Mykorrhizafunktion bei der Konkurrenz um Stickstoff in Kalkbuchenwäldern

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Inhaltsverzeichnis

Abkürzungsverzeichnis

A	Ascomycota
a.s.l.	above see level
Ah	Oberbodenhorizont mit Humusanreicherung
amoA	Ammonia monooxigenase
ANOVA	Analysis of variance
AOA	Ammonia oxidizing archaea
AOB	Ammonia oxidizing bacteria
APE	Atom % Excess
apr	Protease
Asco	Ascomycota
B	Basidiomycota
Basido	Basidiomycota
BSA	Bovine serum albumin
BV	Blind value
C	Kohlenstoff
C.	*Cenococcum*
$CaSO_4$	Calciumsulfat
chiA	Chitinase
CO_2	Kohlenstoffdioxid
$CuSO_4$	Kupfer(II)sulfat
DEEMY	Determination of Ectomycorrhizae (Database)
DIN	Dissolves inorganic N
DMSO	Dimethyl sulfoxid
dNTP	Desoxynucleotide
DOC	Dissolved organic C
DON	Dissolved organic N
EDD	Ectomycorrhizae Descriptions Database
EDTA	Ethylenediaminetetraacetic acid
EM	Ectomycorrhiza
EMF	Ectomycorrhizal fungi
exc.	excavated
FEA	Formalin-ethyl acetate
$FeCl_3$	Eisen(III)chlorid
FR	Fine roots
fw	Fresh weight
GLM	General linear model

H_3BO_3	Borsäure
IAEA	International Atomic Energy Agency
ICPF	International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests
Ident.	Identificated
IPCC	Intergovernmental Panel on Climate Change
IRMS	Isotope ratio mass spectometry
Isol.	Isolated
ITS	Internal transcribed spacer
K_2SO_4	Kaliumsulfat
k_{EN}	Conversion factor
KH_2PO_4	Kaliumdihydrogensulfat
MB	Microbial biomass
MBC	C in microbial biomass
MBN	N in microbial biomass
$MgCl_2$	Magnesiumchlorid
$MgSO_4$	Magnesiumsulfat
Micr.	Microbial
$MnSO_4$	Mangan(II)-sulfat
MT	Morphotyp
N	Stickstoff
N_2	molekularer Stickstoff
$NaMoO_4$	Natriummolybdat
narG	Nitrate reductase
$NaSO_4$	Natriumsulfat
NCBI	National Center for Biotechnology Information
NE	North East
NH_3	Ammoniak
NH_4^+	Ammonium
NH_4Cl	Ammoniumchlorid
nifH	Nitrogenase
nirK	Nitrite reductase
nirS	Nitrite reductase
NM	Non mycorrhizal root tips
NO	Nord Ost
NO	Stickstoffmonoxid
NO_2	Stickstoffdioxid
NO_3^-	Nitrat
nosZ	Nitrous-oxide reductase
NW	Nord West
PAST	Paleontological statistics software package
PCR	Polymerase Chain Reaction
pdw	Plant dry weight
PMSF	Phenylmethylsulfonyl fluoride
PVP	Polyvinylpyrrolidone
RCC	Rhizosphere root complex
rDNA	ribosomale DNA

RFLP	Restriction Fragment Length Polymorphism
RS	Rhizosphere soil
RT-PCR	Real Time - Polymerase Chain Reaction
SDW	Soil dry weight
SE	Standard error
SFW	Soil fresh weight
SPADE	Species Prediction and Diversity Estimation
SRES A2	Special Report on Emissions Scenario A2 (a very heterogeneous world with continuously increasing global population and regionally oriented economic growth)
SW	South West
SWC	Soil water content
t	Timepoint
TN	Total nitrogen
TNb	Total chemically bound N in soil extracts
TOC	Total organic C
TRF	Terminal restriction fragment
TRFLP	Terminal restriction fragment length polymorphism
UNITE	Unified system for the DNA based fungal species linked to the classification
UPLC	Ultra Performance Liquid Chromatography
USGS	United States Geological Survey
VPDB	Vienna Pee Dee Belemnite (IAEA Standard)
WHC	Water holding capacity
WRB	World Reference Base
$ZnSO_4$	Zinksulfat

Abbildungsverzeichnis

Tabellenverzeichnis

Zusammenfassung

Die Buche (*Fagus sylvatica* L.) ist sowohl in ökonomischer als auch in ökologischer Hinsicht in Mitteleuropa eine sehr wichtige Baumart. Häufig sind Buchenwälder auf Böden anzutreffen, auf denen Stickstoff das limitierende Nährelement ist. Auf solchen Böden konkurrieren deshalb Buchen, Ektomykorrhizapilze und Bodenbakterien um den verfügbaren Stickstoff. Der prognostizierte Klimawandel mit steigenden Temperaturen, Trockenperioden und Starkregenereignissen wird den Stickstoffkreislauf und die Konkurrenzverhältnisse beeinflussen, da sich zwei der wichtigsten Bodenparameter, Bodenfeuchte und Bodentemperatur, verändern werden. In diesen Parameter unterscheiden sich auch die beiden Versuchsflächen in Baden-Württemberg in der Nähe von Tuttlingen, die für die Versuche dieser Arbeit ausgewählt wurden. Die Flächen befinden sich gegenüberliegend in einem Tal und sind auf der einen Seite NO bez. NW exponiert und auf der anderen Seite SW exponiert. Der SW-Hang weist im Vergleich zu den nördlich exponierten Hängen eine erhöhte Bodentemperatur und eine verminderte Bodenfeuchte auf und kann damit als Modellstandort für das prognostizierte zukünftige Klima gelten. Wie die Buche mit den erwarteten klimatischen Änderungen umgehen wird, wird unter anderem auch von der Reaktion der Ektomykorrhizapilze abhängen. Diese spielen bei der Stickstoffaufnahme der Buchen eine entscheidende Rolle, da die Wurzelspitzen der Buche in natürlichen Ökosystemen praktisch vollständig mit Ektomykorrhizapilzen kolonisiert sind.

