main effects as shown for the soil layer 0.50 - 0.75 m.

The stem and root biomasses were higher in *Quercus robur* than in *Q. petraea* and *Q. pubescens*, in all combinations of soil and climate treatments (Fig. 3.3, Table 3.2), but the drought-induced growth reduction was stronger in *Q. robur* than in the other two species. Among the four provenances within each species, root and stem masses increased with the aridity of the original provenances sites (Fig. 3.3), using the climatic average balance between precipitation and potential evapotranspiration as aridity indicator (Table 3.1). Generally, the growth of *Q. robur* depended less on the aridity of the provenance site than that of *Q. petraea* and *Q. pubescens*. 
Fig. 3.2 A) Effects of drought and air warming on total root dry mass (kg m\(^{-3}\)) after three growing seasons in acidic (left) and calcareous soil (± SE, \(n = 8\)), pooled over all species and provenances, B) fine root dry mass in the topsoil (upper 0.22 m, kg m\(^{-3}\), \(n = 4\)), C) total root:shoot mass ratio (\(n = 8\)), D) fine root:foliage mass ratio (\(n = 4\)). CO: control, AW: air warming, D: drought, AWD: air warming & drought. Results of statistical analysis are given in Table 3.2.

Differences in growth between the provenances were reduced when water became a limiting factor in the drought treatments. This indicates a higher drought responsiveness of the provenances from the more arid sites than of those from the more humid sites. This observed trend was systematic and consistent in all three species and for both soils. Comparing the two soils, growth on the calcareous soil depended more on the aridity of the provenance site than growth on the acidic soil. The mean growing season temperature at the original provenance site showed no influence on tree growth, neither above nor below ground (not shown).
Fig. 3.3: Relationship of water balances (mm) at the original provenance site (Table 3.1) to root and stem dry weights of the investigated oak provenances in the treatments without (control, air warming) and with droughts (drought, air warming & drought) on acidic and calcareous soil ($n = 16$). Data of corresponding treatments with and without air-warming are pooled as the air-warming treatment had no significant effect on the presented data (Table 3.2). Over all treatments, species, provenances and both soils, the negative slope of the correlation lines was highly significant for root ($R^2 = 0.772, P < 0.001$) and for stem growth ($R^2 = 0.823, P < 0.001$). The Italian provenance Quercus pubescens Arezzo was excluded from this analysis due to missing water balance data.

3.3.2. Effect of drought and soil properties on vertical root mass distribution

The effect of drought-induced root mass reduction decreased with soil depth (Fig. 3.4, Table 3.2). As a result, the centre of mass in the vertical root distribution shifted to lower depths. These downward shifts were found in all three species (Fig. 3.5). In contrast to drought, air warming had no effect on the vertical root mass distributions (Fig. 3.4, Table 3.2). Figure 4 also shows an influence of the soil on the depth distributions of root mass. In the acidic soil, root mass density was highest in the top 0.25 m, averaging 53% of the total root mass over all treatments and species/provenances, whereas only 38% of the root mass was located at 0.25 - 0.50 m and only 8% at 0.50 - 0.75 m depth. Below 0.75 m, there were almost
Fig. 3.4: Effects of drought and air warming on root mass distribution (projected root area, cm$^2$) in four different soil layers, each 0.25 m deep, in either acidic (left) or calcareous soil (right, means of all species and provenances, ± SE, n = 8). Different letters indicate significant differences ($P < 0.05$) between respective treatments in the same soil type and layer. An asterisk indicates a significant difference (*: $P < 0.05$, (*): $P < 0.10$) between acidic and calcareous soils for the respective treatment and soil layer. The pair-wise comparisons in the lowest soil layer (0.75 - 1.00 m) could not be calculated as the model assumptions could not be fulfilled despite square-root-transformations.

no roots in the acidic soil (< 1%). In comparison to the acidic soil, the root mass distribution was shifted to lower depths in the calcareous soil. On average, 41% of the roots were located in the top 0.25 m, 42% at 0.25 - 0.50 m, 16% at 0.50 - 0.75 m and 2% below 0.75 m.

Compared to the Q. robur provenances, the vertical root distribution patterns of Q. petraea and Q. pubescens were more diverse among provenances, especially in the calcareous soil (Fig. 3.5). In the acidic soil, the provenance Arezzo differed most from the other Q. pubescens provenances, with distinctly smaller projected root areas in the upper 0.5 m depth than the other provenances. In the calcareous soil, the Q. petraea and Q. pubescens provenances originating from the drier sites showed a similar downward shift in
Fig. 3.5: Vertical root mass distributions (projected root area, cm²) of the provenances of the three oak species, as affected, by soil type and drought treatment. Data of corresponding treatments with and without air warming are pooled, as the air-warming treatment had no significant effect on root growth (n = 16 for each provenance/soil/drought treatment combination). The provenances are sorted in ascending order by water balances at their sites of origin as in Table 3.1. The abbreviations used for the provenances are the same as given in Table 3.1. Statistical analysis can be found in Table 3.2.

vertical root mass distribution as the *Q. robur* provenances, whereas those from the more humid sites did not.

3.4. Discussion

The correlations between biomass production and climatic water balances of the provenance sites of origin included in this study indicate substantial genetic adaptation of these provenances to local soil humidity conditions. As expected the provenances originating
from drier sites produced under drought conditions more biomass than those from more humid sites. However, provenances from drier sites, with substantially more biomass under well-watered conditions, also showed more biomass reduction than provenances from more humid sites as a consequence of the drought treatment. Therefore, these results were only in partial agreement with the first hypothesis. The similarity of the correlations for both soils suggests that there was little influence of nutrient availability in the soil on the variation in growth among the provenances, while differences on the species level were larger. An improved drought tolerance of the southern oak provenances used in this experiment was also found by Arend et al. (2011), who monitored shoot height and diameters over three growing seasons on acidic soil. It is also consistent with the results of Jensen & Hansen (2010) for Quercus robur and of Bruschi (2010) for Q. petraea. There was no correlation between air temperature at the provenances origins and mass data in the present experiment, as similarly reported for example in Q. petraea by Vitasse et al. (2009). The variation in temperature among the sites was probably too low.

Apart from provenance, also soil water availability had a strong influence on root and shoot growth. As in other oak studies, drought-induced reductions were stronger in shoot than in root mass, leading to an increased root:shoot mass ratio (Broadmeadow & Jackson 2000; Fotelli et al. 2000; Gieger & Thomas 2002; Joslin et al. 2000; Thomas 2000a; Thomas & Gausling 2000). This well-known shift in growth allocation from above to below ground can be interpreted as an acclimatisation to enhance water uptake capacity in relation to transpirational water loss. It is in line with this interpretation that fine root mass was relatively less reduced by drought than total root mass, as fine roots are the most important root fraction for water and nutrient uptake. In agreement with our findings, Fotelli et al. (2000) reported that Q. pubescens roots < 2 mm were proportionally less reduced by drought than roots > 5 mm. Moreover, Thomas (2000a) found that drought had no effect on the fine root mass of
Q. pubescens seedlings and even a positive influence on fine root mass in Q. petraea. In line with these findings, Vogt et al. (1996) derived a weak but significant relationship from a review of literature data according to which fine root mass of trees tends to increase with decreasing precipitation.

Root growth reduction in response to drought was most pronounced in the top 0.25 m of both soils, resulting, in accordance with our second hypothesis, in shifts of the centre of root mass towards lower depths, where water was still more available (Kuster et al. 2012a). The fact that root distributions were also shifted to lower depths in the calcareous soil relative to those in the acidic soil, irrespective of the treatment, indicates that water and nutrient availability differed also between the two soils. In fact, in the acidic soil nutrients such as N, Ca, K and Mn were much more concentrated in the upper 0.15 m and less concentrated at lower depths (Kuster et al. 2012a; Kuster et al. 2012b), leading to a high root density in the acidic topsoil. In contrast in the calcareous soil, nutrients were more evenly distributed over depth, including P and Mn, which were found to be growth limiting in the calcareous soil. We may speculate that in the calcareous soil oak roots were acclimatised to maximise P and Mn mobilisation. According to our tensiometer measurements, water availability was generally sufficiently high for uninhibited root uptake in both soils at all depths in the control treatment and, thus do not explain the observed differences in root distribution between the two soils, although there was a slight gradient to lower availability with depth in the acidic soil and the opposite trend of slightly increasing soil moisture with depth in the calcareous soil (Kuster et al. 2012a).

In contrast to the third hypothesis, the air-warming treatment had no influence on the growth and vertical allocation of roots. With a maximum increase in topsoil temperature of 1 °C during the growing season, the treatment was probably too mild to cause detectable changes in root growth, in particular as temperatures reached up to 20 °C in summer. Thus,
they came already close to the optimum soil temperature for root growth in oaks, which was reported to be about 25 °C (Lyr 1996; Lyr & Garbe 1995). Close to the temperature optimum, measurable responses in tree root growth to temperature changes are expected only if there is no other limiting factor (Pregitzer et al. 2000). Teskey & Hinckley (1981) found in a study on Quercus alba that soil temperature was the most important factor for root growth up to 17 °C, whereas above 17 °C soil water potential became more important. Also in a recent review, Way & Oren (2010) revealed no strong warming effect on tree root growth.

Comparing species responses to drought stress, Q. robur appears to be more sensitive as the absolute reduction in biomass was higher than in Q. petraea and Q. pubescens. Moreover, lower root:shoot mass ratios in Q. robur indicate a minor drought tolerance than in Q. petraea. Other studies on our model ecosystems came to results that are in line with our findings. The drought treatment reduced net photosynthesis more strongly in Q. robur than in the other two species (Arend et al. 2012), and also caused more foliage injury in Q. robur (Günthardt-Goerg et al. 2012). However, regarding the relative abundance of the ectomycorrhizal fungus Cenococcum geophilum, drought generally decreased its abundance on the root system of Q. petraea and Q. pubescens, whereas colonisation of Q. robur roots was not influenced by drought (Herzog et al. 2012). Air warming tended to increase the abundance of C. geophilum on all oak species, also under water limiting conditions.

Apart from heat and drought tolerance, also traits such as frost resistance have to be considered when evaluating oak provenances for future central European forestry. With increased abundance of extreme conditions varying between cold/wet and hot/dry periods in a changing climate (IPCC 2007), tolerance to late frosts requires particular attention in the selection of oak species and provenances. When provenances from sites with milder climates will be selected, they may be less frost resistant than provenances from colder areas of central
Europe (Jensen & Deans 2004). However in the present study, temperature at the selected provenance sites was not correlated to growth response.

