

Effects of climate and forest management on the
water and nitrogen status of European beech
regeneration and understorey vegetation

Inaugural-Dissertation zur
Erlangung der Doktorwürde
der Forstwissenschaftlichen Fakultät
der Albert-Ludwigs-Universität
Freiburg i. Brsg.

vorgelegt von

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Freiburg im Breisgau
2002

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Tag der Bekanntgabe des Prüfungsergebnisses: 19.06.2002

To my family

Contents

Synopsis.....	5
Zusammenfassung.....	7
1. Introduction and aims of the study.....	7
2. N status and growth of beech seedlings	14
2.1 Parameters describing the N status.....	14
2.2 Changes in N metabolism and N uptake due to climate and thinning affect growth of beech seedlings.....	15
2.3 Competitive interference and climate: effects on the N status of beech seedlings	17
2.4 Conclusions	19
3. Water status: effects of climate and thinning.....	21
3.1 Update on the assessment of plant water status.....	21
3.2 Effects of water shortage on water status and competitive ability of beech seedlings.....	23
3.3 Climate and forest management: insights from $\delta^{13}\text{C}$ and sap flow measurements.....	26
3.4 Conclusions	28
4. References.....	29
5. Annex.....	38
6. Acknowledgements.....	39

Synopsis

Human activities result in global climate changes that have various impacts on forest ecosystems. An increase in global surface temperature, compared to pre-industrial levels, is already recorded and further elevation is expected. Extreme events, such as hot and dry periods, would increase in frequency and duration in near future. Due to the relatively low adaptive capacity of forest ecosystems to rapid climate changes, high priority is given to the assessment of their responses. Particularly, studies on forests dominated by the drought susceptible European beech, especially when grown on limestone-derived soils with low water storage capacity, being common in Central and Southern Europe, are of major importance. Moreover, climate models recently emphasize the significance of management practices for the adaptation to the expected changes, since they could contribute to the mitigation of possible adverse effects of climate change.

The present study was aimed at assessing the effects of different climatic conditions on the nitrogen and water status of natural beech regeneration grown in managed (thinned) and not managed (control) stands in a forest in Southern Germany. A SW-exposed site, characterized by low water availability and high temperature and radiation interception, was regarded as representative of the climate conditions expected in the future, due to climate change, whereas a NE-exposed site was used as typical of present climatic conditions. In parallel, greenhouse-experiments conducted under controlled conditions enabled an in depth assessment of the effects of thinning-induced increases in understorey vegetation density on growth of beech regeneration.

The results of this study showed that limited water availability and global warming could result in a suboptimal nitrogen status of beech regeneration. Such climatic conditions mediate low soil N acquisition and force beech seedlings to depend greatly on stored N for new tissues development. Furthermore, N uptake rates and growth are reduced. Simultaneous thinning favors the N status of beech regeneration under the current climatic conditions but would impair it under the expected future climate by intensifying drought. Thinning-induced increased interference by fast growing early successional species can further impair the N status of beech seedlings since they are drought-resistant and able to obtain the available N resources from the soil even when exposed to severe drought.

A high susceptibility of the water status of beech regeneration to decreasing soil water resources has been observed. Under such conditions, application of thinning further impaired the water status by increasing temperature and solar radiation and, thus, inducing plant water loss; still, a more conservative water use of beech seedlings under lower water availability cannot be excluded. Greenhouse experiments showed that under severe water shortage, interference by early successional understorey plants can result in further water depletion of beech seedlings, but clear-cut effects of understorey vegetation on the water status of beech could not be concluded from field studies. Differences in the water status of various understorey plants were rather vegetation type-specific.

Consequently, thinning favours the N and water status of beech regeneration under the current climatic conditions. However, if prolonged drought periods and warming occurs in near future, application of this practise could intensify the reverse effects of such suboptimal conditions and impair the establishment and growth of natural regeneration in European beech forests grown on limestone.

Zusammenfassung

Die globale Klimaveränderung (Global Change), welche durch die vermehrte Freisetzung klimarelevanter Spurengase als Folge menschlichen Handelns verursacht wird, äußert sich im Wandel unterschiedlicher Klimaparameter, die unterschiedliche Effekte auf Waldökosysteme haben können. Zum einen ist eine Zunahme der globalen Oberflächen-Temperatur - im Vergleich zum vorindustriellen Niveau - bereits eingetreten und weitere Zunahmen werden erwartet. Die Häufigkeit und die Dauer von extremen Witterungsereignisse, wie wärmer und trockener Perioden, werden in näherer Zukunft zunehmen. Auf Grund der langen Generationszeiten von Waldbäumen wird angenommen, dass Waldökosysteme eine nur geringe und langsam ablaufende genetisch bedingte Anpassungsfähigkeit an sich ändernde klimatische Rahmenbedingungen besitzen. Folglich sind Untersuchungen der physiologischen Reaktionsfähigkeit von Bäumen auf ein sich veränderndes Klima von entscheidender Bedeutung. Dies gilt insbesondere für Laubwälder, die von der trockenheits-empfindlichen Buche dominiert werden, und deren Verbreitungsgebiet durch Böden vom Rendzina-Typ mit begrenzter Wasserspeicherungskapazität – wie sie in Mittel- und Südeuropa häufig vorkommen – charakterisiert sind. Weiterhin ist das Wissen um die Auswirkungen von Bewirtschaftungsmaßnahmen auf die Anpassungsfähigkeit der Naturverjüngung an die prognostizierten Änderungen der Klimaparameter auf Ebene der Physiologie des Wasser- und Nährstoffhaushalts von entscheidender Bedeutung, da diese Maßnahmen eingesetzt werden könnten, um negative Effekte des Global Change auf die Vitalität von jungen Bäumen zu verringern.

Ziel dieser Arbeit war es, die Effekte unterschiedlicher (meso)klimatischer Bedingungen auf den Stickstoff- und Wasserhaushalt der Buchenaturverjüngung, in bewirtschafteten (Schirmhieb) und nicht bewirtschafteten Beständen eines Buchenwalds in Süd-Deutschland, zu untersuchen. Ein SW-exponierter Hang, der durch limitierte Wasserverfügbarkeit und – im Vergleich zu dem unten charakterisierten NO-Hang – erhöhte Temperatur und Strahlung charakterisiert ist, wurde als Standort, der repräsentativ für die klimatischen Bedingungen, die in der Zukunft als Folge der Klimaveränderung zu erwarten sind, definiert. Ein kühl-feuchter NO-exponierter Hang, konnte als typisch für die derzeitigen klimatischen Bedingungen für Kalkbuchenwälder betrachtet werden.

Die Ergebnisse dieser Arbeit zeigten, dass sich die limitierte Wasserverfügbarkeit und die Erhöhung der Luft- und Bodentemperatur, wie auf dem SW-Hang

beobachtet, ungünstig auf den Stickstoffhaushalt der Buchennaturverjüngung auswirkten. Solche klimatische Bedingungen verursachten eine – im Vergleich zum als Kontrolle dienenden NO-Hang – eine verringerte N-Aufnahme aus dem Boden, die dazu führte, dass das Wachstum der Jungbuchen von gespeichertem N abhing. Als Folge konnte ein reduziertes Wachstum der Jungbuchen festgestellt werden. Die Schirmhiebmaßnahmen wirkten sich unter den als Referenz angesehenen klimatischen Bedingungen des NO-Hangs positiv auf den N Haushalt der Buchennaturverjüngung aus, zeigten aber durch ausgeprägte Trockenheit negative Folgen für den N-Haushalt auf dem SW-Hang. Die durch die Schirmhiebmaßnahmen verstärkte Konkurrenz zwischen Buchen aus der Naturverjüngung und Spezies der Schlagvegetation kann den Stickstoffhaushalt der Jungbuchen weiter verschlechtern, wenn diese Spezies – wie für *Rubus fruticosus* gezeigt - trockenresistent sind und die Fähigkeit besitzen, die im Boden verfügbaren Stickstoffressourcen auch unter starker Wasserknappheit aufzunehmen.