Um die komplex zusammengesetzten Mykorrhizagesellschaften an Buchen charakterisieren und in Bezug auf ihre Funktion analysieren zu können, sind Informationen über die Morphologie und Anatomie der einzelnen Pilzarten notwendig. Selbst in Mitteleuropa fehlen aber für einen Großteil der Ektomykorrhizen exakte wissenschaftliche Beschreibungen. Daher wurde exemplarisch ein mykorrhizaler Morphotyp, der auf den Tuttlinger Versuchsflächen häufig vorkommt, morphologisch und anatomisch beschrieben und gezeichnet. Wegen seiner dicht mit langen Cystiden besetzten Manteloberfläche wird er dem „Short distance“-Explorationstyp zugeordnet. Besonders auffällig sind die drei bis sechsfachen Verzweigungspunkten der Cystiden, die charakteristisch verdickt sind und denen die Mykorrhiza ihren provisorischen Namen „Fagirhiza inflata“ verdankt. Die aufgrund der anatomischen Merkmale und der dextrinoiden Reaktion von Mantel und Cystiden mit Melzers Reagenz angenommene Zugehörigkeit zur Gattung *Sebacina* wurde durch die ITS-Sequenzierung bestätigt.

Bisher war unklar, ob die Stickstoffversorgung von Buchen unter gegebenen Bedingungen von den Bodenmikroben, den Mykorrhizapilzen oder der Herkunft der Bäume abhängt. In einem Experiment sollte untersucht werden, ob und wie sich die unterschiedliche Artenzusammensetzung von Mykorrhizagesellschaften auf die Stickstoffakkumulation in den mykorrhizierten Wurzelspitzen und den Stickstofftransfer zu den Pflanzen auswirkt. Es wurden junge, genetisch ähnliche Buchen mit ihren assoziierten Mykorrhizagesellschaften aus Tuttlingen von einem NO-Hang und einem SW-Hang in einen homogenen Boden transferiert und unter gleichen klimatischen Bedingungen mit ^{13}C und ^{15}N markiert. Die geringe mikrobielle Biomasse im Substrat führte dazu, dass die Konkurrenz um den Stickstoff mit Bakterien stark vermindert wurde. Die nicht mykorrhizierten Wurzelspitzen der NO- und SW-Buchen akkumulierten unter diesen Bedingungen das ^{15}N gleich stark. Im Gegenteil dazu akkumulierten die mykorrhizierten Wurzelspitzen der NW-Buchen das ^{15}N

stark verzögert im Vergleich zu den mykorrhizierten Wurzelspitzen der SW-Buchen. Korrespondierend dazu dauerte bei den NO-Buchen der Transfer des ^{15}N zu den Feinwurzeln und Blättern länger und erfolgte in niedrigeren Raten als bei den SW-Buchen. Daraus folgt, dass die Mykorrhizagesellschaften den N-Transport zur Pflanze kontrollierten. Außerdem zeigen diese Ergebnisse, dass die Mykorrhizapilze, die sich an trockene und warme Bedingungen angepasst haben, die Leistungsfähigkeit von den an moderate Bedingungen angepassten Mykorrhizapilzen in Bezug auf die Akkumulation von Stickstoff sogar übertreffen, wenn die umweltbedingten Einschränkungen wegfallen. Die Ergebnisse legen nahe, dass die Zusammensetzung der Mykorrhizagesellschaften entscheidend für die Zukunftsfähigkeit der Buchenwälder ist.