3.5. Conclusions

Oak saplings are able to acclimatise to water limiting conditions by increasing their root:shoot mass ratio and by shifting the root mass downwards to layers where water is often longer available. We expect that mature oak trees are even more drought tolerant as they are able to grow roots in deeper soil layers (Hanson et al. 2001; Leuzinger et al. 2005). Therefore, we conclude that central European oak species are highly suitable for a future climate, in particular on sites that become too dry for beech or spruce (Leuschner et al. 2001a; Thomas 2000a; van Hees 1997). Thereby, special attention has to be dedicated to the origin of seed material.

Given that the provenances from drier sites, with overall better growth, showed more biomass loss at water limiting conditions than provenances from more humid sites, it remains questionable whether provenances from drier sites are better suited for a future climate.

Acknowledgments

Our particular acknowledgement is for the valuable advice and expertise of Matthias Dobbertin (†). We will keep him in our memories, both as an excellent scientist and as an irreplaceable friend. Furthermore, we thank Peter Bleuler, Martin Keller, Patrick Schleppi, Terry Menard, the internship students, the research unit Forest Soils and Biogeochemistry, the research group Phytopathology and the garden team of WSL for their help in performing the experiment. We also acknowledge Corina Del Fabbro, the Seminar for Statistics of ETHZ and the Central Laboratory of WSL for their support in sample and data analysis. For funding we are gratefully indebted to the Velux foundation (project number: 489).
4. Root and shoot growth patterns of 1st-year *Quercus robur* seedlings as affected by drought conditions

T. M. Kuster, P. Vontobel, M. S. Günthardt-Goerg & R. Schulin
Summary

Seasonal root and shoot growth of young oaks often involves several flushes, separated by periods with no or negligible growth. Previous studies reported alternate phasing of root and shoot growth peaks and explained this pattern by competition between above- and belowground parts within the plant for assimilates. Our hypothesis was that it will become even more distinct when this competition is exacerbated by drought stress, as it is expected to occur more frequently in the future with climate change. We used neutron radiography imaging to investigate the effect of reduced water availability on the phasing of root and shoot growth in 1st-year *Quercus robur* seedlings on two different soils. In contrast to our hypothesis, water availability did not affect root growth, and we found no alternate phasing of root and shoot growth. This indicates that due to the high nutrient contents stored in the acorns, 1st-year oak seedlings have no advantage in alternating assimilate allocation to root and shoot growth and that external factors are overruled by endogenous growth patterns.

*Keywords:* acidic soil, acorn, calcareous soil, climate change, flush, neutron radiography
4.1. Introduction

Climate change scenarios according to IPCC Scenario A2 predict increasing summer temperatures (+3.2 to 4.8 °C) during the 21st century in central Europe (IPCC 2007; Schär et al. 2004). In the meantime with a reduction in summer precipitation of 21 to 28%, drought periods will become more frequent (CH2011 2011). Oak species are known to be tolerant to drought and heat (Ellenberg et al. 2010; Landolt & Bäumler 2010). Their long taproots allow them to take up water from deep soil layers; the xeromorphic structure of their leaves provides efficient water loss reduction and their ability to resume photosynthesis within a few days after re-wetting shows that their physiology can recover quickly from drought stress (Arend et al. 2012; Galle et al. 2007; Günthardt-Goerg et al. 2012; Kubiske & Abrams 1993).

Seasonal shoot growth of young oaks usually occurs in several flushes, separated by stagnancy in shoot growth. Also root growth often shows a rhythmic pattern during the season in oaks, but in contrast to shoot growth, it does not completely stop between the growth peaks (Bédnéau & Auclair 1989; Konopka et al. 2005; Ponti et al. 2004). While some studies found no correlation between above- and belowground growth rates in oaks (Harmer 1990; Pages & Serra 1994), others reported slowed root growth during shoot flushes and vice versa, attributing this pattern to root-shoot competition for assimilates (Reich et al. 1980; Willaume & Pages 2006; Willaume & Pages 2011). Expecting that such competition should become more intense under conditions of limited resource availability, we hypothesised that such alternating root and shoot growth pattern should become even more distinct when assimilation and nutrient uptake are limited by drought stress. To investigate the influence of water deficiency on root and shoot growth patterns we compared the shoot and root growth of 1st-year Quercus robur seedlings growing under drought stress vs. no water stress on either acidic or calcareous soil, using neutron radiography imaging here for the first time to study root growth in oak seedlings.
4.2. Material and methods

4.2.1. Experimental setup

Neutron radiography (NR) imaging is a non-destructive method that is well-suited to monitor root growth over time (Oswald et al. 2008). The imaging was performed at the NEUTRA facility of the Paul Scherrer Institute (PSI), Villigen, Switzerland (47° 32’ 16’’ N, 8° 13’ 26’’ E, 346 m a.s.l.). A detailed description of the facility and its neutron source is given by Lehmann et al. (1999).

In August 2009, we planted single Q. robur acorns into 18 aluminium (Al) containers (size: 64 x 67 x 1.2 cm). The inner surfaces of the container walls were covered with a 50 μm thick Teflon film (Angst + Pfister, Switzerland) in order to prevent Al contamination of the soil. Nine of the containers were filled with acidic loamy sand taken from a Haplic Alisol (pH 4.0), the other 9 containers with calcareous sandy loam taken from a Calcaric Fluvisol (pH 6.9). Nutrient concentrations and other soil properties can be found in detail in Kuster et al. (2012a) and in Kuster et al. (2012b). The seedlings were at first grown in the garden of the Swiss Federal Research Institute WSL (47° 21’ 45’’ N, 8° 27’ 24’’ E, 547 m a.s.l.) at ambient weather conditions. In mid September 2009 they were transferred into a heated greenhouse with artificial light (20 °C/17 °C). One seedling on the acidic soil, which developed only a root but no shoot, was excluded from the experiment.

From October to December 2009, the seedlings were NR imaged in 2-weeks intervals. Two irrigation schemes were applied during this period. One group of containers was watered on average every four days to adjust soil water potential to field capacity (control), while the other group was not watered (drought treatment). In order to control soil moisture, the containers were weighed on a PM30-K balance (Mettler-Toledo, Greifensee, Switzerland) each time before watering (soil water contents see result and discussion sections). For
imaging, the containers were transported from WSL to PSI. Because of the large size of the containers, one radiograph could cover only $\frac{1}{3} \times \frac{1}{3}$ of their lateral view. Thus, each image was assembled from 3 x 3 separately recorded radiographs. Each time the roots were imaged, also shoot heights were measured. In December 2009, the predawn water potential (LWP) was determined in one leaf of each plant, using a Scholander pressure chamber (M 600, Mosler Tech Support, Berlin, Germany) in order to quantify the drought stress of the seedlings at the end of the imaging period (Scholander et al. 1965).

In late April 2010, a new acorn of *Q. robur* was planted into each container for a second round of imaging. The acorns available for planting in 2010 were slightly smaller than those planted in 2009, but as in the previous year they showed little variation in size (Table 4.1). Unfortunately, several seedlings of the second round stopped growing approximately one week after germination and could not be replaced (Table 4.1). As only 3 seedlings survived on the calcareous soil, the experiment was discontinued for this soil type. All containers with surviving seedlings growing in acidic soil were moved to PSI again in May 2010, where they were kept in a greenhouse between NR image recordings. In comparison to the first round, control seedlings were only watered every 2nd week, whereas the drought-treated seedlings received no water during the imaging period, except on 6 July 2010, when they were irrigated to avoid wilting. Otherwise, the imaging and irrigation schemes were the same as in the first round. In August 2010, after measuring LWP, we disassembled all containers, separated the roots of the second round from the soil, scanned them using an Epson Expression 10000 XL scanner (Epson, Japan), and analysed the scans by means of WinRhizo 2009c (Regent Instruments, Québec, Canada).

The NR radiographs were processed and analysed as follows. At first, flat and dark current corrections were performed, as described by Oswald et al. (2008), to eliminate
Table 4.1: Treatment and soil effects on acorn size, plant growth parameters, soil water content and predawn leaf water potential (LWP) at the end of the measuring periods in 2009 and 2010 (means ± SE). Different letters indicate a significant (P < 0.05) drought effect within a given soil type and measuring period, * indicates a significant soil type effect within a given treatment in the measuring period 2009.

<table>
<thead>
<tr>
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<th>2009</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>acidic</td>
<td>drought</td>
<td>calcareous</td>
<td>drought</td>
</tr>
<tr>
<td>Number of repetitions</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Acorn Size (cm³)</td>
<td>4.0± 0.2</td>
<td>4.0± 0.5</td>
<td>4.0± 0.3</td>
<td>3.8± 0.6</td>
</tr>
<tr>
<td>Total root length (cm)</td>
<td>313.6± 137.1</td>
<td>*399.8± 41.2</td>
<td>201.0± 33.4</td>
<td>*183.4± 15.1</td>
</tr>
<tr>
<td>Shoot height (cm)</td>
<td>10.8± 1.9</td>
<td>*14.1± 1.7</td>
<td>9.8± 1.5</td>
<td>*8.5± 1.0</td>
</tr>
<tr>
<td>Root:shoot length</td>
<td>30.0± 12.2</td>
<td>28.9± 2.9</td>
<td>23.6± 6.1</td>
<td>22.5± 3.2</td>
</tr>
<tr>
<td>Number of flushes</td>
<td>2.0± 0.6</td>
<td>2.3± 0.3</td>
<td>1.4± 0.4</td>
<td>2.0± 0.4</td>
</tr>
<tr>
<td>Soil water content (%)</td>
<td>19.9± 0.6</td>
<td>*9.3± 0.6</td>
<td>*16.7± 0.4</td>
<td>*11.3± 1.0</td>
</tr>
<tr>
<td>LWP (MPa)</td>
<td>-0.2± 0.1</td>
<td>*-1.2± 0.1</td>
<td>*-0.3± 0.1</td>
<td>-0.6± 0.2</td>
</tr>
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<td>-0.5± 0.1</td>
<td>-1.1± 0.4</td>
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* indicates a significant soil type effect within a given treatment in the measuring period 2009.
Drought effects on root and shoot growth of pedunculate oak

Fig. 4.1: Example of a root growth series of the neutron radiography experiment with Quercus robur, grown in well-watered acidic soil during the second growth period in 2010, and corresponding total root length at each measuring date. Root growth between 26 April 2010 and 3 May 2010 was only small with a total root length of 0.5 cm (not shown).

artefacts due to beam variations and camera noise, using the QNI-Quantitative Neutron Imaging software developed by Hassanein (2006). After adjusting brightness, the 9 radiographs taken from each container at a given time were stitched together using IDL 7.1 (ITT Visual Information Solutions, Boulder, Colorado, USA). Because of partially low contrast between soil and roots in the radiographs, the roots were tracked manually in the resulting images, using Adobe Photoshop CS5 (Adobe, San Jose, California, USA, see example shown in Fig. 4.1). Total root length was determined by means of WinRhizo 2009c. Comparing the scans with the last NR images taken before the roots were sampled and scanned revealed that roots with a diameter > 1 mm were detected in the NR images with the same degree of reliability as in the scans. For smaller roots the recovery rate decreased with diameter: from 88% for > 0.75 mm to 75% for > 0.5 mm. Roots < 0.2 mm were not detectable with NR, as noted before by Moradi et al. (2009).
4.2.2. Statistical analysis

For statistical analyses we used R 2.11.1 (R Development Core Team, Vienna, AT). The data presented in Table 4.1 were separately analysed for the two growing periods after log-transformation by multivariate ANOVA using a linear model. Selected differences between water treatment and soil were tested pair-wise using contrasts based on t-tests and considered significant at P < 0.05 (Crawley 2009).