Auf der SW-exponierten Hangseite führten die Schirmhiebmaßnahmen zu erhöhter Temperatur und Strahlung und, als Folge, zu einem verstärkten Wasserverlust der Pflanzen. Trotzdem konnten bei reduzierter Bodenwasserverfügbarkeit Hinweise auf eine verbesserte Wassernutzungseffizienz (water use efficiency) bei den Jungbuchen gefunden werden. Ergänzende Experimente im Gewächshaus zeigten, dass unter extremem Wassermangel die Konkurrenz von Schlagvegetationsspezies zu weiterer Wasserknappheit und reduziertem Wachstum für die Jungbuchen führen kann. Unter Freilandbedingungen konnten jedoch keine deutlichen Einflüsse des Unterwuchses auf den Wasserhaushalt der Buchennaturverjüngung festgestellt werden.

Zusammenfassend lässt sich sagen, dass Schirmhiebmaßnahmen als waldbauliches Instrument zur Förderung der Naturverjüngung zur Verbesserung des Stickstoff- und Wasserhaushalts der jungen Buchen auf dem NO-Hang – der als repräsentativ für die aktuellen klimatischen Bedingungen angesehen wird – führten. Wenn in Zukunft, wie durch die aktuellen Klimamodelle vorhergesagt, ausgeprägte Trockenperioden und Temperaturerhöhungen eintreten sollten, könnte die Anwendung solcher Schirmhiebmaßnahmen die negativen Effekte der Klimaveränderung verstärken und die Vitalität und das Wachstum der Naturverjüngung in Buchenwäldern reduzieren.

1. Introduction and aims of the study

The need for understanding the responses of forest ecosystems to climate change at various geographical scales is of high priority for narrowing the gap between current knowledge and policymaking (IPCC, 2001). Accumulation of CO₂, methane, nitrogen oxides and other gases in the atmosphere is the basis of climate change (e.g. LAWLOR, 1998; PALMER & RÄISÄNEN, 2002) and results in several alterations. Among these, global warming is caused by the characteristic of such gases to absorb long-wave radiation emitted from the earth's surface and warming, thus, the atmosphere. The average global surface temperature has already increased by ca. 0.6°C over the 20th century and is expected to reach 1.4 to 5.8°C higher values relative to 1990 by 2100 (IPCC, 2001). This great range of temperature increase indicates that warming would vary among different regions. Greater elevations in temperature are prognosticated for high and temperate latitudes (LINDER *et al.*, 1996; LAWLOR, 1998), but a ca. 0.4°C increase in average temperature per decade is also expected in the Mediterranean (PEÑUELAS, 1996). Furthermore, extreme events such as hot and dry periods in summer, flooding, fires and late spring frosts will increase in frequency and duration (LINDER *et al.*, 1996; PEÑUELAS, 1996; SPIECKER, 1999a; SAXE *et al.*, 2001; MILLY *et al.*, 2002).

Already in the past, global surface temperature increased and required the vegetation to adapt (SAXE *et al.*, 2001). However, climate change and warming are now taking place at such a rate that it might be difficult for forest species to adjust their physiological apparatus to the new conditions and/or to adapt genetically by evolutionary change, due to their long generation time (e.g. PEÑUELAS, 1996; SAXE *et al.*, 2001). This limited adaptive capacity of forest ecosystems makes them particularly vulnerable to climate change (IPCC, 2001). Therefore, the assessment of the responses of trees to global warming is important, particularly since species- and region-specific effects of elevated temperatures on physiological processes and on growth of trees have been reported (SAXE *et al.*, 2001).

Consequently, one of the forest species that captured the interest of researchers in Europe is the drought-sensitive European beech (*Fagus sylvatica* L.), the most important deciduous tree species of the potential natural vegetation in Central Europe. The geographical distribution of this species can be limited by restricted water availability (ELLENBERG, 1992). Particularly when growing on soils of low water storage capacity such as the shallow rendzina soils derived from limestone

which are common in Central and Southern Europe (e.g. Swabian and Frankian Alb, French Jura, Swiss Jura, several areas in Northern and Central Greece), the impacts of prolonged drought periods during the growing season on growth of European beech can be intensified. Furthermore, within forest ecosystems dominated by European beech, its natural regeneration would be even more susceptible to the prognosticated climate changes.

Elevated temperatures and limited water availability can impair directly the water status of beech seedlings, but may also interact with their N balance by decreasing pedospheric N uptake and altering, thus, their N metabolism. Consequently, growth of beech seedlings may be restricted (MARSCHNER, 1995; FOTELLI *et al.*, 2000), while patterns of interspecific competitive interactions with neighbouring vegetation and/or the ability of beech regeneration to cope with such interference might be altered (PEÑUELAS, 1996). Another factor playing an important role for the N metabolism and growth of beech forests in Central Europe is the substantial increase in atmospheric N deposition, due to human activities, which results in enhanced foliar N uptake (e.g. RENNENBERG & GESSLER, 1999). Increased N deposition could partially compensate for the negative effects of drought and warming on the N status of a beech ecosystem.

Generally, global warming is expected to result in a positive growth response up to an optimum, due to the ability of forests to store increasing amounts of carbon (SAXE *et al.*, 2001). Still, most of such expected responses are reported from greenhouse-experiments and not from field studies. Although the causes of a putative increased growth are not completely assessed, recent field studies confirm an increase of growth in many forests across Europe (SPIECKER, 1996; SPIECKER, 1999a, b). This increase is expected to continue, if increases in temperature are to be of less than a few °C (IPCC, 2001), but it is smaller than indicated by pot-experiments. However, there may be secondary effects of global warming that decrease tree growth, as for example drought. In Central Europe drought resulted in growth recession in the late 1940s and mid 1970s (SPIECKER, 1999a). Moreover, a low gain from warmer temperatures is prognosticated for the drought-sensitive European beech (VON HEINS DORF, 1999; SAXE *et al.*, 2001). Still, forest management could have a greater effect on the productivity of forests than climate change (IPCC, 2001) and could mitigate possible negative effects of climate change (LOEHLE, 1996; LINDNER, 1999). Consequently, management strategies are increasingly included into climate models and are assessed for a positive response of forest growth to

global warming (SAXE *et al.*, 2001). In the light of the expected changes in near future, there is a need to re-evaluate commonly applied forest management practises and to develop adaptive management of beech forests according to the conditions expected in the future (UNTHEIM, 1996). Among silvicultural treatments, thinning, by means of selective felling, is widely applied in Northern and Central Europe in order to promote the natural regeneration of deciduous species, and particularly of European beech, and the development of mixed beech-dominated forests (DERTZ, 1996; MINISTERIUM FÜR LÄNDLICHEN RAUM, ERNÄHRUNG, LANDWIRTSCHAFT UND FORSTEN IN BADEN WÜRTTEMBERG, 1997; MINISTERIUM FÜR LÄNDLICHEN RAUM, ERNÄHRUNG, LANDWIRTSCHAFT UND FORSTEN IN BADEN WÜRTTEMBERG, 1999; TARP *et al.*, 2000).