Um die Auswirkungen des prognostizierten Klimawandels (erhöhte Temperatur und niedrigere Bodenfeuchte) auf die Stickstoffversorgung von Buchen abschätzen zu können, wurde Buchennaturverjüngung mit umgebendem Boden (Mesokosmen) in Tuttlingen von dem NW-Hang auf den gegenüberliegenden SW-Hang ("Klimawandel" Behandlung) oder von dem NW auf den NW Hang (Kontrolle) transferiert. Die Buchen wurden für ein Jahr unter diesen Bedingungen kultiviert. In der nachfolgenden Vegetationsperiode wurde nach Injizieren von ^{15}N markierten Stickstoffformen (Glutamin, Ammonium, Nitrat) in den Boden an mehreren Zeitpunkten geerntet. Anhand der ^{15}N-Aufnahmeraten stellte sich Nitrat als die dominierende Stickstoffquelle für die Buchen heraus. Die klimatischen Bedingungen auf der SW-Seite führten zu einem Einbruch bei der Nitratbereitstellung durch die Bodenbakterien und damit zu Nitratmangel und nachfolgend zu einer Reduktion der Biomasse der Buchennaturverjüngung. In den mykorrhizierten Wurzelspitzen hingegen zeigte sich durchgängig, dass ^{15}N aus Ammonium am stärksten akkumuliert wurde, gefolgt von Nitrat und Glutamin. In den meisten Fällen wurde auf der SW-Seite signifikant oder tendenziell weniger ^{15}N akkumuliert als auf der NW-Seite. Intaktes Glutamin wurde weder in den mykorrhizierten Wurzelspitzen noch in den Buchen festgestellt, was auf eine sehr geringe Bedeutung von organischen Stickstoffformen für die Stickstoffversorgung der Buchen schließen lässt. Die Ergebnisse lassen befürchten, dass es in Zukunft große Probleme bei der Stickstoffversorgung der Buche geben wird. Vermutlich sind diese Restriktionen in der Stickstoffversorgung eine der Ursache für die prognostizierte erhebliche Reduktion der Kalkbuchenwälder bis zum Ende des 21. Jahrhunderts.

Abstract

Beech (*Fagus sylvatica* L.) is a very important tree species in Central Europe due to its economical value and ecological services. Beech forests are often growing on soils, where nitrogen is the growth limiting factor. Under these conditions beech trees, ectomycorrhizal fungi and soil bacteria are competing for the bioavailable nitrogen in the soil. The predicted climate change will influence the nitrogen cycle and competition for nitrogen, because the two main soil parameters, soil humidity and soil moisture, will change. These two parameters are different at the two experimental sites near Tuttlingen (SW Germany). The two sites are divided by a narrow valley and are on the one hand side NE or respectively NW exposed and on the other side SW exposed. Compared to the northern exposed slopes, the SW slope shows higher soil temperature and lower soil humidity. Therefore the SW-slope can be taken as a model site for future climate conditions. The reactions of beech forests to climate change are, among other factors, dependent on the reaction of ectomycorrhizal fungal to climate change. These fungi are very important for nitrogen nutrition of beech, because nearly all root tips are colonized by these fungi.

For analyzing complex assembled mycorrhizal communities and characterizing their functions, information about morphology and anatomy of single species are necessary. But even in Central Europe scientific descriptions for most of the ectomycorrhizal fungi are lacking. Because of this lacking knowledge, a common mycorrhizal morphotype of the experimental sites in Tuttlingen was morphological and anatomical described and drawn. The new morphotyp is densely covered with cystidia and is therefore determined as a “Short distance”-Exploration type. Characteristic are the inflated three to six fold polytomies of the cystidia. They lead to the temporary name “Fagirhiza inflata”. Anatomical features and dextrinoide reaction with Melzer’s reagent suggested this fungus belonging to the order *Sebacina*, which was confirmed via ITS sequencing.

Until now it was not clear, if nitrogen nutrition of beech trees is dependent on soil bacteria, mycorrhizal fungi or origin of the trees. In an experiment the influence of different mycorrhizal assemblages on nitrogen accumulation in root tips and subsequent transfer to plants was examined. Young, genetically similar beech trees including associated mycorrhizal assemblages from Tuttlingen NE and SW slope were transferred to a homogenous soil. The trees were cultivated under equal climate conditions and labeled with ^{13}C and ^{15}N before harvest. Because of very low microbial biomass in the soil, competitive strength of microbial biomass was strongly reduced. ^{15}N accumulation in non mycorrhizal root tips from NE and SW beeches was similar under these conditions. In contrast to this result, mycorrhizal root tips of SW trees accumulated more ^{15}N than mycorrhizal root tips from NE beech trees. Corresponding to this ^{15}N transfer to fine roots and leaves was faster in SW trees compared to NE trees. This result shows that mycorrhizal fungi are controlling the N transfer to the plant. Furthermore these results show, that drought and warm conditions adapted mycorrhizal fungi are more efficient under mild conditions than the mycorrhizal fungi adapted to the mild conditions, if climatically constraints do not exist. These findings suggest that community structure of ectomycorrhizal fungi is crucial for sustainability of beech forests.

To estimate impacts of the predicted climate change (higher temperature and lower soil moisture) on nitrogen nutrition of beech trees an experiment under field conditions was

conducted. Beech soil systems (mesocosms) were transferred from a NW to a SW slope (climate change treatment) and from a NW to a NW slope (control). The beech trees were cultivated under site specific condition for one year. In the following vegetation period beech trees were harvested after injections with ^{15}N labelled nitrogen forms (glutamine, ammonium and nitrate) at several time points. Climatic constraints lead to a strong reduction of soil bacteria which can provide nitrate. As a consequence beech trees showed a reduction in biomass because nitrate was the dominant nitrogen source for beech nutrition, indicated by highest ^{15}N accumulation of all three nitrogen forms. In contrast to beech trees mycorrhizal root tips showed highest ^{15}N accumulation derived from ammonium, followed by nitrate and glutamine. In most cases, accumulation in mycorrhizal root tips on the SW slope was significant or by trend lower than on the NW slope. Intact glutamine was found neither in mycorrhizal root tips nor in beech trees. This means organic nitrogen is not important for beech nutrition.