4.3. Results

In the first round of the experiment, the non-irrigated calcareous soil had only 70% of the soil water content (SWC) of the irrigated calcareous soil, and the non-irrigated acidic soil had even less than half the SWC of the irrigated acidic soil (Table 4.1). This was in line with measurements of the predawn leaf water potential (LWP), indicating that the drought stress was more severe in Quercus robur seedlings growing on non-irrigated acidic than on non-irrigated calcareous soil. Unexpectedly, some seedlings grew initially faster on both soils without than with irrigation in the first imaging round (Fig. 4.2). This indicates a growth inhibition in the control treatment that was due to impaired soil aeration resulting from excessive soil wetness, as the effect did not occur in the second round, when the irrigation rate in the control treatment was reduced (see below). Despite the stronger reduction in shoot growth rates of the drought-treated seedlings at later stages of the first growing period, there was neither a drought effect on total root length nor on shoot height (Table 4.1). In agreement with the results of a model ecosystem experiment using the same soils (Kuster et al. 2012a), the seedlings grew faster on the acidic than on the calcareous soil, above and below ground.

In the second round of the NR experiment, the irrigated containers had lower SWCs than the irrigated containers of the first round, as they were only watered every 2nd week
Fig. 4.2: Mean root (solid lines/black squares) and shoot (dashed lines/white circles) growth patterns (cm day\(^{-1}\)) during the first measuring period in autumn 2009 (top) and during the second measuring period in summer 2010 (below), separately shown for each drought treatment and soil type combination. Number of repetitions and shoot length at the end of each growing period are shown in Table 4.1.

(Table 4.1). As a result, the differences in SWC and LWP between irrigated and non-irrigated containers were smaller than in the first round. But nonetheless, shoot growth was much stronger in the second than in the first round, while it was the same on the acidic soil in both rounds in the drought treatments. In contrast to the first imaging period, shoot heights were reduced by the drought treatment at the end of the second imaging period. Due to an outlier in the drought treatment, this effect only came close to the level of significance (P = 0.057). In contrast to shoot growth, total root length growth was much smaller in 2010 than in 2009, which may be explained, at least partially, by the smaller size of the acorns planted in 2010 (Table 4.1).

There were also less 2\(^{nd}\) and 3\(^{rd}\) shoot growth flushes in the second round. In 2009, 6 out of 17 seedlings flushed once, 7 twice and 4 even three times. In 2010, 1 of 7 seedlings flushed once and the other 6 twice. Flushing occurred in close synchrony on the two soils, and we
found no influence of the drought treatment on the frequency and timing of flushes (Table 4.1, Fig. 4.2). Also root growth rates showed fluctuations. These did not alternate with the shoot growth flushes; they rather showed a parallel course, especially in the control treatment. Furthermore, the fluctuations in shoot growth rates appeared to superimpose a trend of decline after the first flush in both experimental rounds, reflected in a steady decrease of flush size over time. Root growth, on the other hand, rather showed the opposite trend in the second round and at best a very slight trend to decrease in the drought treatment in the first round.

4.4. Discussion and conclusions

The observed synchrony in the pattern of root and shoot growth was in contrast to our hypothesis and in line with the findings of Harmer (1990) and Pages & Serra (1994). The experiment also revealed no influence of the drought treatment on the phasing of root and shoot growth spurts. Peaks and dips of shoot and root growth occurred at the same time in both water treatments and soils. The lack of a drought effect may have been related to the fact that this experiment was performed with first-year seedlings. During their first year, oak seedlings mainly depend on assimilates and nutrients stored in the acorns for their initial growth and less on soil nutrients, as shown for example for N uptake by *Quercus ilex* (Villar-Salvador *et al.* 2010) and for N and P uptake by *Q. robur* (Newton & Pigott 1991). This may also explain why root and shoot growth did not show alternate phasing. Alternating assimilate allocation to root and shoot growth, as suggested by Willaume & Pages (2011) for *Q. pubescens* seedlings, seems therefore not to be a sufficient advantage as long as reserves from acorns are available. It seems to be more important for the seedlings to develop water and nutrient supply capacities below ground in synchrony with capacities for photosynthesis and carbon assimilation above ground. While our results indicate that during the first period after germination drought stress conditions were overruled by endogenous growth patterns, we
cannot rule out that external factors may come into play at later stages when energy and nutrient reserves stored in the acorns are depleted. Villar-Salvador et al. (2010) reported that the relative amount of N taken up by roots in relation to N stored in the acorns increased from 25 - 38% during the first shoot flush to 32 - 54 % during the second shoot flush. Patterns of alternately phased root and shoot growth were reported for two-year-old Quercus alba saplings (Reich et al. 1980) and first-year seedlings of Q. pubescens (Willaume & Pages 2006). While this does not necessarily mean that growth patterns can change with age, it suggests that they differ among species.

With climate change the frequency of dry summers is predicted to increase (CH2011 2011; IPCC 2007). The high contents of nutrients and assimilates stored in oak acorns are advantageous for germination under conditions of low soil moisture and limited external nutrient supply, even though extended drought periods may lead to smaller acorns. Slight reductions in acorn size were not found to affect root and shoot growth patterns in the present study.

In this study NR imaging was used for the first time to monitor root growth in oak seedlings over time. As this non-invasive method depends on the contrast in water content between soil and roots it is particularly well suited to study root growth in soils with coarse texture and low organic matter content in response to drought conditions (Menon et al. 2007).

Acknowledgments

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Menard, the internship students and the garden team of WSL for their help in performing the experiment. We express gratitude to the Velux foundation for funding (project number: 489).
5. Nitrogen dynamics in oak model ecosystems subjected to air warming and drought on two different soils

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Summary

Being tolerant to heat and drought, oaks are promising candidates for future forestry in view of climate change in Central Europe. Air warming is expected to increase and drought decrease soil N availability and thus N supply to the trees. Here, we conducted a model ecosystem experiment, in which mixed stands of young oaks (Quercus robur, Q. petraea and Q. pubescens) were grown on two different soils and subjected to four climate treatments during three growing seasons: air warming by 1 to 2 °C, drought periods (average reduction of precipitation by 43% to 60%), a combination of these two treatments, and a control. In contrast to our hypotheses, neither air warming nor drought significantly affected N availability, whereas total amounts, vertical distribution and availability of soil N showed substantial differences between the two soils. While air warming had no effect on tree growth and N accumulation, the drought treatment reduced tree growth and increased or tended to increase N accumulation in the reduced biomass, indicating that growth was not limited by N. Furthermore, $^{15}$N-labelling revealed that this accumulation was associated with an increased uptake of nitrate. On the basis of our results, climate change effects on N dynamics are expected to be less important in oak stands than reduced soil water availability.

Keywords: Quercus petraea, Q. pubescens, Q. robur, $^{15}$N tracer, ammonium, nitrate, recovery rate, climate change
5.1. Introduction

Climate change scenarios predict an increase in air temperatures by 3.2 to 4.8 °C and an increased frequency of severe dry periods (-21 to -28% precipitation) during the 21st century for Central Europe (CH2011 2011; IPCC 2007). Oak trees are relatively tolerant to heat and drought (Ellenberg et al. 2010; Landolt & Bäumler 2010) and are therefore expected to be more competitive at low altitudes, as these may become too warm and dry for other species, in particular for beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) (Gessler et al. 2007; Leuschner et al. 2001a). Species of oaks are therefore considered as promising candidates in future central European forestry.

Apart from direct drought and heat stresses, oaks may also have to cope with the indirect effects of the predicted climate change, e.g. on the nitrogen (N) cycle. As oaks often grow on acidic soil rather poor in nutrients (Landolt & Bäumler 2010) and as fixation of atmospheric N\(_2\) by legumes and alders is not common in oak stands, N availability may also play an important role in oak forests. Apart from atmospheric deposition, bio-available N in oak stands is provided primarily through microbial decomposition of organic matter, ammonification and nitrification; the rates of these processes depend on soil temperature (Zhang et al. 2008). With increasing temperature, the supply of inorganic N to plant roots was generally found to increase (Rennenberg et al. 2009; Sardans et al. 2008a; Saxe et al. 2001). However, elevated air and soil temperatures may at the same time increase N demand by accelerating tree growth (Gessler et al. 1998; Saxe et al. 2001; Way & Oren 2010); and if carbon inputs into soil increase more than the N supply, a decrease in available soil N due to increased N immobilisation may result, as discussed by Bardgett (2005). In contrast to increased temperatures, droughts reduce litter decomposition, N mineralisation and nitrification rates (Chen et al. 2011; Sardans et al. 2008b). While microbial activities are reduced when water availability is low, drought periods can lead to N mobilisation when
decayed microbial biomass is decomposed after soil re-wetting (Borken & Matzner 2009; Schimel et al. 2007).