However, thinning affects growth conditions for tree seedlings in different ways. It improves abiotic conditions (light intensity, water and nutrient availability, soil and air temperature; e.g. BREDA *et al.*, 1995; AUSSENAC, 2000; MIZUNAGA, 2000; THIBODEAU *et al.*, 2000) for tree seedlings resulting in increased nutrient uptake and growth. On the other hand, thinning may induce growth not only of young trees but also of woody and herbaceous plants occurring in the forest understorey. Such a response may, therefore, impair growth of tree seedlings by increasing the competitive interference or by altering the patterns of interspecific competition between young tree seedlings and fast growing understorey species (MADSEN, 1995; LOF, 2000). Therefore, the effects of thinning on the conditions in the forest understorey have to be assessed under the aspect of climate change in order to test whether such silvicultural treatments promote, indeed, the natural regeneration of beech under the conditions expected in future.

The present study aimed at assessing the effects of different climatic conditions and opening of the mature canopy on the nitrogen and water status, and, thus, on the establishment and growth, of natural beech regeneration. The work was initiated within the frame of the SFB-433 interdisciplinary research project of the Deutsche Forschungs-gemeinschaft (DFG) entitled “Buchendominierte Laubwälder unter dem Einfluss von Klima und Bewirtschaftung – Beech-dominated deciduous forests under the influence of climate and management”. For the purposes of this study, advantage was taken of the opportunity provided by a beech forest in Southern Germany that is characterized by (1) different meso – climatic conditions, as induced by variations in the exposure (northeast vs. southwest), and (2) varying canopy density conditions, as modulated by partial thinning of its mature canopy. Furthermore, the studied

beech ecosystem is not characterised by high atmospheric N load. Previous investigations revealed that the SW-exposed site receives more radiation and has, thus, higher air and soil temperatures, while it is also characterised by lower soil water storage capacity, compared to the NE-exposed site. These conditions on the SW-exposed site result in water shortage of adult beech trees during low rainfall periods in summer (GESSLER *et al.*, 2001). Therefore, the difference in local climatic conditions between the two sites was regarded suitable for simulating the effects of global warming. The NE-exposed site was used as model site for typical beech forests growing on limestone under the present climatic conditions and the SW-exposed site as representative of the climate expected in near future.

Furthermore, due to the great complexity of interactions in a forest understorey in the field, the effects of climate and thinning on the potential interference of understorey species on beech seedlings were studied under controlled conditions. For this purpose, an early successional fast-growing species, *Rubus fruticosus*, growing in the understorey of the beech forest studied, was chosen as a model-competitor for assessing in detail the effects of interference on the nitrogen and water status of beech seedlings in greenhouse experiments which were conducted under well-defined growth conditions.

For validating the effects of climate and thinning on the N and water status of the studied plants, numerous parameters were included in the methodology of the present work. The N status was characterised by means of:

- (a) measurement of free amino acids-N comprising the pool of total soluble nonprotein nitrogen (TSNN) in leaves, fine roots, and wood of beech seedlings. This parameter was already found to be a sensitive indicator of changes in the N balance of tree species under conditions of high atmospheric N loads (SCHNEIDER *et al.*, 1996; GESSLER *et al.*, 1998a) and has been validated for its usefulness in studying effects of varying climatic and growth conditions on the nitrogen status of plants
- (b) the commonly used determination of total N contents in the respective plant tissues of beech and in above- and below-ground biomass of neighbouring vegetation
- (c) tracing experiments with the use of the stable isotope ^{15}N being increasingly used during the last years for such purposes (e.g. GEBAUER *et al.*, 2000). This

enabled the assessment of N uptake rates and of partitioning of currently acquired N within both beech seedlings and neighbouring understorey vegetation, as well as between neighbouring plants.

Similarly, the parameters used for characterising the plant water status covered different aspects:

- (a) At the whole plant-level, the plant water potential of beech seedlings and their sap flow rates, based on the Steady State Heating Method (FICHTNER & SCHULZE, 1990), were determined.
- (b) On the leaf level, gas exchange measurements were conducted.
- (c) Furthermore, the abundance of the stable isotope ^{13}C provided information on the conditions of CO_2 assimilation under varying influencing factors and for different time intervals (e.g. FARQUHAR *et al.*, 1989; BERRY *et al.*, 1997; ADAMS & GRIERSON, 2001). ^{13}C abundance has been used as indicator of the water status of beech seedlings and its relationship to numerous environmental and physiological parameters has been tested.

Furthermore, phenological parameters such as the root-to-shoot ratio and the total biomass of the studied plants were determined for assessing morphological and structural responses to climate change and for enabling assessment of all above-mentioned parameters at the whole-plant level.

Two chapters corresponding to effects of climate and management practises (1) on the N status, and (2) on the water status of the plants, respectively, follow. Each chapter begins with a short introduction on the importance of the subject and continues with an overview about current knowledge on the insights that each parameter offers in tree physiology and ecophysiology. Subsequently, the key-findings are separated in thematic units accompanied by the abstracts of the respective original publications conducted and are followed by the main conclusions. The experiments shown in the attached original publications have been conducted within the frame of the present study.

2. N status and growth of beech seedlings

Nitrogen is of major importance for trees since rates of nitrogen mineralization are often so slow that most forest ecosystems, apart from those subjected to high atmospheric loads of N, are N-limited (e.g. TAMM, 1991; MARTIN & LORILLOU, 1997; RENNENBERG *et al.*, 1998). The N status of European beech seedlings occurring in the natural regeneration of a beech forest in Southern Germany has been assessed by means of various physiological parameters and it will be discussed in this chapter in the light of climate change and forest management. Moreover, the role of neighboring understorey vegetation in acquiring N resources, as assessed in the field and particularly with controlled greenhouse-experiments, will be discussed.

2.1 Parameters describing the N status

Despite the generally low contribution of amino-nitrogen to total N content in leaves and roots (<5%) of coniferous and deciduous tree species (e.g. FOTELLI *et al.*, 2002a, b), it is of great physiological importance. Particularly in European beech, numerous studies with young and adult trees conclude that total soluble nonprotein nitrogen (TSNN) is sensitive to changes in N availability (GESSLER *et al.*, 1998b; GESSLER & RENNENBERG 2000) and N demand (RENNENBERG & GESSLER 1999). Therefore, TSNN is a reliable indicator of internal N status, even when total N in various plant tissues remains constant (BAUER *et al.*, 2000), as found for example over a wide range of nutritional and climatic conditions (RENNENBERG *et al.*, 1998; GESSLER & RENNENBERG, 2000). On the other hand, total N contents have been commonly used as a parameter describing the N status of the plants (e.g. MILLARD 1989; NAMBIAR & SANDS, 1993; BAUER *et al.*, 2000; COTRUFO *et al.*, 2000) and there is a great availability on such information from varying ecosystems enabling comparability of results with those found in other ecosystems of the same vegetation zone or in ecosystems belonging to different vegetation zones and grown under varying climatic conditions. Furthermore, the use of ¹⁵N-enriched solutions as tracers offers another insight into the N dynamics and ecological processes by enabling determination of N uptake rates (e.g. MEAD & PRESTON, 1994; AMMANN *et al.*, 1999) and assessing the competitive interactions among neighbouring plants sharing

finite resources (e.g. CLINTON & MEAD, 1994; CHANG *et al.*, 1996; CHANG & PRESTON, 2000).

2.2 Changes in N metabolism and N uptake due to climate and thinning affect growth of beech seedlings

FOTELLI MN, NAHM M, HEIDENFELDER A, PAPAN H, RENNENBERG H, GESSLER A. 2002. Soluble nonprotein nitrogen compounds indicate changes in the nitrogen status of beech seedlings due to climate and thinning. *New Phytologist* 154: 85-97 [ANNEX 1].