In conclusion, these results show, that in future serious problems in nitrogen nutrition of beech trees can occur. A model, which predicts a strong reduction of beech forest in Central Europe at the end of the 21th century, takes into account the change of soil parameters shown by the experiments described here.

1 Einleitung

1.1 Mykorrhiza – eine Symbiose zwischen Pflanze und Pilz

Unter einer Mykorrhiza versteht man eine im Bereich der Feinwurzeln angesiedelte, meist mutualistische Beziehung zwischen einer Gefäßpflanze und einem Pilz. Der Pilz hat durch sein oft weit verzweigtes, aus dünnen Hyphen bestehendes, extramatrikales Mycel Zugriff auf Nährstoff- und Wasserressourcen, die für die Pflanze mit ihren im Vergleich zu den Hyphen sehr voluminösen Feinwurzeln nicht erreichbar sind. Im Austausch mit photosynthetisch gewonnenen Kohlenhydrate gibt der Pilz Nährstoffe wie Stickstoff oder Phosphat an die Pflanze weiter (Smith and Read, 2010).

Die evolutionäre Entwicklung dieser Symbiose ist eng an die Entwicklung der Landpflanzen geknüpft (Brundrett, 2002). Bereits im Devon vor ca. 400 Mio. Jahren traten zusammen mit den ersten Bryophyten arbuskuläre Mykorrhizen auf (Taylor et al., 1995). Im Laufe der Zeit haben sich verschiedene Typen von Mykorrhizen entwickelt, die sich bezüglich ihrer Morphologie und ihrer Wirtspflanzen unterscheiden. Die zur heutigen Zeit in den temperaten und borealen Wäldern dominierende Form der Ektomykorrhiza trat vermutlich vor ca. 180 Mio. Jahren das erste Mal auf (Berbee and Taylor, 1993). Die ältesten fossilen Nachweise von Ektomykorrhizen an einer Laubbaumart stammen aus dem Unteren Eozän und sind ca. 52 Millionen Jahre alt (Beimforde et al., 2011).

Theodor Hartig beschrieb 1840 als Erster die Strukturen des äußeren Hyphenmantels und der interzellulären Hyphen, ohne jedoch zu erkennen, dass es sich um Pilze handelt (Trappe, 2005). Nach ihm ist das „Hartigsche Netz“ benannt, das als Austauschorgan zwischen Pilz und Pflanze dient. Nachdem Bruchmann (1874) erkannt hatte, dass diese Strukturen pilzlichen Ursprungs sind, prägte Albert Bernhard Frank (1885) den Begriff Mykorrhiza. Er fertigte detaillierte Zeichnungen an und stellte die wegweisende Hypothese auf, dass es sich um eine symbiotische Beziehung handelt und dass der Pilz zur Ernährung der Pflanze beiträgt. Es dauerte jedoch über 50 Jahre, bis seine Hypothese bestätigt werden konnte (Trappe, 2005).

Heutige Schätzungen gehen von 7000 – 10000 Pilzarten aus, die in der Lage sind, Ektomykorrhizen auszubilden (Rinaldi, 2008; Taylor and Alexander, 2005). Der mit 95 % größte Anteil gehört zum Phylum der Basidiomyceten. 4,8 % gehören zum Phylum der Ascomyceten und die verbleibenden 0,2 % zum Phylum der Zygomyceten (Molina et al., 1992). Ca. 8000 verholzende Pflanzen kommen als Partner in Frage (Meyer, 1973). Die Buche, der durch ihre vorherrschende Stellung in der potentiellen natürlichen Vegetation Mitteleuropas (Ellenberg and Leuschner, 1996) eine besondere Bedeutung zukommt, ist zu nahezu 100 % mykorrhiziert und wird innerhalb eines Bestandes von ca. 60 - 90 Pilzarten besiedelt (Buée et al., 2005; Pena et al., 2010).

1.2 Die Anatomie und Morphologie von Ektomykorrhizen

Ektomykorrhizen lassen sich in die anatomisch sehr unterschiedlichen Organe Hartigsches Netz, Mantel und extramatrikales Mycel einteilen (abziehende Hyphen und Rhizomorphen) (Abbildung 1.1). Das Hartigsche Netz ist der Bereich, über den der Stoffaustausch zwischen den beiden Organismen stattfindet. Bei Angiospermen beschränkt sich der besiedelte Bereich in der Regel auf die Epidermis (Godbout and Fortin, 1983). Es gibt aber Mykorrhizapilze z.B. an der Buche, die auch die Cortex- und Endodermiszellen besiedeln (Clowes, 1951). Bei Gymnospermen werden in der Regel die Cortexzellen und teilweise auch die Endodermiszellen umwachsen (Smith and Read, 2010). Überwiegend findet radiales und nur sehr eingeschränkt longitudinales Hyphenwachstum statt (Kottke and Oberwinkler, 1986). Die Hyphen verzweigen sich im Interzellularraum, anders als im Mantel und im extramatrikalen Mycel, extrem häufig (Mangin, 1910) und liegen dicht gepackt (Smith and Read, 2010). Dadurch entsteht eine große Kontaktfläche, über die der Stoffaustausch stattfinden kann. Sowohl Basidiomyceten als auch Ascomyceten bilden, auch an verschiedenen Wirtspflanzen, sehr ähnliche Strukturen (Blasius et al., 1986).