Nitrogen is taken up by plants mainly in the form of NH$_4^+$ and NO$_3^-$; but also the uptake of free amino acids by tree roots has been reported and may play a major role (Stoelken et al. 2010), especially when organic matter mineralisation and nitrification are inhibited. Nitrate and ammonium uptake rates vary with their availability. In a study on Pinus sylvestris, enhanced uptake of nitrate-N versus ammonium-N was found in NO$_3^-$-enriched soils (Schulz et al. 2011), while the opposite was true in a soil with high NH$_4^+$ concentration (Nordin et al. 2001). Notwithstanding this flexibility, there may be considerable differences among oak species in their preference for different N forms. Quercus petraea appeared to prefer NO$_3^-$-N when equal amounts of both inorganic forms were supplied (Berger & Glatzel 2001; Schulz et al. 2011), whereas no significant preference for either NO$_3^-$-N or NH$_4^+$-N was found in Q. robur (Stadler et al. 1993). Given that nitrification is generally more sensitive to environmental conditions than N mineralisation, climate change may not only affect the total amount of available N in a soil, but also lead to a substantial shift in the ratio between different N forms. In turn, this shift in N availability may have important impacts on the growth and competitiveness of oaks (Rennenberg et al. 2009; Saxe et al. 2001). Apart from water availability and temperature, also soil pH and carbonate content can strongly influence NH$_4^+$ and NO$_3^-$ availability and shift the ratio between N forms in plant uptake. For example, Berger & Glatzel (2001) found higher leaf N concentrations in Q. petraea growing on a calcareous soil fertilised with NO$_3^-$ than with NH$_4^+$, whereas no differences between these treatments were found on an acidic soil.

In the study presented here we used the opportunity, provided by a factorial model ecosystem experiment in which mixed stands of young Q. robur, Q. petraea and Q. pubescens trees were subjected during three growing seasons to different enhanced air temperatures and
drought treatments on acidic and calcareous soil, to investigate the effects of climate change on soil N availability and uptake by young oaks. Based on the literature presented above, our hypotheses were (i) that air warming increases and drought decreases the availability of NH$_4^+$ and NO$_3^-$ in soil, (ii) that these effects are stronger on NO$_3^-$ than on NH$_4^+$ availability, and (iii) that N uptake by oak trees responds more strongly to changes in soil NO$_3^-$ than in NH$_4^+$. To determine the effects of supplied N forms on N uptake, we conducted a tracer experiment in which $^{15}$N was applied in form of nitrate on half of the stands and in form of ammonium on the other half.

5.2. Material and methods

5.2.1. Study site and experimental design

The experiment was performed at the model ecosystem facility of the Swiss Federal Research Institute WSL, Birmensdorf, Switzerland (47°21’48” N, 8°27’23” E, 545 m a.s.l., Kuster et al. 2012a). The facility consists of 16 hexagonal chambers of 3 m height. Using a Latin square design with four replicates of each treatment, one of the following four treatments was applied from spring 2007 to October 2009 to each chamber: air warming (AW), drought (D), the combination of air warming and drought (AWD), and a control treatment (CO). Each chamber was split below ground into two 1.5 m deep concrete-walled lysimeters with a surface area of 3 m$^2$, allowing the use of two different soils in each chamber (soil analysis: statistical unit = soil type, n = 4). On each of these lysimeters, 3 different oak species were planted (plant analysis: statistical unit = oak species, n = 4).

The lysimeters were filled in spring 2005 with a 0.5 m drainage packing (consisting of 3 layers of pure quartz sand of decreasing grain size) and a 1 m soil layer. One soil in each chamber consisted of two layers of acidic loamy sands taken from a Haplic Alisol (pH 4.0,
subsoil 15-100 cm, topsoil 0-15 cm) and the other of a single layer of calcareous sandy loam taken from a Calcaric Fluvisol (pH 6.9). Both soils, originating from mixed oak forests, were described in detail by Kuster et al. (2012a). A woodchip layer on top of these soils reduced weed germination and protected the soil surface against rainfall splash. In spring 2006, two-year-old saplings of *Quercus robur*, *Q. petraea* and *Q. pubescens* were planted, each species was represented by eight trees on each lysimeter, and grown with adequate water supply at ambient air temperatures during the growing season of 2006.

The air warming treatment consisted in closing the side glass walls of the chambers more than those of the control treatment, which passively increased the daytime air temperature by 1 – 2 °C and consequently the soil temperature at a depth of 12 cm by 0.5 – 1 °C (Kuster et al. 2012a). Drought periods were imposed by suspending irrigation for several consecutive weeks during two periods in each growing season (April to October). Drought-treated oak stands received 43% (2008 & 2009) to 60% (2007) less water compared to the long-term annual mean precipitation of the site. During growing seasons, automatically closing roofs excluded natural rainfall.

5.2.2. \(^{15}\text{N} \) labelling and measurement of \(^{15}\text{N}, \text{NH}_4^+ \) and \(\text{NO}_3^- \) in soil, biomass and discharge water

In spring 2008 (May 23rd) 99-atom-% \(^{15}\text{N} \) was applied as K\(^{15}\text{NO}_3\) on two of the four replicate chambers of each treatment and as \(^{15}\text{NH}_4\text{Cl}\) on the other two. The application rate was 2 mmol m\(^{-2}\) N-15 (30 mg m\(^{-2}\)) in both cases. The tracers were dissolved in deionised water (5 l m\(^{-2}\)) and sprayed with a backpack sprayer as homogenously onto the soil surface as possible, followed by 2 x 15 l m\(^{-2}\) of deionised water to flush the tracers into the soil.

In September 2009, *i.e.* before the start of autumnal leaf senescence, 5 leaves were collected from two trees per oak species, soil and climate treatments and pooled for laboratory
analysis. The leaves were chosen to represent the foliage of each tree by flush, colour, stress symptoms, leaf size and location in the canopy. In October 2009, the entire aboveground biomass was harvested per tree and the foliage was separated from the twigs. In spring 2010, all root stocks were excavated, and a fine root (diameter < 2 mm) sub-sample was taken from each stock. All samples were dried at 65 °C. The samples of each plant part (leaves, stem + twigs and fine roots) obtained from 2 trees of the same species on each lysimeter, i.e. same climate treatment and same soil, were pooled for further analysis. Dried leaf and fine root samples were ground using a ball mill (MM400 & MM2000, Retsch, Haan, Germany), while a vibrating cup mill was used to grind the dried stem + twigs samples (Pulverisette 9, Fritsch, Idar-Oberstein, Germany). $^{15}$N and total N concentrations were determined by using an isotope-ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and an elemental analyser (EuroVector EA3000, HEKAtech GmbH, Wegberg, Germany). Tracer fractions, defined as molar ratios of tracer-N to total N, were calculated from the measured $\delta^{15}$N values as described in detail by Providoli et al. (2005). Reference values were obtained from ambient, non-labelled lysimeters with the same two soils outside the chamber facility. Recovery rates, i.e. the ratios between concentrations of tracer added in 2008 and tracer found in 2009, were calculated according to Providoli et al. (2005).

Soil samples (each pooled from 5 soil cores of 3 cm diameter) were taken at three different depths from each subplot (top soil: 0-15 cm, sub soil: 30-45 cm and 60-75 cm) in each chamber. To check the influence of water availability within the drought treatment, we took soil samples at the end of the 1st drought period in 2009 (June 30th), 13 days after re-watering started (only drought treated soils, July 14th) and at the end of the experiment in autumn 2009 (October 26th – November 5th). Because the subsoil was too dry, no samples could be taken from the two lowest sampling depths during and immediately after drought.
periods. Soil samples were freed from stones and organic debris, ground and sieved (< 1 mm). Organic and inorganic nitrogen of bulk soil samples were conjointly analysed for $^{15}$N and total N concentrations with the same instruments as used for plant samples. For measuring available soil NO$_3^-$ and NH$_4^+$ concentrations, fresh samples were extracted with 1 M KCl on the day of sampling according to Carter (2008). The extracts were analysed for NH$_4^+$ by means of flow injection analysis and detection by spectrophotometry (Perkin-Elmer FIAS-300, Waltham, Massachusetts, USA) at 589 nm. Nitrate was analysed using the method of Norman & Stucki (1981), as modified by Schleppi et al. (2012). The method involved two steps. In a first step the absorbance of NO$_3^-$ was measured conjointly with other, non-nitrate species in the extracts, using a Cary 50 UV-VIS spectrophotometer (Varian, Palo Alto, California, USA) at 215 nm. In a second step NO$_3^-$ was reduced using CuSO$_4$-coated zinc granules and the absorbance of the remaining non-nitrate species measured. Nitrate concentrations were then calculated from the differences in absorbance between the two steps. Soil organic carbon (C$_{org}$) was measured using the Swiss reference method for soil analyses (Eidg. Forschungsanstalt für Pflanzenbau FAP 1996). In this method, an excess amount of K$_2$Cr$_2$O$_7$ is used to oxidise the organic matter of a sample. The remaining K$_2$Cr$_2$O$_7$ is determined by means of an Fe$^{2+}$-solution containing a ferroin indicator. The difference between applied and remaining K$_2$Cr$_2$O$_7$ is used to calculate the C$_{org}$ content of the sample.

For determining microbial carbon (C$_{mic}$) and nitrogen (N$_{mic}$) at the end of the drying-rewatering cycles (19th October 2009), we used the method of Dannenmann et al. (2006). Two sets of soil samples, each representing a pooled sample from all three soil layers, were extracted with 0.5 M K$_2$SO$_4$ for 60 min at a soil:solution ratio of 1:2. One set of sub-samples was fumigated with chloroform in a vacuum/release purging-cycle desiccator for 24 h before K$_2$SO$_4$ extraction - the other not. After determination of total organic carbon (TOC) as the difference between total carbon and total inorganic carbon and of total N (N$_{tot}$) in the extracts,
microbial biomass C and N were calculated as the respective differences in TOC and total N between fumigated and non-fumigated samples. The differences were divided by the correction factors \( k_{\text{EN}} = 0.54 \) and \( k_{\text{EC}} = 0.38 \) to estimate the microbial biomass C and N values, as described by Dannenmann et al. (2006).