We assessed the effect of climatic and canopy density changes on the seasonal patterns of total soluble nonprotein N (TSNN) in naturally regenerated beech seedlings grown on limestone.

Leaves, roots, wood and phloem from seedlings grown in control and thinned stands on a dry-warm SW-exposed site and a moist-cooler NE-exposed site were examined. The concentrations of amino compounds, ammonium and nitrate, comprising TSNN were determined in May (leaves expansion), July (mid-summer) and September (end of the growing season).

In May, Asn was augmented in leaves and roots on the NE site, whereas Arg dominated in leaves and phloem on the SW site. In July, all TSNN compounds declined, independent of site and treatment. In September, TSNN, particularly Arg, increased in roots, phloem and wood on the SW site, compared to the NE.

TSNN indicates changes in the N status of beech seedlings, due to altered growth conditions. Drier and warmer climate on the SW site, relative to the NE, resulted in earlier N remobilization in spring and storage in autumn. Thinning tended to improve the N status on the NE site, and to impair it on the SW site by affecting differently the climate and soil nutrient balance of each site.

FOTELLI MN, RIENKS M, RENNENBERG H, GESSLER. 2002. Climate and management practices affect ¹⁵N-uptake, N balance and biomass of European beech seedlings. *Plant, Cell and Environment*, submitted [ANNEX 3].

We assessed the effect of different local climate and thinning of the adult canopy on growth and N status of naturally regenerated European beech seedlings, as well as on the partitioning of N between beech and other understorey species.

At the beginning of the growing season, ¹⁵N-labeled solutions were applied to the soil around beech seedlings and understorey vegetation grown in control and thinned stands on a dry-warm SW-exposed site and a moist-cooler NE-exposed site. Harvest of labeled plants took place three times during the growing season and biomass, ¹⁵N uptake, and N contents were determined.

Site and thinning produced clear differences, particularly at the end of the growing season. Then, biomass and cumulative ¹⁵N uptake of beech seedlings increased due to thinning on the NE site and decreased on the SW site. Total N in leaves, roots and stems of beech seedlings responded similarly. Most of ¹⁵N accumulated in biomass during the entire growing season was found in beech seedlings. Accumulation in the biomass of the herbaceous vegetation was substantial only in July and decreased thereafter.

Growth and N status of beech seedlings are favored by thinning under cool-moist conditions. However, under the climatic conditions expected in future, i.e. higher temperature and reduced water availability, thinning can have adverse effects on beech regeneration.

These experiments showed that TSNN was sensitive to changes in the N status of beech seedlings due to altered growth conditions, as modulated by climate and thinning (FOTELLI *et al.*, 2002a). By assessing the N status of seedlings of the natural regeneration, TSNN reflected mainly the pattern of soil nitrate concentration which was substantially affected by the varying growth conditions. Furthermore, individual amino acids being of major importance as signals regulating the N balance or as transport and storage forms of N within the plant (KREUZWIESER *et al.*, 1997; GESSLER *et al.*, 1998a, b), also indicated different metabolic responses of beech seedlings under the varying growth conditions. For example, patterns of Arginine (Arg), being the amino acid mostly responsible for storage, remobilization and transport of N (FLAIG & MOHR, 1992; GEZELIUS & NÄSHOLM, 1993; GESSLER *et al.*, 1998a, SCHMIDT & STEWART, 1998), indicated that remobilization of N from

storage tissues was particularly important for seedlings on the warmer and drier SW site during new leaves development (FOTELLI *et al.*, 2002a). On the contrary, seedlings grown on the cooler and moister NE site could depend to a greater extent on N being actively taken up from the soil, due to higher soil nitrate availability; a fact further supported by glutamic acid (Glu), the primary product of nitrate assimilation in mycorrhizal roots (MARTIN & LORILLOU, 1997), being mostly augmented in the roots. The in detail study of the composition of TSNN from major amino acids helped explaining how is the N metabolism of beech regeneration affected and which physiological processes took place under different environmental conditions, whereas total N contents provided us with a more general picture of their N status.

Furthermore, TSNN proved to be a reliable tool for forest practitioners by reflecting the effects of thinning on the N status of beech regeneration and, thus, on the establishment of natural regeneration under different climatic conditions. Contrary to what might have been expected, thinning impaired the N status of seedlings grown on the warmer and drier SW site, compared to the cooler and moist NE (FOTELLI *et al.*, 2002a). This was demonstrated by decreased TSNN content in leaves at the end of the growing season, while Arg was augmented; an indication of earlier initiation of remobilization to storage tissues due to suboptimal conditions. Furthermore, the reliability of these results is confirmed by consistently lower nitrate uptake rates and total N contents, and thus growth of beech seedlings grown on the warm and dry site (FOTELLI *et al.*, 2002c). Therefore, since the conditions expected in the near future include elevated temperatures (IPCC, 2001), it can be hypothesized that application of thinning may have adverse effects on the natural regeneration by further increasing radiation interception and temperature on already warmer sites.

2.3 Competitive interference and climate: effects on the N status of beech seedlings

Application of ^{15}N tracing in the field showed that it is difficult to assess possible interference by neighboring vegetation on beech seedlings under different climatic and canopy density conditions, because of the great complexity of interactions occurring in the forest understorey (FOTELLI *et al.*, 2002c). However, differences in the seasonal pattern of N distribution within functional groups of understorey plants can be assessed. It was shown for example that the relative importance of herbaceous vegetation in terms of N acquisition is greater until the middle of the

growing season and decreases later on, but still no safe conclusions could be reached regarding interference (FOTELLI *et al.*, 2002c). Consequently, experiments assessing interference for certain N resources conducted under carefully controlled conditions were required.

FOTELLI MN, RENNENBERG H, GESSLER A. 2002. Effects of drought on the competitive interference of an early successional species (*Rubus fruticosus*) on *Fagus sylvatica* L. seedlings: ¹⁵N uptake and partitioning, responses of amino acids and other N compounds. *Plant Biology* 4: 311-320 [ANNEX 2].

We assessed the role of water availability, as a factor regulating the ability of beech seedlings to cope with competitive interference by an early successional species (*Rubus fruticosus*) for nitrogen resources.

A glasshouse experiment was performed with 2 levels of interference (beech with and without *R. fruticosus*) and three levels of irrigation (high, intermediate, none). ¹⁵N uptake and partitioning of both species, and composition of N pools in leaves, roots and phloem of beech, were determined.

Under all irrigation regimes, ¹⁵N uptake by beech seedlings decreased when grown together with *R. fruticosus*. *R. fruticosus* presented higher ¹⁵N uptake rates than beech, under all water supply levels. When irrigation was reduced, a substantial decrease in ¹⁵N uptake of beech seedlings and a concurrent increase in ¹⁵N uptake by *R. fruticosus* were observed. Interference by *R. fruticosus* and low irrigation also affected the ¹⁵N partitioning in beech seedlings and resulted in reduced allocation of ¹⁵N to the roots. The combination of competitive interference and lack of irrigation led to an increase in soluble nonprotein N in roots and leaves of beech, due to protein degradation. This response was attributed to an increase in levels of amino acids serving as osmoprotectants under these conditions. The concentration of proline in leaves of beech was negatively correlated to shoot water potential. A competition-induced reduction of total N in leaves of beech, under high and intermediate irrigation was found. These results illustrate (1) the advantage of *R. fruticosus* in terms of N uptake, compared to young beech, particularly under inadequate water supply, and (2) the changes in N composition of beech seedlings, in order to cope with reduced soil water and interference by *R. fruticosus*.