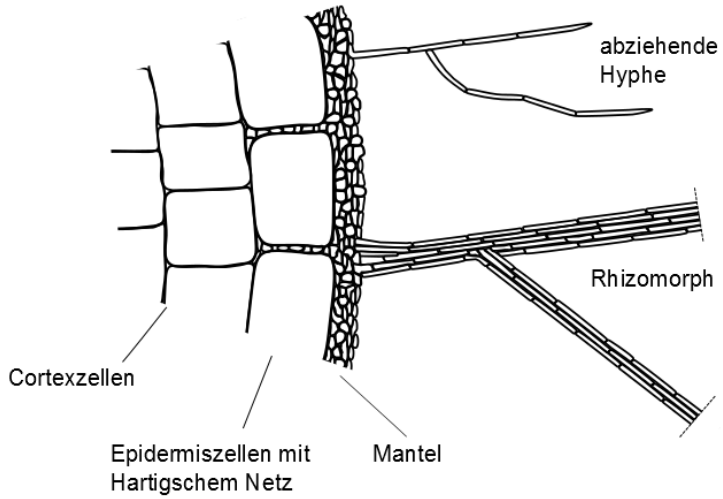

Abbildung 1.1: Schematischer Aufbau einer Ektomykorrhiza mit pflanzlichen (Cortex- und Epidermiszellen) und pilzlichen Geweben (Hartigsches Netz, Mantel, abziehender Hyphe und Rhizomorph)

Der Mantel umhüllt die mykorrhizierte Wurzelspitze und ist das Bindeglied zwischen dem extramatrikalen Mycel und dem Hartischen Netz. Je nach Mächtigkeit und Struktur liegt der Gewichtsanteil des Mantels an der mykorrhizierten Wurzelspitze zwischen 20 % (Vogt et al., 1982, 1991) und 40 % (Harley and McCready, 1952). Man unterscheidet zwischen plectenchymatischen und pseudoparenchymatischen Manteltypen. Ein pseudo-parenchymatischer Mantel besteht aus isodiametrischen bis unregelmäßig puzzleartigen Zellen, die kaum noch an die fadenartigen Strukturen von Hyphen erinnern. In einem plectenchymatischen Mantel sind die fadenartigen Hyphen als solche noch erkennbar. Es können größere Interhyphalräume auftreten. Teilweise sind die Zwischenräume auch mit

einer Matrix gefüllt (Godbout and Fortin, 1983; Kottke and Oberwinkler, 1986). Ein Mykorrhizamantel ist oft in mehrere Schichten gegliedert, die unterschiedliche anatomische Merkmale besitzen (Agerer, 1987-2006). Plectenchymatische und pseudoparenchymatische Schichten können sich dabei abwechseln. Häufig kommen auch spezielle Strukturen wie Setae (sehr spitze, dickwandige und häufig stark melanisierte Zellen), Cystiden (teils mit giftigen Substanzen gefüllte, dünnwandige Zellen), Milchröhren und pigmentierte Hyphen vor (Agerer, 1986; Peyronel, 1960; Taylor and Alexander, 2005). Mantelstrukturen sind zum Teil spezifisch und können daher sehr gut zur Bestimmung von Mykorrhizen herangezogen werden (Agerer, 1995). Die Hyphenoberflächen werden auch von Bakterien besiedelt, die die Funktion der Symbiose unterstützen können (Frey-Klett et al., 2007).
Die Funktion des Mantels ist primär die Speicherung von Nährstoffen wie Phosphor- und Stickstoffverbindungen, z.B. für die Fruchtkörperproduktion, bei der in einem kurzen Zeitfenster große Mengen an Nährstoffen benötigt werden (Taylor and Alexander, 2005). Die P- und N-Konzentrationen sind vier- bis fünffach höher als im Pflanzengewebe (Vogt et al., 1981) und scheinen auch an den Nährstoffbedarf der Wirtspflanze gekoppelt zu sein (Genet et al., 2000; Lussenhop and Fogel, 1999). Um die Speicherfunktionen erfüllen zu können, müssen die Mäntel relativ langlebig sein. Die Lebensdauer der mykorrhizierten Spitzen reicht von einigen Monaten bis hin zu 2,5 Jahren (Withington et al., 2006). Der Mantel wird z.B. mechanisch durch Setae und chemisch durch Cystiden vor Fraß geschützt (Taylor and Alexander, 2005). Zusätzlich können die Hyphen des Mantels und auch des extramatrikalen Myzels mit Kristallen aus z.B. Calciumoxalat besetzt sein (Brand, 1991).