Drainage water was sampled from the bottom outflow of the lysimeters cumulatively at intervals of 3 weeks. After filtering (0.45 \( \mu \)m, ME25, Whatman, Maidstone, UK), the samples were analysed for NH\(_4^+\) using the same instruments as for soil-NH\(_4^+\) and for NO\(_3^-\) with ion chromatography (DX-120, Dionex, Sunnyvale, California, USA). Discharge samples collected between August and November 2008 were also analysed for total N, using chemiluminescence detection (Shimadzu Corporation, Kyoto, Japan). The labelled ions \( ^{15}\text{NH}_4^+ \) and \( ^{15}\text{NO}_3^- \) were captured together on glass microfibre filters (GF/F 25 mm, Whatman, Maidstone, UK), as described in detail by Providoli et al. (2005) and Schleppi et al. (2006), and analysed using the same instruments as for plant sample analysis. \( \delta^{15}\text{N} \) values of some reference water samples from the calcareous soil were higher before than after \( ^{15}\text{N} \) addition. It can be assumed that only low amounts of labelled \( ^{15}\text{N} \) were translocated into lower soil layers and into the drainage water in relation to background \( ^{15}\text{N} \), yielding negative tracer recovery values. For this reason, we present absolute \( \delta^{15}\text{N} \) values in the case of drainage water instead of calculated tracer fractions, i.e. background values were not considered.

5.2.3. Soil respiration

Soil respiration was measured several times in 2009 during the growing season at permanent docking cylinders (diameter = 10 cm) in each soil subplot using a 6400-09 soil CO\(_2\) flux chamber connected to an LI-6400 infrared gas analyser (both LI-Cor Bioscience Inc, Lincoln, Nebraska, USA). Each measurement was conducted three times in a row to average out short-term variations.
5.2.4. Statistical analysis

All statistical analyses were based on the statistical units “oak species” for plant analysis and on “soil type” for soil analysis. They were carried out using R 2.11.1 (R Development Core Team, Vienna, AT). Treatment and interaction effects were analysed using a multivariate ANOVA model and tested for a significance level of $P < 0.05$ (linear mixed-effect model), accounting for the split-plot design with two soils in each chamber. Concentrations were log-transformed and percentages arcsin-square-root-transformed before analysis in order to fulfil the assumptions of normal distribution. Significant differences between treatments, soils, species, depths and applied types of tracer were pair-wise tested using selected contrasts based on $t$-tests (Crawley 2009).

5.3. Results

5.3.1. Root and shoot biomass

Whereas the air-warming treatment had no significant effect on biomass (Kuster et al. 2012a), the drought treatment reduced tree biomass on average by about one third (Fig. 5.1). Biomass was more reduced above than below ground. As a result, the root:shoot biomass ratios increased under drought conditions. Generally, more biomass was produced below and above ground on acidic than on calcareous soil under well-watered conditions, but not in the treatments with drought (Kuster et al. 2012a). At the species level, no soil effect on biomass was found in the control treatment for the roots and foliage of $Q. \text{robur}$ and for the roots and stems + twigs of $Q. \text{pubescens}$. $Quercus \text{petraea}$ produced more biomass on the acidic than on the calcareous soil - also in the drought treatment. The biomass of $Q. \text{robur}$ was, in all treatments and on both soils, significantly higher than that of $Q. \text{petraea}$ and $Q. \text{pubescens}$. 
Fig. 5.1: Average coarse root, stem + twigs and foliage biomass (g tree$^{-1}$) of Quercus robur, Q. petraea and Q. pubescens on either acidic (aci) or calcareous soils (cal) at the end of the experiment, separately shown without (CO) and with drought (D) treatment (alone and in combination with air warming), n = 8. The air-warming treatment had no effect on the biomass of any plant part, neither under well-watered nor under drought conditions, and is therefore not shown separately. Error bars (± SE) refer to either root or shoot (stem + twigs and foliage) biomass. Different letters indicate significant differences (P < 0.05) between treatments/soils within the same plant part and species.

5.3.2. Soil respiration

Soil respiration was reduced by 60 to 70 % in both soils during the drought periods (Fig. 5.2). Considering that root biomass was reduced in average only by about 20% in the drought treatments (Fig. 5.1), this reduction in soil respiration can be attributed primarily to a decrease in microbial activity. After re-watering, soil respiration returned to control values within a few days, indicating a fast recovery of microbial activity. There was a small air-warming effect on soil respiration in the acidic, but not in the calcareous soil at the end of the first drought period in June 2009. The effect was opposite for treatments with and without drought, i.e. soil respiration was higher in the air-warming treatment (AW) than in the control (CO), but lower
Fig. 5.2: Average soil respiration (µmol CO₂ m⁻² s⁻¹) during the growing season in 2009 in either acidic (left) or calcareous soil (right), separately shown for the control (CO), and the treatments air warming (AW), drought (D) and the combination of air warming & drought (AWD), n = 4. Bold lines on the x-axis indicate periods when all chambers were irrigated. An asterisk * indicates a significant difference (P < 0.05), an asterisk in brackets (*) a tendency towards a difference (P < 0.10) between the two soils within the same treatment at a given time. Different letters indicate significant differences (P < 0.05) between treatments within the same soil.

in the combined air-warming/drought treatment (AWD) than in the drought (D) treatment alone. Soil respiration rates were on average 20% higher in calcareous than in acidic soils. This soil effect was especially pronounced in the AWD treatment. Given that there was more root biomass in the acidic than in the calcareous soil (Fig. 5.1), the higher soil respiration rate in the calcareous as compared to the acidic soil was likely due to enhanced microbial activity.

5.3.3. Nitrogen in soil and microbial biomass

In both soils, total N (Nₜₒₜ) concentrations were considerably higher in the top 15 cm than below at the end of the experiment in autumn 2009. Furthermore, Nₜₒₜ was higher (1.6 times) in the acidic than in the calcareous topsoil, whereas below 30 cm Nₜₒₜ was 1.7 times higher in the calcareous than in the acidic soil (Fig. 5.4). Nitrate concentrations were about twice as high in the upper 15 cm as at the lower sampling depths in the acidic soil, but did not vary significantly with depth in the calcareous soil (Tables 5.1 and 5.2). They were generally
N dynamics in model oak ecosystems subjected to air warming and drought

higher in the calcareous than in the acidic soil, especially towards the end of the growing season and at lower depths (Table 5.2). In contrast, there was no soil or depth effect on available NH$_4^+$. Neither air warming nor drought was found to influence N$_{tot}$, NH$_4^+$ or NO$_3^-$ concentrations. Furthermore, there were no differences in available NH$_4^+$ and NO$_3^-$ concentrations between the three sampling times (Table 5.2). Averaged over all treatments, the C$_{org}$:N$_{tot}$ ratio tended to be slightly higher in the acidic (15.8 ± 0.5) than in the calcareous topsoil (13.9 ± 0.7).

Also microbial C and N were not influenced by the air-warming and drought treatments (Fig. 5.3). While the difference between the two soils was small for C$_{mic}$ (P = 0.023), there was on average 1.5 times more N$_{mic}$ in the calcareous (15.9 mg kg$^{-1}$) than in the acidic soils (10.8 mg kg$^{-1}$, P = 0.005). In the AW treatment, there was even 2.5 times as much N$_{mic}$ in the calcareous as in the acidic soil. As the soil had a stronger influence on N$_{mic}$ than on C$_{mic}$, also the microbial C:N ratio differed between the calcareous (C:N=$35 ± 5$) and the acidic soil.

Fig. 5.3: Average microbial C (top) and N (below) concentration (mg kg$^{-1}$, ± SE, n = 4), separately shown for each treatment (CO: control, AW: air warming, D: drought, AWD: air warming & drought) in either acidic (left) and calcareous soils (right). An asterisk * indicates a significant difference (P < 0.05) between the two soils within the same treatment. The air-warming and drought treatments had no effect on microbial C and N concentrations.
Table 5.1: Main effects of soil (acidic vs. calcareous), drought (D, discontinuous vs. continuous irrigation), air warming (AW, elevated vs. ambient air temperature), applied nitrogen form (NO$_3^-$ vs. NH$_4^+$), species (Q. robur, Q. petraea and Q. pubescens), soil depth (0-15 cm, 30-45 cm and 60-75 cm) and selected 2-way interactions between factors (other interactions were not significant) on the N concentration (n = 4), tracer fraction (n = 2) and partitioning of applied tracer (n = 2) in soil and plant (fine root, stem + twigs and leaf) samples in autumn 2009. Bold numbers represent significant effects (P < 0.05) which are presented in Table 5.2, Fig. 5.4 and Fig. 5.6.

<table>
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<td>0.398</td>
<td>0.012</td>
<td>-</td>
</tr>
<tr>
<td>Soil*Depth</td>
<td>&lt;0.001</td>
<td>0.269</td>
<td>0.053</td>
<td>-</td>
</tr>
<tr>
<td>Soil*N-form</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 5.2: Mean NO$_3^-$ and NH$_4^+$ concentrations (mg kg$^{-1}$) at three different depths (0-15 cm, 30-45 cm and 60-75 cm) measured on three different dates (± SE, n= 8). Significant differences between respective depths in the same soil and at the same date are indicated by different letters. An asterisk (*) indicates a significant difference between acidic and calcareous soil for the same treatment and depth at a given date (P < 0.05). The factors date, drought and air warming had no significant effects on NO$_3^-$ and NH$_4^+$ concentrations (Table 5.1).

<table>
<thead>
<tr>
<th></th>
<th>NO$_3^-$ (mg kg$^{-1}$)</th>
<th>NH$_4^+$ (mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3^-$-N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO &amp; AW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-15 cm</td>
<td>1.7 ± 0.1 a</td>
<td>-</td>
</tr>
<tr>
<td>30-45 cm</td>
<td>* 1.2 ± 0.5 b</td>
<td>-</td>
</tr>
<tr>
<td>60-75 cm</td>
<td>* 0.9 ± 0.5 b</td>
<td>-</td>
</tr>
<tr>
<td>D &amp; AWD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-15 cm</td>
<td>2.2 ± 0.2</td>
<td>2.3 ± 0.4 a</td>
</tr>
<tr>
<td>30-45 cm</td>
<td>-</td>
<td>* 0.5 ± 0.3 b</td>
</tr>
<tr>
<td>60-75 cm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NH$_4^+$-N</td>
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</tr>
<tr>
<td>CO &amp; AW</td>
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<td></td>
</tr>
<tr>
<td>0-15 cm</td>
<td>1.9 ± 0.3</td>
<td>-</td>
</tr>
<tr>
<td>30-45 cm</td>
<td>1.6 ± 0.2</td>
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<tr>
<td>60-75 cm</td>
<td>1.7 ± 0.3</td>
<td>-</td>
</tr>
<tr>
<td>D &amp; AWD</td>
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<tr>
<td>0-15 cm</td>
<td>2.2 ± 0.3</td>
<td>2.2 ± 0.2</td>
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<tr>
<td>30-45 cm</td>
<td>-</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>60-75 cm</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>


(C:N=51 ± 8). The rather high microbial C:N ratios could indicate that standard correction factors k$_{EN}$ and k$_{EC}$ applied here were probably inappropriate for the investigated soil, i. e. there was a higher extraction efficiency for microbial C than for microbial N.