Thus, the results of the greenhouse experiments showed that under severe water depletion, interference by a fast growing early successional species, like *Rubus fruticosus*, results in N shortage for young beech seedlings sharing the same N resources (FOTELLI *et al.*, 2002b). Being drought resistant and maintaining internal water status and growth almost unaffected by soil water limitation (FOTELLI *et al.*, 2001), *R. fruticosus* was able to use more of the available N resources. This was demonstrated by increasing nitrate uptake rates as water depletion proceeded, while the respective uptake rates of beech seedlings were substantially lower (FOTELLI *et al.*, 2002b). Furthermore, the combination of limited water availability and interference led to decreased total N contents and to concurrent increase of soluble nonprotein N (TSNN), resulting from protein degradation due to suboptimal conditions and/or the need for accumulation of amino acids enhancing tolerance of plant cells to water depletion (mainly proline and subsequently glutamine and asparagine; HANDA *et al.*, 1986; HEUER, 1994; GIROUSSE *et al.*, 1996; McNEIL *et al.*, 1999).

These results, further, support the hypothesis that opening of the mature canopy in beech stands susceptible to water limitation may impair the N status of the regeneration, as found in the field (FOTELLI *et al.*, 2002a, c). Particularly during the initial phase of regeneration following thinning, the simultaneous enhanced growth of such fast growing understorey species could substantially impair the success of the regeneration, if unfavorable environmental conditions, like prolonged drought periods, are expected (e.g. IPCC, 2001). When overcoming this initial stage, the low N-demanding beech could regain advantage over such competitors having high energy costs for maintaining high N uptake rates (AERTS, 1999), particularly in nutrient-poor environments like the studied field site.

2.4 Conclusions

Warming and prolonged drought periods in Southern Germany can impair the N status of beech regeneration. This was demonstrated by the dependence of new leaves development on stored forms of N, reduced N uptake rates and limited growth. Simultaneous thinning-induced increased interference by understorey vegetation might intensify the phenomena at early phases of regeneration by deriving N resources beech regeneration; still clear conclusions on the effects of interference were reached only under controlled conditions. Thinning alone impairs the N status of

beech regeneration on already drier sites, by further increasing water shortage, temperatures and radiation interception.

3. Water status: effects of climate and thinning

The sensitivity of natural ecosystems towards climate change is mainly ruled by the limitation of water resources (IPCC, 2001). Elevated temperatures combined with reduced soil water availability could result in increased vulnerability of forest ecosystems not adjusted to high frequency of such drought events, due to the generally lower adaptive capacity of forests. However, plants of arid regions, adapted to summer drought, are capable of maintaining high water use efficiency and an unimpaired physiological function. How temperate beech forests respond to such conditions and, furthermore, how traditionally applied management practices would alter the effect of climate change by altering climate on a local scale is a question.

3.1 Update on the assessment of plant water status

Leaf gas exchange measurements determining stomatal conductance, transpiration and assimilation rates etc., are routinely used in the field of ecophysiology. Particularly the use of portable equipments being suitable also for field studies contributes greatly to the acquisition of information on stomatal functioning under natural conditions. Still, information at the leaf level alone is not adequate for describing the responses to water depletion at the whole plant level. The modification of the Heat Balance Method measuring whole plant sap flow rates of adult trees (ČERMÁK *et al.*, 1973; KUČERA *et al.*, 1977) by FICHTNER & SCHULZE (1990) enabled such measurements with young seedlings. Furthermore, sap flow measurements can be conducted in the field for longer time periods covering entire growing seasons and can be directly recorded with a data logger. This approach comprises an important tool in ecophysiological studies to plant water status (NADEZHINA, 1999) in relation to environmental conditions in the field. Also, measurement of plant water potential with a pressure chamber (SCHOLANDER *et al.*, 1965) provides a direct picture of the internal water status of the plant at the whole plant level. Currently, mass spectrometry opened new aspects for environmental biology. Among the stable isotopes, the ^{13}C isotope is an information-rich signal providing enlightening insights on the conditions under which CO_2 assimilation occurred by covering various time intervals (from seconds with on-line measurements to years with analysis of tree rings; e.g. SCHLESER *et al.*, 1999; ADAMS & GRIERSON, 2001; GESSLER *et al.*, 2001). Moreover, being dependent

upon the difference between stomatal and air CO₂ concentration, ¹³C abundance has become a well-established indicator of plant water status and water use efficiency (e.g. FARQUHAR *et al.*, 1989; DAMESIN *et al.*, 1998; FOTELLI *et al.*, 2001), while a continuously increasing database on its relation to several climatic and physiological parameters is developing in the last years (e.g. LIVINGSTON *et al.*, 1998; KOROL *et al.*, 1999; BONAL *et al.*, 2000a; SCHEIDEGGER *et al.*, 2000; PORTÉ & LOUSTAU, 2001; WARREN *et al.*, 2001, FOTELLI *et al.*, 2002d). Consequently, this parameter contributes greatly to the assessment of the responses of forest species to climate change which can be studied not only at the plant level but, further, on the ecosystem level since determination of responses of entire functional groups is also possible (e.g. BROOKS *et al.*, 1997; GUEHL *et al.*, 1998; BONAL *et al.*, 2000b, FOTELLI *et al.*, 2002d).

FOTELLI MN, GESSLER A, PEUKE AD, RENNENBERG H. 2001. Drought affects the competition between *Fagus sylvatica* L. seedlings and an early successional species (*Rubus fruticosus*): growth, water status and $\delta^{13}\text{C}$ composition. *New Phytologist* 151: 427-435 [ANNEX 4].

Competitive interactions between European beech (*Fagus sylvatica* L.) seedlings and the early successional species *Rubus fruticosus*, and the role of water availability, are reported and discussed in relation to management practices and climatic changes in beech ecosystems of Central Europe.

Responses of growth, water status, gas exchange and carbon isotope composition ($\delta^{13}\text{C}$) to two competition and three irrigation treatments were examined in a factorial-design glasshouse study.

Under regular irrigation, coexistence with *R. fruticosus* did not significantly affect growth, water potential and gas exchange of beech seedlings. However, moderate water shortage caused a twofold reduction in beech biomass and changes in root : shoot ratios. Drought lowered transpiration rates and predawn water potentials (below the xylem embolism threshold) for *F. sylvatica*; $\delta^{13}\text{C}$ of leaves and fine roots increased (discrimination was reduced). By contrast, significantly lower $\delta^{13}\text{C}$ of *R. fruticosus* foliage indicated an improved water status. Competitive interference intensified the effects of reduced irrigation.

Water availability regulates the competitive interactions between beech seedlings and *R. fruticosus*. Natural regeneration of beech seedlings may be inhibited by interference from a

species such as *R. fruticosus*, especially during summer drought, as predicted by actual climate models.

FOTELLI MN, RENNENBERG H, HOLST T, MAYER H, GESSLER A. 2002d. Effects of climate and silviculture on the carbon isotope composition of understory species in a beech (*Fagus sylvatica* L.) forest. *New Phytologist*, submitted. [ANNEX 5].

We assessed the effects of climate and thinning on $\delta^{13}\text{C}$ signatures of different plant-groups in the understory of a beech forest in Southern Germany, and the relationship to environmental parameters.

$\delta^{13}\text{C}$ was measured in different tissues of beech regeneration, herbaceous and woody vegetation grown in thinned and control stands on two sites differing in climatic conditions (drier, warmer SW vs. moist, cooler NE).

In most cases, all tissues had lower $\delta^{13}\text{C}$ on the NE site, compared to the SW site and higher $\delta^{13}\text{C}$ in thinned stands. Variation in $\delta^{13}\text{C}$ was generally accounted for by soil water potential and in the control stands by radiation. Only $\delta^{13}\text{C}$ in wood of beech was mainly determined by temperature. $\delta^{13}\text{C}$ signatures of woody and herbaceous plants were higher and their relationship to environmental parameters was poor, compared to beech.