Vom Mantel ziehen sehr häufig Hyphen und Rhizomorphen (aus mehreren Hyphen zusammengesetzte Mycelstränge) in den Boden ab und bilden gemeinsam das extramatrikale Mycel. Dieses Pilzmycel wirkt stark oberflächenvergrößernd und erschließt Bodenregionen, die nicht im direkten Kontakt zum Mantel stehen. Neben den einzelnen Hyphen werden 6 verschiedene Rhizomorphentypen unterschieden (Agerer, 1991, 1995). Die Formen reichen von lose verwobenen Hyphen bis hin zu kompakten, differenzierten, im Inneren mit großen, gefäßartig aufgebauten Hyphen ohne Septen, die einen Transport von Nährstoffen und Wasser über große Distanzen gewährleisten. Für Stabilität sorgen verschiedene Typen von Anastomosen (Verbindung zwischen zwei Hyphen), die offen oder septiert sein können, gelatinöse Zellwände und zurück- und vorwärtsgerichtete Verzweigungen (Agerer 1992). Während die Transportorgane überwiegend hydrophob sind, sind die Spitzen der Hyphen, oder die aufgefächerten Enden der Rhizomorphen meist hydrophil, um Nährstoffe und Wasser besser aufnehmen zu können (Unestam and Sun, 1995). Da die Strukturen eine Vielzahl an Merkmalen bieten, die teilweise charakteristisch für Gattungen oder Arten sind, können Rhizomorphen und abziehende Hyphen ebenfalls gut zur Bestimmung von Ektomykorrhizen verwendet werden (Agerer, 1987-2006).

Eng verknüpft mit den Formen des extramatriaklen Mycels ist das Konzept der Explorationstypen (Agerer, 2001). Je nach Ausprägung der Strukturen werden Bodenvolumina in der Nähe oder in weiterer Entfernung von der Wurzelspitze erschlossen und unterschiedliche ökologische Nischen besetzt. Der Mantel von Ektomykorrhizen des „contact“-Typs, z.B. *Russula* sp. und *Lactarius* sp., ist glatt und besitzt nur sehr vereinzelt abziehende Hyphen (Agerer, 2006). Die Nährstoffaufnahme erfolgt über die Manteloberfläche (Harley and Smith, 1983). Ektomykorrhizen vom Typ „short distance“ haben eine große Anzahl an Hyphen mit einer Länge von bis zu ca. 0,5 cm, die das nahe

Umfeld der Wurzelspitze ausbeuten können. Ein prominentes Beispiel ist *Cenococcum geophilum* (Agerer, 2001). „Medium distance“ Typen sind durch relativ undifferenzierte Rhizomorphen mit einer Reichweite im Zentimeterbereich charakterisiert. Es werden die Untertypen „fringe“ mit fächerartig vom Mantel abziehende Hyphen und vielfach verzweigten, undifferenzierten Rhizmorphen, „mat“ mit großflächigem, mattenartigem Myzel und „smooth“ mit glatten Mantel und meist undifferenzierten Rhizomorphen unterschieden. „Long distance“ Typen bilden differenzierte Rhizomorphen, die Wasser und Nährstoffe über weite Strecken transportieren können. Bei *Pisolithus tinctorius* wurden zum Beispiel 42 cm Rhizomorphenlänge gemessen (Schramm, 1966). Pilze der Gattungen *Boletus*, *Paxillus* und *Suillus* sind typische Vertreter dieses Typs (Agerer, 2006). Es kommt also für die Nährstoffversorgung des Wirtes nicht nur auf die Abundanz eines Morphotypes an (Thomson et al., 1994), sondern auch auf die Menge und Ausprägung des extramatrikalen Systems (Kammerbauer et al., 1989; Rousseau et al., 1992). Zusätzlich zur Exploration ist das extramatrikale Myzel auch für die Besiedelung von unmykorrhizierten Wurzelspitzen verantwortlich (Brundrett, 1991; Read, 1992).

1.3 Die Rolle der Mykorrhiza im Stickstoffkreislauf der Waldökosysteme

Waldökosysteme sind häufig durch Stickstoffarmut geprägt, mit der Folge, dass das Wachstum der Bäume stickstofflimitiert ist (Chapman et al., 2006; Lovett et al., 2004; Rennenberg et al., 2009). Wegen der geringen N-Zufuhr sind diese Ökosysteme auf ein effizientes Recycling und gleichzeitig auf geringe N-Verluste angewiesen (Schimel and Bennett, 2004). Die grundlegenden Prozesse des Stickstoffkreislaufes sind in Abbildung 1.2 dargestellt.