5.3.4. Nitrogen losses with discharge

The concentrations of NH$_4^+$ and NO$_3^-$ in the discharge from the lysimeters were low and generally close to the detection limit (Fig. 5.5B/C). When calcareous soils were intensively
watered after drought periods (grey bars in Fig. 5.5), NO$_3^-$ concentrations quickly peaked for a short time. No such effect was found in November 2008, when watering intensity was low and cold temperature inhibited microbial activity. In the discharge from the acidic soil, nitrate peaks were found only after the second drought in 2008. The re-watering effect on NH$_4^+$ concentrations was weak, and the fact that peaks in discharge from drought-treated soils were often paralleled by peaks in the discharge from well-watered soils indicated that these peaks were not due to re-wetting. After the first re-watering in 2009, the NH$_4^+$ peak appeared with a delay of one sampling interval (3 weeks) compared to the NO$_3^-$ peak, which is in line with stronger retention of NH$_4^+$ than of NO$_3^-$ in soil. The discharge from drought-treated soils was very low throughout the growing seasons (Fig. 5.5A), while nitrate and ammonium concentrations were of similar magnitudes as in the control treatments. This means that much less NH$_4^+$ and NO$_3^-$ was leached from the drought treated than the control lysimeters. Approximately 4 times more inorganic N was leached over the entire measuring period (July 2008 - November 2009) from calcareous (25 mg m$^{-2}$ NH$_4^+$-N + 18 mg m$^{-2}$ NO$_3^-$-N) than from

**Fig. 5.4:** Average nitrogen concentration (g kg$^{-1}$, n = 8) and average tracer fraction (µmol mol$^{-1}$, n = 4) ± SE at the end of the experiment in soil (organic and inorganic N conjointly analysed), fine roots, stem + twigs and leaves either in/on acidic (left) or calcareous soils (right), separately shown for each species (Qrobur: Q. robur, Qpetraea: Q. petraea and Qpubesc: Q. pubescens), depth (0-15 cm, 30-45 cm and 60-75 cm), type of applied tracer ($^{15}$NO$_3^-$ vs. $^{15}$NH$_4^+$) with and without drought treatment (alone and in combination with air warming). An asterisk * indicates a significant difference (P < 0.05), an asterisk in brackets (*) a tendency towards a difference (P < 0.10) between well-watered and drought-treated samples for the same species/depth. An asterisk * in the line between $^{15}$NO$_3^-$ and $^{15}$NH$_4^+$ tracer fractions indicates a significant difference between the applied N forms. S indicates a significant difference (P < 0.05), and $ a tendency towards a difference (P < 0.10) between the two soils for the respective combination of species or depth and type of applied tracer. Different lowercase letters indicate significant differences between depths or species in the control treatment on the same soil, different uppercase letters differences in the drought treatment. There was no air-warming effect on any of the presented parameters (Table 5.1).
acidic soil ($7 + 3$ mg m$^{-2}$) in the drought treatments and even 9 times more in the treatments without drought ($58 + 82$ mg m$^{-2}$ vs. $12 + 4$ mg m$^{-2}$). Total amounts of N losses with discharge, including inorganic and organic N, were determined only for the period between August and November 2008. The respective treatment averages ranged from 7 (D & AWD) to 33 mg m$^{-2}$ (CO & AW) for the acidic soil and from 6 to 108 mg m$^{-2}$ for the calcareous soil for this period. The total amount of N leaching averaged four times that of inorganic N. Extrapolating these rates to the entire study period from July 2008 until November 2009, total N losses with leaching did not exceed more than a few grams per m$^{2}$. The air-warming treatment had no effect on the N concentrations of the drainage water.

5.3.5. Total N concentration in fine roots, stem + twigs and leaves

Air warming also had no effect on biomass N concentrations (Table 5.1). The drought treatment increased the N concentration of fine roots in acidic soil and of stem + twigs on both soils (Fig. 5.4), but had no effect on leaf N concentrations. The N concentration of fine roots was 1.2 times higher in calcareous than in acidic soil under well-watered conditions (Fig. 5.4). There was a trend towards higher aboveground biomass N concentrations on acidic than on calcareous soils. In the absence of drought stress, N concentrations were on average higher in $Q. petraea$ than in $Q. pubescens$ and $Q. robur$, especially in the aboveground biomass (Table 5.1, Fig. 5.4).

5.3.6. $^{15}$N tracer fractions in drainage water, soil, and tree biomass

The $\delta^{15}$N values of the drainage water were analysed together for NH$_4^+$ and NO$_3^-$. In discharge from acidic soil, $\delta^{15}$N decreased during the growing season 2008 and then increased again after leaf senescence in October 2008 (Fig. 5.5D). The initially high values in the drainage water can likely be explained by a $^{15}$N-pulse after the start of the labelling experiment. Later, the values in the drainage water decreased, as $^{15}$N in the soil solution was
presumably taken up by roots. After leaf senescence, uptake of $^{15}$N decreased, and therefore more $^{15}$N was leached in autumn. In contrast, $\delta^{15}$N fluctuated around zero without a clear trend in the discharge from calcareous soil. Most likely, background $^{15}$N values interfered with $^{15}$N values from the labelling experiment.

Two growing seasons after application, there was still little $^{15}$N tracer below 30 cm depth in both soils (Fig. 5.4). This means that only a small amount of the applied tracer was translocated into deeper soil layers, which is consistent with the very low N concentrations of the drainage water. At the uppermost sampling depth, the form in which the tracer was applied had the opposite effect on the $^{15}$N fractions in both soils: In the acidic topsoil, tracer

![Fig. 5.5: A) Average drainage volume (l m$^{-2}$), B) average NO$_3^-$ concentration, C) average NH$_4^+$ concentration and D) average $\delta^{15}$N values (‰) in the drainage water from 2008 until 2009, from either acidic (left) or calcareous soil (right), all n = 8. The type of applied tracer and the air-warming treatment had no effect on the presented parameters. Bold lines on the x-axis indicate periods when all chambers were irrigated, grey bars indicate the intensive re-watering periods with which the two drought periods in each growing season were ended.](image)
fractions were about twice as high when $^{15}$NO$_3^-$ was applied than in the application of $^{15}$NH$_4^+$. In contrast, tracer fractions were higher in the calcareous topsoil when the tracer was applied as $^{15}$NH$_4^+$ compared to $^{15}$NO$_3^-$. Comparing the two soils, higher tracer fractions were recovered in the upper 15 cm of the calcareous soil than in the acidic topsoil when $^{15}$NH$_4^+$ was used as a tracer, whereas there were no differences between the two soils for the $^{15}$NO$_3^-$ tracer. Neither AW nor D affected the abundance and distribution of $^{15}$N in soil (Table 5.1).

The differences between the two soils in total N and $^{15}$N tracer concentrations did not translate into aboveground $^{15}$N accumulation by the trees (Table 5.1). The only significant soil effect was that $^{15}$N tracer fractions were higher in fine roots in calcareous than in acidic soils (Fig. 5.4). Also AW had no effect on tracer fractions in the biomass, whereas D significantly decreased the accumulation of $^{15}$N when it was applied as $^{15}$NH$_4^+$ (Fig. 5.4). Pair-wise comparisons revealed that this drought-induced decrease in the accumulation of $^{15}$NH$_4^+$-applied tracer N was particularly strong in $Q.$ petraea. Drought had no significant effect on $^{15}$N accumulation when the tracer was applied in form of nitrate. The fractions of $^{15}$N accumulated in the biomass were generally higher after application in form of $^{15}$NO$_3^-$ than in form of $^{15}$NH$_4^+$. These differences between application forms were particularly pronounced in the drought treatments and on the calcareous soil. There were significant species differences in the $^{15}$N fractions of the aboveground biomass, but not in the roots. In general, lower tracer fractions were found in $Q.$ robur than in $Q.$ petraea and $Q.$ pubescens (Fig. 5.4), especially under well-watered conditions and on calcareous soils. This species effect was more pronounced for $^{15}$NH$_4^+$ than for $^{15}$NO$_3^-$ tracer application.

5.3.7. $^{15}$N recovery

With an estimated bulk density of 1.35 t m$^{-3}$, the total mass of soil was about 500 times larger than the biomass of the trees harvested at the end of the experiment. As a consequence,
Fig. 5.6: Average total recovery rates (% ± SE, n = 4) after two growing seasons and partitioning of the applied \( ^{15}NH_4^+ \) and \( ^{15}NO_3^- \) tracers found in soil (approximation of the mass), root (approximation from fine root samples), stem + twigs and leaf biomass under well-watered and drought conditions, separately shown for acidic and calcareous soil. The air-warming and drought treatments and the soil had no effect on the total recovery rates (Table 5.1).

More \( ^{15}N \) was recovered from the soils (between 32 and 72%), despite their low tracer fractions, than from the biomass of the trees (6 to 29%, Fig. 5.6). Overall, lower total recovery rates were obtained for tracer applied as \( ^{15}NH_4^+ \) (average over all treatments: 55%) than for tracer applied as \( ^{15}NO_3^- \) (79%, Fig. 5.6). On average, our tracer recovery rates are consistent with those of other N-labelling studies of forest soils. While there was no air warming, drought or soil effect on total \( ^{15}N \) recovery, there was a drought effect on the total amount of tracers recovered from the biomass of the trees. The biomass of drought-treated trees was about one third lower than that of well-watered trees in 2009 (Fig. 5.1), and given that tracer fractions were similar in drought and well-watered trees, the total tracer mass taken up by drought-treated trees was thus lower than uptake by well-watered trees. In line with this
difference, a larger fraction of recovered $^{15}$N rather remained in drought-treated than in control soils.