Higher $\delta^{13}\text{C}$ signatures of all plants on the SW, compared to the NE site, were mostly due to lower water availability, and may be indicative of more conservative water use, on the SW site. However, in the light-limited unthinned stands, radiation became the main determinant of $\delta^{13}\text{C}$. Wood $\delta^{13}\text{C}$ in beech reflected the long-term difference in soil temperature between the two sites. Higher $\delta^{13}\text{C}$ signatures in woody and herbaceous vegetation were rather vegetation type-specific.

3.2 Effects of water shortage on water status and competitive ability of beech seedlings

From the results of the greenhouse experiments it is concluded that beech seedlings are rather susceptible to limited soil water, as induced by controlled water withdrawal. This was demonstrated by reduced gas exchange, more negative plant water

potential and finally substantially inhibited growth, at levels of soil water potential not lower than -0.6 MPa (FOTELLI *et al.*, 2001).

However, in the field site even a considerably smaller reduction in soil water potential to levels >-0.05 MPa, on the SW site, compared to the almost optimal water availability on the NE site, was sufficient for causing water depletion-responses of beech seedlings, such as e.g. reduced plant water potential (Fig. 1). Moreover, the higher radiation interception on the SW-exposed site (GESSLER *et al.*, 2001) probably had an additional adverse effect on the water status of beech seedlings grown on this site, since higher radiation stimulated sap flow and, thus, the loss of water through transpiration (Fig. 2). These findings are indicative of the high vulnerability of beech regeneration to the prolonged drought periods expected during the growing season in future (IPCC, 2001). However, carbon isotope analysis of various plant tissues revealed higher $\delta^{13}\text{C}$ signatures of beech seedlings grown on the warmer and drier SW site (FOTELLI *et al.*, 2002d); a response related (a) to lower plant water potential (e.g. DAMESIN *et al.*, 1998; FOTELLI *et al.*, 2001), and (b) to higher water use efficiency (e.g. PORTÉ & LOUSTAU, 2001). Therefore, it is also possible that the warmer and drier conditions of the SW site resulted on the long-term in more conservative water use of beech seedlings; a response indicating more optimal coordination of the photosynthetic apparatus and the water status, and adjustment to suboptimal climatic conditions (e.g. LI, 1999; ARNDT *et al.*, 2000).

Interference by a fast growing, drought-tolerant species like *R. fruticosus* in greenhouse experiments intensified the drought-induced responses of beech seedlings, i.e. low plant water potential, limited gas exchange, higher carbon isotope composition, and led to irreversible physiological damages to beech seedlings within six weeks (FOTELLI *et al.*, 2001). These findings indicate that water limitation under natural conditions, results in a competition by early successional neighboring vegetation for finite water resources that is disadvantageous for beech regeneration. Still, no such effect of neighboring vegetation on the water status of beech seedlings could be found in the field, probably due to the substantially greater soil water availability in the field, compared to that applied under controlled conditions. In addition, the great complexity of interactions between environmental parameters in the forest understorey under natural conditions, as also indicated by $\delta^{13}\text{C}$ analysis (FOTELLI *et al.*, 2002d), makes it difficult to detect competitive interferences between plant functional groups. On the contrary, the differences in $\delta^{13}\text{C}$ between beech

regeneration and functional groups of understorey plants where more likely species- or vegetation-type specific (FOTELLI *et al.*, 2002d).

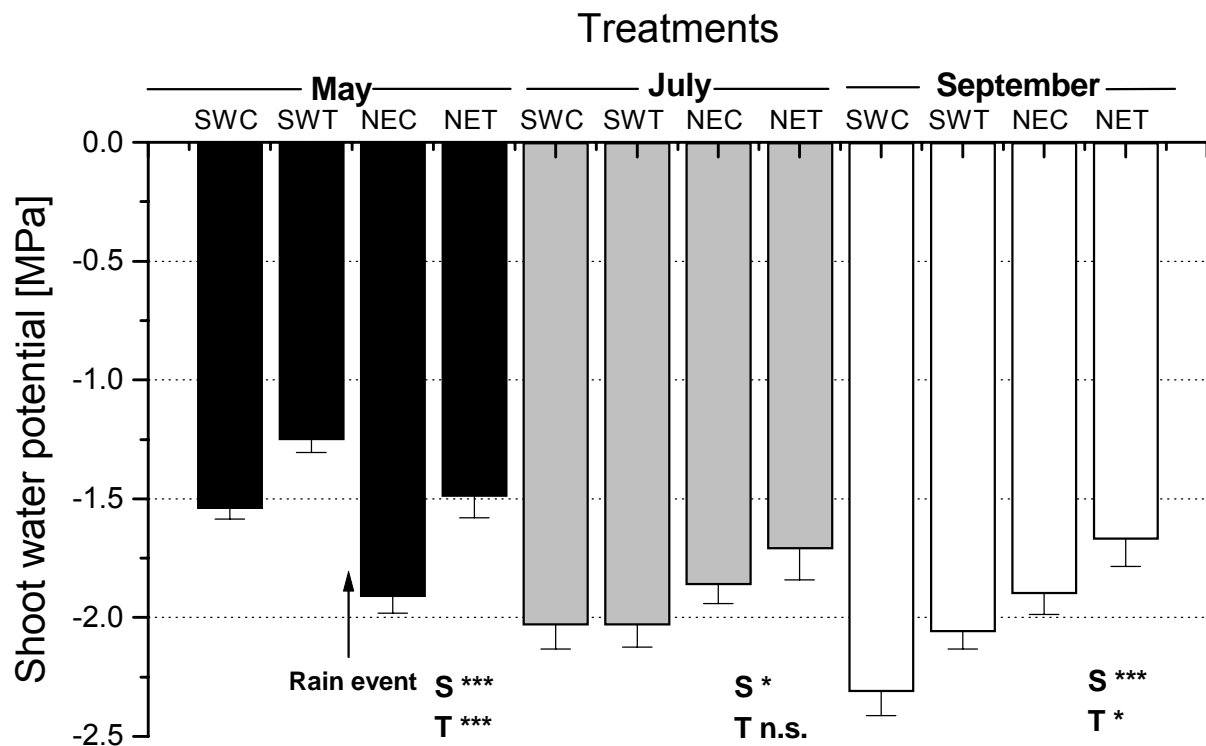


Figure 1. Shoot water potential of beech seedlings occurring in the natural regeneration and grown in differently treated stands (T-thinned vs. C-control) on two opposite sites (SW-exposed vs. NE-exposed). Shoot water potential was measured during three measuring campaigns in May (at the beginning of the growing season), in July (in mid-summer), and in September (at the end of the growing season) with the Scholander pressure chamber. One, two or three asterisks correspond to site (S) or thinning (T) effects on a level of significance of 95%, 99% or 99.9%, respectively, while n.s. designates no significant effects. Values shown are means (+SE) of 7 seedlings.