Die Zufuhr von Stickstoff in den Kreislauf erfolgt entweder über atmosphärische Deposition oder über die Fixierung von atmosphärischem Stickstoff durch Mikroorganismen (Rennenberg et al., 2009). Die Deposition ist anthropogen beeinflusst und kann in stark belasteten Gebieten bis zu 60 kg N ha-1 Jahr-1 (Tietema and Beier, 1995). Sie erfolgt in Form von NO_2, NO, NO_3^-, NH_3 und NH_4^+ (Rennenberg and Gessler, 1999). Im Vergleich dazu fällt die N_2-Fixierung in den im Rahmen dieser Dissertation betrachteten temperaten Wäldern Mitteleuropas mit weniger als 3 kg N ha^{-1} $Jahr^{-1}$ sehr gering aus (Cleveland et al., 1999). Sterben die N-fixierenden Mikroorganismen ab, werden deren komplexe organische Verbindungen depolymerisiert und stehen danach als monomere Verbindung den Pilzen, Pflanzen oder ammonifizierenden Mikroorganismen zur Verfügung (Rennenberg et al., 2009). Depolymerisation ist auch der erste Schritt beim Abbau abgestorbener pflanzlicher Biomasse, d.h. insbesondere von Blättern und Feinwurzeln. Insbesondere saprotrophisch lebende Pilze sind durch eine geeignete Ausstattung an extrazellulären Enzymen in der Lage, komplexe organische Stickstoffquellen zu erschließen (Bending and Read, 1996a, 1996b; Dighton, 2007). Nach der Depolymerisation können sowohl Mikroorganismen und Pflanzen auf die organischen Verbindungen zugreifen und stehen somit in Konkurrenz zueinander (Rennenberg et al., 2009). Teilweise können auch Mykorrhizapilze die organischen Stickstoffquellen abbauen und damit die Ammonifizierung und Nitrifizierung durch andere Mikroben umgehen (Cullings and Courty, 2009; Read and Perez-Moreno, 2003). Es wurde gezeigt, dass Pflanzen sowohl ohne Mykorrhiza (Abuzinadah and Read, 1986; Sangwanit, 1986) als auch mykorrhiziert unter natürlichen Bedingungen organische

Stickstoffverbindungen aufnehmen können (Dannenmann et al., 2009; Finzi and Berthrong, 2005; Näsholm et al., 1998; Schmidt and Stewart, 1999). In Reinkulturen der Pilze wurde die Aufnahme von Aminosäuren vielfach nachgewiesen (Abuzinadah and Read, 1989; Anderson et al., 1999; Dannenmann et al., 2009; Jackson et al., 2008; Keller, 1996; Martin et al., 2008). Ob die direkte Aufnahme organischer Verbindungen allerdings in temperaten Wäldern *in situ*, d.h. insbesondere unter dem Einfluss der Konkurrenz von mineralisierenden Bakterien, eine wesentliche Rolle spielt, ist unklar. Werden die monomeren organischen Stickstoffverbindungen (Aminosäuren, Aminozucker und Nukleotide) nicht durch Mykorrhizapilze oder direkt von der Pflanze aufgenommen, werden sie über die Prozesse der Ammonifikation und der Nitrifikation zuerst zu Ammonium und dann zu Nitrat reduziert (Abb. 1.2). Die Stickstoffformen Ammonium und Nitrat werden von den mykorrhizierten Wurzelspitzen bei den im Boden vorkommenden N Konzentrationen meist aktiv aufgenommen(Glass et al., 1992). Die Ammoniumaufnahmeraten von mykorrhizierten Wurzelspitzen der Buche liegen zwischen 0,1 – 0,7 µmol g^{-1} fw h^{-1}, die Nitrataufnahmeraten zwischen 0,02 – 0,17 µmol g^{-1} fw h^{-1} (Geßler et al., 2005). Die Aufnahmeraten und das Verhältnis von Ammonium- zu Nitrataufnahme sind stark von der Temperatur abhängig (Geßler et al., 2005). In Experimenten mit markiertem Stickstoff wurde festgestellt, dass je nach Mykorrhizapilzart unterschiedlich viel Stickstoff in den Wurzelspitzen akkumuliert wird und sich damit deren Bedeutung für die Stickstoffversorgung der Pflanze unterscheidet (Pena and Polle, 2013). Da Mykorrhizagesellschaften üblicherweise aus Pilzen unterschiedlicher Explorationstypen gebildet werden, wird eine räumlich (Agerer et al., 2002) und zeitlich (Pena et al., 2013a) gestaffelte Ausbeutung der N-Quellen ermöglicht. Der Kreislauf beginnt erneut, wenn Organismen oder Teile davon absterben und recycelt werden.

Dennoch entstehen zum einen durch Denitrifizierung über die Atmosphäre und zum anderen durch Auswaschung über die Hydrosphäre Stickstoffverluste. Ausgewaschen wird N überwiegend in Form von DON (gelöster organischer Stickstoff) oder leicht löslichem Nitrat (Gessler et al., 1998). Insbesondere nach starken Regenfällen mit vorausgehender Trockenheit kann es zu erheblichen N Verlusten durch Auswaschung kommen, da durch die abgestorbenen Mikroorganismen viel DON freigesetzt wird (Borken and Matzner, 2009). Ammonium findet sich nur in geringen Mengen in der Bodenlösung, da die Ammonifikation langsamer verläuft als die Nitrifikation (Horn et al., 2010). Zusätzlich werden die positiv geladenen Ammoniumionen häufig an Tonmineralen fixiert und sind damit weniger mobil als Nitrationen (Horn et al., 2010). Die Auswaschungsverluste durch Ammonium sind deshalb gering. Die atmosphärischen Stickstoffverluste erfolgen in den verschiedenen Stufen der Denitrifizierung in Form von NH_3, NO, N_2O und N_2 (Abb. 1.2). Für einen Kalkbuchenwald wurde eine Verlustrate von 1,8 - 6 kg N_2 ha^{-1} $Jahr^{-1}$ durch die Denitrifikation ermittelt (Dannenmann et al., 2008).