5.4. Discussion

5.4.1. N status of the experimental trees

Although soil N concentrations were in the range of most forest soils in Europe (Vanmechelen et al. 1997), leaf N concentrations (14 to 17 g kg$^{-1}$) were below the N sufficiency level given as 20 to 30 g kg$^{-1}$ for oaks by Bergmann (1993). They were also lower than leaf N concentrations reported in other oak studies: 21 g kg$^{-1}$ in 15-to-25-years-old Q. pubescens trees (Tognetti et al. 1998), 22 and 24 g kg$^{-1}$ in 2-years-old Q. petraea and Q. robur seedlings, respectively, grown on artificial soil (Thomas & Ahlers 1999), and 21 – 22 g kg$^{-1}$ in 85-year-old Q. petraea and Q. robur trees (Karolewski et al. 2005). However, the narrow C$_{org}$:N$_{tot}$ ratios in the topsoils of our study indicate that organic matter decomposition was not limited by N availability. Thus, we suggest that N supply through microbial N mineralisation was not a major limitation for tree growth, despite low soil N concentrations. Based on the results of Luster et al. (2008), who studied N leaching in a prior experiment using the same soils and lysimeters as our study, we can safely assume that initial soil N mobilisation effects due to disturbances in soil structure associated with the filling of the lysimeters had levelled out well before our study started and thus played no further role in our experiment.

5.4.2. Air-warming effects

The lack of air-warming effects in this study is in contrast to our hypotheses. Despite the small temperature difference between air-warming and control treatment and the apparently rather good supply of soil N for plant uptake, some effects on soil N turnover
processes might nonetheless have been expected in the air-warming treatments. This was in fact the case in a Mediterranean shrubland study in which Sardans et al. (2008a) increased air and soil temperatures by about 1 °C over 6 years and found a significant increase in the concentration of soil NO$_3^-$ during the summer and autumn, while there was only a minor effect on soil NH$_4^+$ and no effect on N$_{tot}$. Small, but measurable warming effects were also found in other studies, as reviewed by Rennenberg et al. (2009), Rustad et al. (2001) and Saxe et al. (2001). On the other hand, also several studies were cited in these reviews that did not find any temperature effects on soil N processes. Thus, we may conclude that more than marginal warming effects on forest soil N pools will in general require a temperature increase that substantially exceeds 2 °C.

5.4.3. Drought effects

Also the drought treatment had no significant effect on any of the soil N parameters studied, although the substantially reduced soil respiration indicated strongly inhibited microbial activity. While the air warming treatment was mild, the drought treatment was drastic compared to climate change scenarios (CH2011 2011; IPCC 2007; Schär et al. 2004). The fact that there were no lasting drying-rewetting effects on soil respiration and microbial C and N, although the treatments had been applied during three consecutive growing seasons, indicated that the microbial communities in the experimental soils were well adapted to drought stress. The nitrate pulses in the discharge after re-wetting indicated that still a large fraction of the microbial biomass did not survive and was mineralised as soon as soil moisture conditions improved again (Borken & Matzner 2009; Dannenmann et al. 2009). In line with our findings, also Pesaro et al. (2004) reported that microbial activity recovered from drought within 6 days after re-wetting, but also that some microbial groups remained depressed for a longer time. While microbial activity may recover quickly from short-term disturbances, there
may still be significant long-term effects if drought and re-wetting cycles occur repeatedly over many years (Johnson et al. 2002; Johnson et al. 2008; Sardans et al. 2008b).

Drought treatment was sufficiently severe to cause a substantial reduction in the growth of the experimental trees. The fact that N concentrations in the biomass of the trees did not decrease but rather increase with drought indicates that N was not the limiting factor for tree growth under drought. Additionally, the lack of drought effects on soil N in our results is in line with this conclusion if we assume that the N demand of the plants was reduced at least as much as the availability of N in the soil. Our results are comparable to those of Sardans et al. (2008b) who found that water limitation increased N accumulation in Q. ilex roots by 22 % but did not reduce it in leaves and aboveground wood biomass. Also Broadmeadow & Jackson (2000) found no drought effect on leaf N in Q. petraea, while Picon et al. (1996) found that leaf N concentrations of drought-treated trees were lower than those of well-watered Q. robur trees, indicating increased growth limitation due to insufficient N supply.

5.4.4. Nitrate vs. ammonium uptake

The fact that 15N accumulation by the trees was reduced under drought conditions when the tracer had been applied as 15NH4+, but not after application of 15NO3-, suggests that N was taken up primarily in the form of nitrate, in agreement with findings of Berger & Glatzel (2001) and Schulz et al. (2011). Even if nitrification transformed a substantial fraction of the applied 15NH4+ into nitrate, it is plausible that a larger fraction of the tracer remained in the form of ammonium in the drought treatment than in the control. Although we found no significant drought effects on soil 15NH4+ and 15NO3− concentrations, nitrification may have been inhibited over shorter periods. In agreement with Berger & Glatzel (2001) and in line with the enhanced microbial activity observed in the calcareous soil, N uptake in form of NO3− was more important on the calcareous than on the acidic soil.
Summarizing, the findings agree with our hypothesis that N uptake by oak trees is more responsive to nitrate than to ammonium availability. However, oaks may not only depend on the supply of inorganic N forms. Stoelken et al. (2010) observed that uptake of NO$_3^-$ and NH$_4^+$ by *F. sylvatica* was reduced and uptake of amino acids increased with rising N availability in an organic form. Possibly, oak trees can respond in a similar way to increased availability of dissolved organic soil N and reduced availability of mineral soil N.

### 5.5. Conclusion

Our results suggest that central European oaks will suffer less from climate change effects on soil N availability and uptake than from drought-induced growth reduction. As our model ecosystem experiments were performed with plantations of young trees, the results may apply only to a reforestation situation. However, given that mature trees generally have a more developed rooting system for nutrient and water uptake and larger nutrient storage capacities than saplings, mature oak stands may be even less sensitive to changes in N availability under the predicted future climate conditions.

### Acknowledgements

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6. Synthesis

6.1. General conclusions

6.1.1. Drought effects

The drought treatment applied in the model ecosystem experiment was strong compared to the IPCC climate change Scenario A2 (CH2011 2011; IPCC 2007). As a result, root, stem and foliage biomass were significantly reduced after three growing seasons. However, none of the trees died during the experimental period, although predawn leaf water potentials were very low during drought periods (-3 to -4 MPa), and many drought-stressed trees showed leaf injuries (Günthardt-Goerg et al. 2012). With soil re-wetting, evapotranspiration and photosynthesis recovered within a few days after the end of a drought period (Arend et al. 2012).

The impressive capability to survive droughts can be related to xeromorphic leaf structures (Günthardt-Goerg et al. 2012; Kubiske & Abrams 1993) as well as to adaptive responses in the allocation of growth. The vertical distribution of root mass allocation showed a shift to lower depth under drought conditions, where soil water was still more available. Furthermore, biomass was less reduced below than above ground, leading to an increased root:shoot mass ratio. This response also occurs in other species but is generally less pronounced in more drought-sensitive species such as whitebeam (Sorbus aria), ash (Fraxinus excelsior) and beech (Fagus sylvatica) (Broadmeadow & Jackson 2000; Thomas 2000a). This suggests that relatively more assimilates were invested into parts required for water and nutrient uptake than into the parts required for photosynthesis and assimilation, in order to balance water supply with losses through transpiration under conditions of decreased availability. The fact that we found only a minor reduction in fine root mass in drought-
treated trees can be interpreted along the same lines. Also Thomas (2000a) and Fotelli et al. (2000) found that drought affected fine root mass less than that of coarser roots in *Quercus petraea* and *Q. pubescens*.

Whereas root-shoot growth patterns in oaks under well-watered conditions have been measured already in other studies (Harmer 1990; Pages & Serra 1994; Reich et al. 1980; Willaume & Pages 2006), we evaluated for the first time the influence of water and nutrient availability on these patterns in an additional neutron radiography experiment. The synchronous growth of shoot and root was, however, neither influenced by drought nor by soil properties as peaks and dips of shoot and root growth rates occurred at the same time in both water treatments and soil types. Therefore, we conclude that during the first year after germination environmental conditions were overruled by endogenous growth patterns. With high amounts of nutrients and assimilates stored in the acorns, there is no advantage in alternating root and shoot growth, whereas it is important to quickly develop capacities for both water and nutrient uptake as well as for photosynthesis and carbon assimilation. Similar growth patterns have also been found for first-year seedlings of *Q. robur* by Harmer (1990) and Pages & Serra (1994). We cannot rule out that external factors as water and nutrient availability may come into play at later stages, when energy and nutrient reserves stored in the acorns are depleted. For example Reich et al. (1980) reported for two-year-old *Q. alba* saplings alternating patterns of root and shoot growth, indicating root-shoot competition for assimilates.

Due to reduced evaporative cooling the drought treatment alone had a similar effect on air and soil temperatures as the air-warming treatment without drought, and an added temperature effect in combination with the air-warming treatment. This finding shows that potentially substantial feedback effects from changes in soil moisture on temperatures must be taken into account in climate change scenarios, as discussed by Seneviratne et al. (2006),
Fischer et al. (2007), Seneviratne et al. (2010) and Hirschi et al. (2011). Forests are known to mitigate heat waves by evaporative cooling (Renaud et al. 2010; Renaud & Rebetez 2009). By reducing soil water availability, droughts decrease this capacity for temperature regulation.

In contrast to our expectations, drought effects on nitrogen (N) availability and uptake were minor. Although microbial activity was reduced during drought periods, microbial biomass and concentrations of inorganic soil N were not different from the control treatment after three growing seasons with periodic droughts. Moreover, soil microbial activity recovered within few days, indicating that not only the oak trees, but also the entire ecosystem was well adapted to low soil moisture. Nitrogen concentrations in the tree biomass increased with droughts. As a highlight of this study, we therefore report that soil N supply became not a limiting growth factor under low water availability. Prolonged drought-rewetting cycles over several decades might reduce nitrate availability by depressing the abundance of nitrifiers in soil microbial communities, because nitrifiers are among the most drought-sensitive micro organisms (Borken & Matzner 2009; Schimel et al. 2007). A higher reduction in nitrate compared to ammonium availability may negatively influence the growth of oaks, as they seem to prefer nitrate in comparison to ammonium uptake.