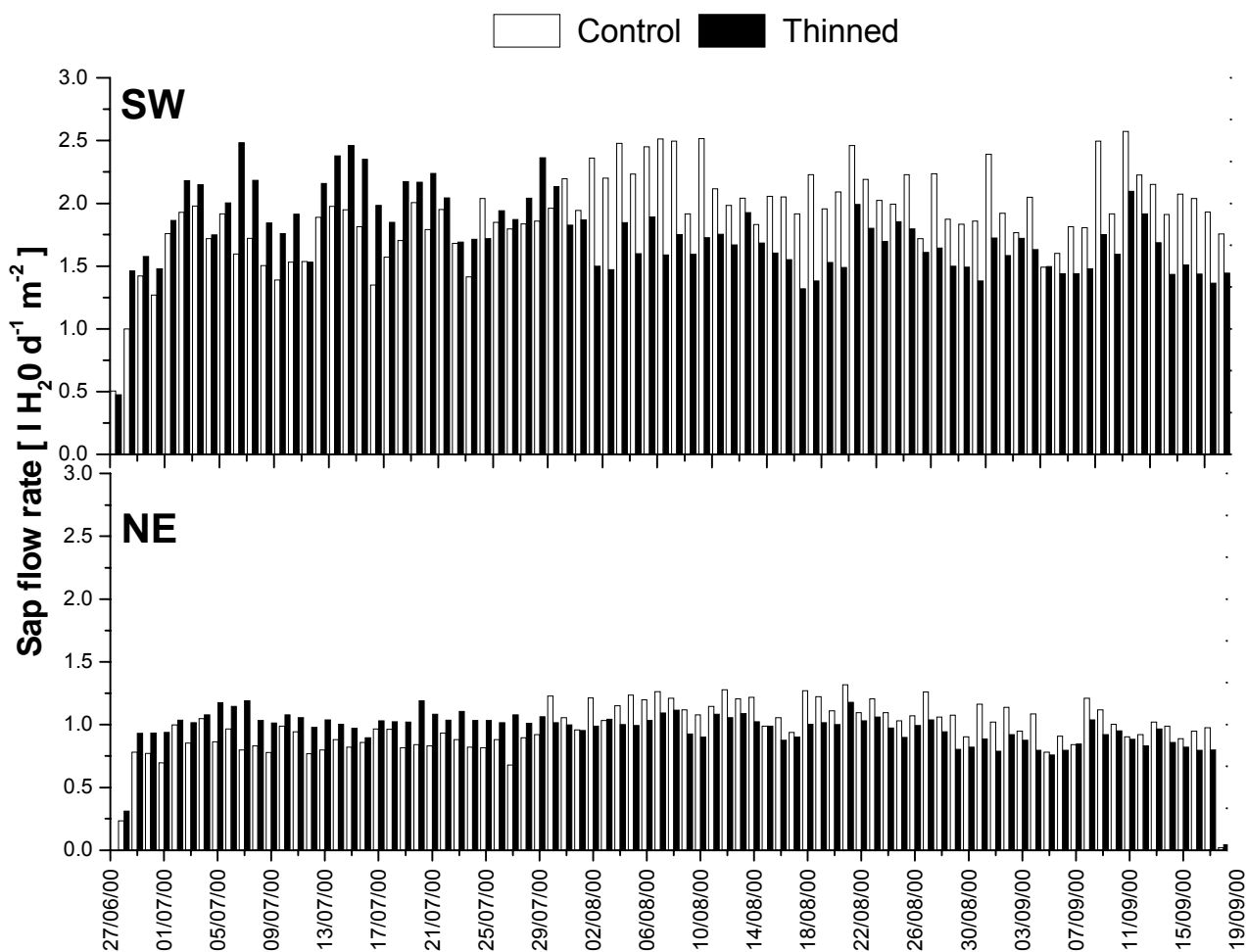


Figure 2. Daily sums of sap flow rates of beech seedlings occurring in the natural regeneration and grown in differently treated stands (Thinned vs. Control) on two opposite sites (SW-exposed vs. NE-exposed). The presented data were measured during the period 28.06.00-17.09.00 and are means of 2-3 seedlings.

3.3 Climate and forest management: insights from $\delta^{13}\text{C}$ and sap flow measurements

Soil water availability was the major determinant of foliar and root $\delta^{13}\text{C}$ in beech seedlings and of shoot $\delta^{13}\text{C}$ in woody and herbaceous understorey vegetation, respectively, and accounted for higher $\delta^{13}\text{C}$ signatures on the warmer and drier SW

site (FOTELLI *et al.*, 2002d). Carbon isotopic signature in wood of beech seedlings was mainly affected by the long-term difference in soil temperature between the two sites (ca. 0.8 °C higher mean daily soil temperature on the SW site during the growing season). These findings further emphasise the importance of the expected prolonged drought periods and of global warming and the great influence they would have on stomatal activity and water status of natural beech regeneration.

However, forest management also has a great potential in terms of manipulating the local climate in beech stands, as outlined by carbon isotope analysis. It was shown that canopy density - induced differences in environmental conditions among the stands, particularly in terms of radiation interception (GESSLER *et al.*, 2001) produced strong relationships between $\delta^{13}\text{C}$ and light intensity which were stand-specific (FOTELLI *et al.*, 2002d). For example, in the unthinned stand of the NE site the limited light intensity mostly controlled foliar $\delta^{13}\text{C}$ of beech seedlings and $\delta^{13}\text{C}$ in shoots of herbaceous vegetation, and accounted for the very low $\delta^{13}\text{C}$ (e.g. FARQUHAR *et al.*, 1989; BROADMEADOW & GRIFFITHS, 1993; LIVINGSTON & SPITTLEHOUSE, 1996).

The differential effect of forest management in forest stands is further emphasised by the sap flow rates of beech seedlings (Fig. 2). For example, it was found that during precipitation - rich periods (like July in the 2000 growing season) the higher radiation interception, temperature and precipitation throughfall reaching the regeneration in the thinned stands resulted in higher sap flow rates, compared to the control stands. On the contrary, during dry periods (like August 2000) the sap flow of beech seedlings grown under high solar radiation was decreased, probably due to lower soil water availability caused by increased evaporation under these conditions (ČERMÁK *et al.*, 1993).

Consequently, forest management, in this case thinning, does indeed alter the growth conditions for beech seedlings and affects their physiological apparatus, and it could be a valuable tool for maintaining favourable regeneration conditions despite the expected climate change. Therefore, actual silvicultural practises have to be further evaluated and adapted to climate change in order to provide optimum growth conditions for beech regeneration in future.

3.4 Conclusions

Even slight decreases in soil water availability may result in water shortage of the drought susceptible beech regeneration. Moreover, higher solar radiation and temperature in dry sites can have an additional adverse effect on the water status of the regeneration by causing higher transpiration rates and thus, additional water loss. However, beech seedlings may develop a more conservative water use strategy under long term low water availability, as indicated by carbon isotope analysis. Thinning - induced increase in the density of drought-resistant understorey vegetation could cause further water depletion to beech seedlings, as concluded from greenhouse experiments. Still, the differences found in the water status of various plant groups in the field were rather vegetation type-specific. The high dependence of carbon isotope signatures on radiation interception, when the latter is a limiting factor, indicates the great influence that thinning can have on the growth conditions for beech regeneration, and the possibilities it offers in mitigating adverse effects of global climate change on beech forests.

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5. Annex

Within the present PhD study the following original publications were conducted:

ANNEX 1 FOTELLI MN, NAHM M, HEIDENFELDER A, PAPEN H, RENNENBERG H, GESSLER A. 2002. Soluble nonprotein nitrogen compounds indicate changes in the nitrogen status of beech seedlings due to climate and thinning. *New Phytologist* **154**: 85-97.

ANNEX 2 FOTELLI MN, RENNENBERG H, GESSLER A. 2002. Effects of drought on the competitive interference of an early successional species (*Rubus fruticosus*) on *Fagus sylvatica* L. seedlings: ¹⁵N uptake and partitioning, responses of amino acids and other N compounds. *Plant Biology* **4**: 311-320.