When discussing the relevance of these results for forestry under climate change conditions, it must be kept in mind that the experiments were performed with young oak trees. The results do not necessarily apply also to adult trees, although we believe that mature oaks are even less vulnerable to drought, as they can tap into even deeper water reservoirs with their more developed taproots (Hanson et al. 2001; Leuzinger et al. 2005). Furthermore, it must be considered that our experiments were performed at the current ambient atmospheric CO$_2$ concentration, because treatments with artificially elevated atmospheric CO$_2$ levels were beyond the possibilities of the available financial and infrastructural resources. As water use
efficiency is generally increased at elevated CO\textsubscript{2} concentrations, we may expect that reduced precipitation will result in less severe drought stress than under current CO\textsubscript{2} levels (Bucher-Wallin et al. 2000; Leuzinger & Körner 2007). Thus, we believe that oaks are well adapted to cope with the increasing frequency of drought and heat spells predicted by climate change scenarios. They will be able to occupy the expanding areas of arid niches that are too dry for other trees such as beech or spruce (Leuschner et al. 2001a; Schumweber et al. 2011; Thomas 2000a; van Hees 1997). Even the rather drought-tolerant Scots pine is expected to be replaced by \textit{Q. pubescens} in very dry regions (Wohlgemuth 2006). On the one hand, Scots pine is known to be slightly more drought-sensitive than \textit{Q. pubescens} (Poyatos et al. 2008; Weber et al. 2007), on the other hand, attacks by mistletoe, beetles and pathogens were found to increase with higher air temperatures and drought in particular in \textit{Pinus sylvestris}. (Dobbertin et al. 2005; Rebetez & Dobbertin 2004). Moreover, historical forest management practices, which favoured the light demanding Scots pine during the last centuries for example in the Swiss Rhone valley, were discontinued (Rigling et al. 2006).

6.1.2. Air-warming effects

The air warming treatment increased air temperatures by 1 - 2 °C during daytime in the growing season. This increase had almost no effects on ecosystem water regime and tree growth. While tree height was slightly increased by the additional warming (Arend et al. 2011), there was no air-warming effect on stem biomass, because tree diameter was reduced at the same time. Surprisingly, air-warming had also no effect on water losses through evapotranspiration and on foliage biomass, while net photosynthesis and transpiration were only marginally increased (Arend et al. 2012). As another highlight of our results, we would like to emphasise that the air-warming treatment also did not exacerbate the effects of the drought treatment when both treatments were applied in combination.
The air-warming treatment increased topsoil temperatures by 0.5 to 1.0 °C, which led to a slight increase in soil microbial activity, but did not affect soil N availability. With no effects on tree growth and soil N availability, the air-warming treatment also did not affect the N nutrition status of the trees. We conclude that the air-warming treatment should have been more drastic to produce measurable effects.

6.1.3. Interaction of soil types with climate treatments

We found in the present experiment more water consumption and tree growth on the acidic than on the calcareous soil. This is in agreement with the experimental study conducted by Burger (1931) who reported higher growth and leaf vitality on soils with a low calcium concentration than on soils with a high calcium concentration. Moreover, the oak’s preference for acidic soil is also reflected by indicator values for soil reaction given by Landolt & Bäumler (2010). As water supply, light conditions and temperature were the same for both soils, another factor must have limited tree growth on the calcareous soil. The higher root-to-shoot ratio of trees growing on the calcareous than on the acidic soil suggests that the calcareous soil was deficient in one or more nutrients. The availability of inorganic N was not lower but rather higher in the calcareous than in the acidic soil, although total N concentrations were higher in the acidic soil; and leaf N concentrations did not differ between trees grown on the two soils at the end of the experiment. Thus we conclude that another nutrient than N was responsible for the reported differences in oak growth between these two soils.

We suspect that low solubility of phosphate or manganese, or of both together, were responsible for the reduced growth on the calcareous soil. These were the only nutrients with higher availability in the acidic than in the calcareous soil. Taking the relationship between the ratios of nutrient concentration to nutrient content in the leaf biomass of trees grown on
the acidic and trees grown on the calcareous soil as an indicator of nutrient deficiency, following the concept proposed by Salifu & Timmer (2003), both P and Mn could have limited tree growth on the calcareous soil in 2008 and 2009 (Fig. 6.1). Furthermore, with increasing distance to the reference, this deficiency in P and Mn increased from season to season. Averaged over all treatments and provenances, leaf Mn concentrations in 2009 (23 mg kg$^{-1}$) were clearly below deficiency levels given for oak leaves by Bergmann (1993; 35 - 100 mg kg$^{-1}$), whereas P concentrations in 2009 (2.97 g kg$^{-1}$) almost reached the threshold of sufficient supply (1.5 - 3 g kg$^{-1}$). In line with this, the distance between reference and symbols displayed in Fig. 6.1 indicate a much larger difference between the two soils in Mn than in P availability. Mn deficiency has been reported to limit growth in oaks growing on alkaline soils by (Messenger 1986; Thomas et al. 1998). It is not likely, however, that differences in nutrient uptake can explain also the observed differences in growth among species and provenances. A preliminary inspection of the data revealed no consistent differences in nutrient uptake between provenances and species.

There were no differences in growth and evapotranspiration between trees growing on the two soils under drought conditions. As a major finding of this study, this means that there was no cumulative effect of drought and nutrient limitation. Correspondingly, the distance between reference and symbols in Figure 6.1 indicated less P and Mn deficiency than for well-watered conditions. Thus, we would like to highlight that the choice of soil may be less important for the cultivation of oaks in a future forestry than today, if drought conditions prevail. Furthermore, these findings show that soil properties have to be considered studying drought effects on oaks, as the relative reduction in growth can be very different on different soils.
General conclusions

Fig. 6.1: Oak leaf P (left panel) and Mn (right panel) concentrations (amount of leaf nutrient per mass unit, y-axis) vs. contents (total amount of nutrient in the foliage biomass of a tree, x-axis) for treatments without (CO & AW, filled symbols) and with droughts (D & AWD, open symbols) in the experimental years 2007 (rhomboids), 2008 (upward triangles) and 2009 (downward triangles). Concentrations and contents of each combination of treatment and experimental year on calcareous soil were normalised to 1 (grey square). For each of these combinations of treatment and experimental year, the shift of concentrations and contents on acidic soil in relation to these references is shown with a different symbol. The different species and provenances on each soil compartment were pooled before analysis (n = 8). A symbol laying right of the dashed line indicates that trees on the calcareous soil were insufficiently supplied with the respective nutrient compared to trees on the acidic soil, whereas a symbol left of the dashed line indicates excess uptake. Distance between reference and symbol reflects the magnitude of the difference between calcareous and acidic soil, including interaction effects of the experimental year and drought treatment. A detailed explanation of this concept is given by Salifu & Timmer (2003).

6.2. Which oak species/provenance is the future forest tree?

Irrespective of climate treatment and soil, Q. robur always produced more biomass than Q. petraea and Q. pubescens. However, the differences in growth between these species were smaller under drought conditions than in absence of drought stress, indicating that Q. robur
was more sensitive to soil water availability. This finding is in line with the results of other studies in the “Querco” project. Thus, *Q. petraea* and *Q. pubescens* appeared to be more drought-tolerant than *Q. robur* also with respect to photosynthesis (Arend et al. 2012) and stress-induced leaf injuries (Günthardt-Goerg et al. 2012). Comparing wood anatomical (Fonti et al. 2012) and leaf N responses (Hu et al. 2012), the highest drought tolerance among the three species was found in *Q. pubescens*. *Q. robur* proved to be less drought-tolerant than in the other oak species furthermore in the studies of Scharnweber et al. (2011) and Thomas et al. (2002). Therefore, we consider *Q. robur* the most promising species among these three oaks for sites where drought conditions remain comparatively mild, whereas *Q. petraea* and *Q. pubescens* are better suited for sites with long and severe drought periods. As demonstrated by the increasing abundance in the Swiss Rhone valley (Brändli 2010; Wohlgemuth 2006), *Q. pubescens* is the oak species that is best suited on the driest and warmest sites in Central Europe.

In all three oak species, provenances originating from more xeric sites produced more biomass than provenances from more humid sites, irrespective of climate treatment and soil. The conclusion that foresters therefore should always prefer provenances from drier sites may be premature, however, as provenances from drier sites reacted more sensitive to water limiting conditions than those from more humid sites. As demonstrated by the example of the Italian provenance Arezzo in our study, there may be major deviations from the general trend, indicating that also other factors need to be taken into account. Furthermore, provenances from warmer and drier sites might be more at risk of late frosts (Jensen & Deans 2004) as they are also expected to increase in frequency with climate change (IPCC 2007).

In conclusion, we demonstrated for the first time by means of controlled experiments and the use of two different soils that young saplings of all three central European oak species can successfully cope with drought and increased air temperatures. Therefore, we can expect
that their high drought-tolerance will enable them to occupy the increasing area of sites that are currently still dominated by other forest tree species, in particular beech, but will become too xeric for them in the future. Although all oak species are more drought-tolerant than beech and spruce, differences in ecophysiological adaptations between species and provenances should be taken into account when choosing a suitable oak genotype for planting on a specific site.

6.3. Outlook

While many questions about the ability of oaks to cope with climate change could be answered in the “Querco” project, the following are major questions that remained open:

(i) _Performance of oak in competition with beech and spruce_

While it is known that oaks are more tolerant to drought than beech and spruce, it would be interesting to determine below which thresholds of water availability oaks become more competitive than beech or spruce and how these thresholds depend on site factors.

(ii) _Effect of drought on wood quality_

Whereas the results of this study clearly confirm the ability of oaks to sustain even severe droughts, effects on wood quality were not investigated because the seedlings studied here are not representative for trees at harvest. In forestry, wood quality is a very important aspect, however.

(iii) _What is the optimum temperature of oak ecosystems?_

The mild air-warming treatment in the “Querco” experiment had only minor effects on tree growth, water consumption and N nutrition status. As temperatures
are likely to increase more than in this treatment with climate change, it would be interesting to investigate how higher temperatures will affect these processes in oak ecosystems.

(iv) Uptake of nitrogen in organic form

The concentration of organic N in form of amino acids and peptides is expected to increase, whereas inorganic N availability may decrease under water limiting conditions. While it is known that beech is able to directly take up amino acids, no studies have been done on oaks so far. Uptake of amino acids or peptides could be a way to mitigate possible inorganic N limitations under drought conditions.

(v) Frost tolerance

Provenances from more xeric sites seem to be predestined for future forestry. However, there is not much known about their frost tolerance. Future experiments should therefore investigate the relationships between adaptations to drought stress, heat tolerance and frost sensitivity.
References


References


Appendix

Other manuscripts of the “Querco” project


Kuster T.M., Vontobel P., Günthardt-Goerg M.S., Schulin R. (in preparation) Root and shoot growth patterns of 1st-year *Quercus robur* seedlings as affected by drought conditions (chapter 4)


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