ANNEX 3 FOTELLI MN, RIENKS M, RENNENBERG H, GESSLER. 2002. Climate and management practices affect ¹⁵N-uptake, N balance and biomass of European beech seedlings. *Plant, Cell and Environment*, submitted.

ANNEX 4 FOTELLI MN, GESSLER A, PEUKE AD, RENNENBERG H. 2001. Drought affects the competition between *Fagus sylvatica* L. seedlings and an early successional species (*Rubus fruticosus*): growth, water status and $\delta^{13}\text{C}$ composition. *New Phytologist* **151**: 427-435.

ANNEX 5 FOTELLI MN, RENNENBERG H, HOLST T, MAYER A, GESSLER A. 2002. Effects of climate and silviculture on the carbon isotope composition of understorey species in a beech (*Fagus sylvatica* L.) forest. *New Phytologist*, submitted

6. Acknowledgements

I am grateful to Prof. Dr. Heinz Rennenberg for accepting me as a Ph.D. student at the Chair of Tree Physiology of the University of Freiburg, for providing me with the subject of my Ph.D., for his helpful and critical supervision, and for undertaking the correction of this work. Furthermore, I would like to thank him for the financial support from resources of the Chair of Tree Physiology and for supporting my application for the scholarship from LGFG (Landesgraduiertenförderungsgesetz).

I thank a lot Dr. Arthur Geßler for the direct supervision and his great help on the realization of the present work. I am especially thankful to him for listening without complaints to my numerous questions, for the constructive discussions and his creative way of thinking that often gave solutions to many occurring problems. I have learnt a lot from him.

I thank Prof. Dr. Siegfried Fink, Chair of Forest Botany, for undertaking the examination of the present work and for supporting my application for the scholarship from LGFG (Landesgraduiertenförderungsgesetz).

I thank Prof. Dr. Heinrich Spiecker, Institute for Forest Growth, for undertaking the oral examination of the present work and providing me with material during the writing of this study.

I would like to thank Dr. Andreas D. Peuke for being always willing to help, for his statistical advices and his remarkable humor, which made the late-evening working overtimes enjoyable.

I am particularly thankful to Dr. Stanislav Kopriva for introducing me to the world of molecular biology, for his patience while teaching me the methods, as well as for the interesting discussions.

I thank Prof. Dr. Mark A. Adams, University of Mellburn, Australia, for his helpful criticism and discussions during the writing of the first publication within this work.

I thank Thomas Holst and Prof. Dr. Helmut Mayer, Institute of Meteorology, for providing me with meteorological data (precipitation, soil and air temperature, relative humidity, light intensity) and phenological data from the field site. Moreover, I thank Dr. Sabine Augustin and Prof. Dr. Ernst E. Hildebrand, Institute of Soil Science and Forest Nutrition, for providing me with data on soil water potential from the field site. Furthermore, they are all acknowledged for their co-operation and interest during the preparation of the last publication within the present work.

I thank Anita Heidenfelder, and Prof. Dr. Hans Papen, Institute of Meteorology and Climate Research – Atmospheric Environmental Research, for providing me with data on soil nitrate concentrations, as well as for their co-operation during the preparation of the third publication completed within this PhD work. Furthermore, I thank Dr. Rainer Gasche for providing me with information on the patterns of soil respiration in the field site.

I thank Thomas Paul and Prof. Dr. Albert Reif, Institute of Vegetation Science, for providing me with data on the coverage of understorey vegetation in the field site, and Björn Conrad for providing me with data on the density of beech regeneration in the field site.

I would also like to thank Dr. Jürgen Kreuzwieser for his interest and the helpful suggestions when problems came up.

I am particularly thankful to Michael Rienks (Michi) for being always willing to help to all kinds of technical problems coming up and for his expertise in everything that has to do with computers, thanks to which I often avoided any waste of time. Especially, I want to thank him for his help in developing the technique for the application of the ^{15}N -tracer solution in the field.

Many thanks go to Michael Nahm (Mischka) for his great help during the measuring campaigns following the greenhouse experiments, even though quite

early in the morning. Moreover, I thank him for his great contribution during my measuring campaigns in the field site, for willing to spend many consecutive days, and most of all weekends, in the forest and for making working pleasant even under unfavorable weather conditions. I would also like to thank Michael Pfeifer for his help and for introducing me to secrets of lab work at the very beginning of my PhD.

I am thankful to Peter Escher for his technical assistance during the measurements of stable isotopes and also for giving priority to my work contributing, thus, to its early completion. I also thank him for the nice conversations we had together. Moreover, I thank a lot Ilka Hetzger for the introduction in the procedure of amino acids measurements and for her great help with ASA, each time that problems came up.

I am thankful to Renate Nitschke for her willingness to help whenever I asked her to, and particularly for her help “fighting” any “enemies” that from time to time threatened my plants in the greenhouse. Many thanks go also to Monika Eiblmeier for helping me often in the lab with technical problems, as well for her help in searching and ordering chemicals and equipment which I have needed during my work.

Special thanks go to the secretaries of the Chair of Tree Physiology, Annette Schlierenkamp and Sabine Pregger for their valuable help in many bureaucratic procedures, within and out of my work, which I would not have been able to put in order by myself. I also thank them for the nice small chat-breaks during the work.

I thank a lot all colleges of the Chair of Tree Physiology during the last almost two and half years for the pleasant working atmosphere and all the nice time we had together. Particularly, I would like to thank the “group from above”; Tanja Hartmann, Jost Hauberg, Eleni Papadopoulou, Claudia Keitel, Petra Hönicke, Susanne Mult, Jörg Kruse (Bobby), Nacer Missoum for the good understanding during the many hours of work each day.

I am grateful to my teachers in Greece, Dr. Kalliopi Radoglou and Prof. Dr. Eleni-Isis Constantinidou for preparing me properly for the PhD studies, as well as for their great interest on the progress of my work here.

For the nice time we spent together, I am grateful to all new friends I have made in Germany. Many thanks go to Despina, Spyros, Savvas, Fabio, Kai, Anja and Kassa for making the first months in Freiburg much easier for me, as well as to Thorsten, Muriel, Karin and Maria for the nice evenings we spent together. Finally, I cannot thank enough Nektaria, Angela, Nikos and Vassilis for becoming a kind of a “family” for me here, for the unforgettable time we had together and for always being there for me.

I also thank a lot my old friends in Greece, and particularly Giwrgos, Sapfo and Maria, who kept in touch during these years and who so generously took care of everything that needed to be arranged for me during my absence from Greece and who helped me in many ways during my stays in Thessaloniki.

My family, and especially my parents and my brothers, I will always thank for their love, and help by all means every time I needed it. Especially, I am thankful to my mother for supporting my decision to come to Germany, under rather difficult conditions at that time. Probably I wouldn't be here if it wasn't her.

Last, but not the least, I thank Miltiadis for coming all the way from Greece to help me to my first measuring campaigns in the greenhouse and in the field site. I am also grateful that he took care of the bureaucratic matters that had to be settled during my absence from Greece and my settlement again back in Thessaloniki. But most of all I thank him for his love, support, trust and endless patience during these years and for always standing by me, despite the great distance. Finally, I thank him for being a great motivation for the early completion of this work.

This work was initiated within the field of the SFB-433 interdisciplinary project “Buchendominierte Laubwälder unter dem Einfluss von Klima und Bewirtschaftung - Beech-dominated deciduous forests under the influence of climate and management” funded by the DFG (Deutsche Forschungs-gemeinschaft). This PhD was financially supported by the DAAD (Deutscher Akademischer Austauschdienst) and by the LGFG (Landesgraduiertenförderungsgesetz), as well as by means of the Chair of Tree Physiology, University of Freiburg.