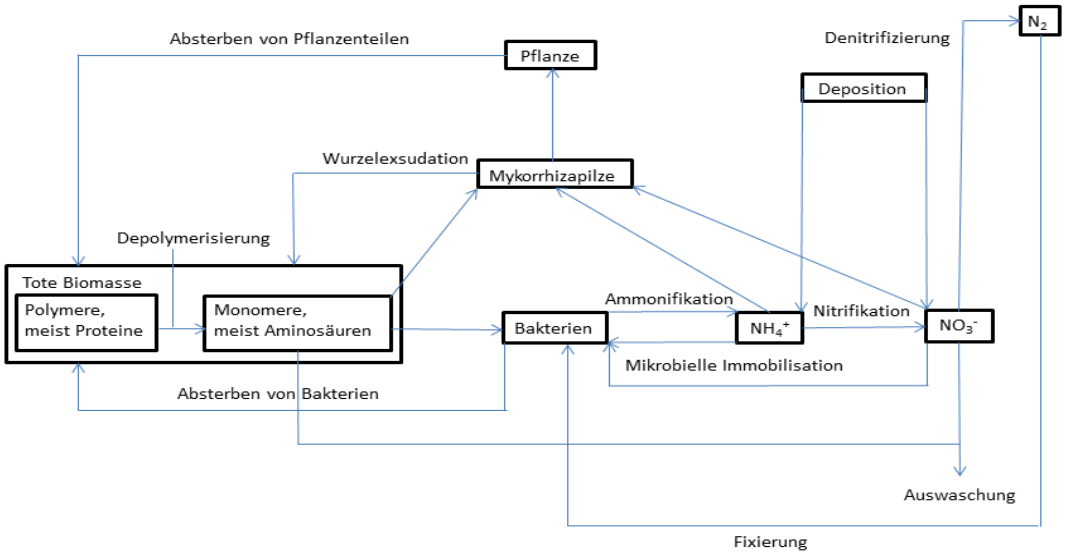

Abbildung 1.2: Der Stickstoffkreislauf in Waldökosystemen (modifiziert nach Rennenberg et al., 2009)

1.4 Klimawandelbedingte Änderungen im Stickstoffkreislauf der Waldböden können sich auf die Verbreitung und Produktivität der Buchenwälder auswirken

Die Rotbuche, Fagus sylvatica L., ist in Mitteleuropa weit verbreitet und dominiert die Wälder der potentiellen natürlichen Vegetation (Ellenberg and Leuschner, 1996). Sie ist deshalb nicht nur für die Forstwirtschaft von großer Bedeutung, sondern auch ein wichtiger Bestandteil der Kulturlandschaft (Schraml and Volz, 2004). Sie ist als Schattbaumart sehr konkurrenzstark gegenüber anderen Baumarten und akzeptiert eine große Bandbreite an bodenphysikalischen und –chemischen Beschaffenheiten: Sowohl auf basischen als auch auf sauren Böden, auf Böden mit unterschiedlich guter Nährstoffverfügbarkeit (Leuschner et al., 1993, 2006), in Gebieten mit einer mittleren Jahrestemperatur von 4 bis 12°C und einem Jahresniederschlag von 460 bis 2000 mm (Leuschner, 1998) ist sie bestandsbildend präsent. Ihre geographische Verbreitung umfasst West- und Mitteleuropa und die montane Stufe in Süd- und Südosteuropa, wobei sie auf der iberischen Halbinsel und in Griechenland nur in den nördlichen Teilen anzutreffen ist (Röhrig and Dengler, 1992).
In den letzten drei Jahrzehnten wurde die Buche in Deutschland im Rahmen des Waldumbaus von Nadelwäldern stark durch die forstpolitischen Vorgaben gefördert (Tarp et al., 2000). Das Ziel war, einen Wald zu formen, der mit einem hohen Buchenanteil der potentiellen natürlichen Vegetation näher kommt als die bisherigen nadelholzdominierten Wälder. Allerdings beruht das Konzept der potentiellen natürlichen Vegetation auf der Annahme, dass die klimatischen Verhältnisse stabil bleiben (Manthey et al., 2007). Dieses scheint für die Zukunft nicht mehr gegeben zu sein (IPCC, 2007). Die ansteigenden Temperaturen und die zunehmende Frequenz und Länge der Trockenperioden, gefolgt von starken Niederschlägen, können sich auf den Wasser- und Nährstoffhaushalt, auf den Zuwachs und auf das Konkurrenzverhalten von Buchen auswirken (Geßler et al., 2007). Insbesondere Standorte, auf denen das Wachstum der Buchen stickstofflimitiert ist und die Wasserverfügbarkeit niedrig ist, wie auf vielen Kalk- und Sandstandorten, könnten davon

betroffen sein (Geßler et al., 2007). Dabei wird die erhöhte Jahrestemperatur an sich nicht als vorrangiges Problem angesehen (Bolte, 2005), da z.B. die Ammonifikation und die Nitrifikation bei steigender Temperatur schneller ablaufen (Domisch et al., 2006; Emmett et al., 2004; Hart and Perry, 1999; Harte and Shaw, 1995; Stark and Firestone, 1996). Rustard et al. (2001) stellten in einer Metaanalyse fest, dass bei Bodenerwärmung mit steigender Netto-N-Mineralisierung und damit einhergehendem höheren N-Angebot zu rechnen ist, um das die beteiligten Organismen des Stickstoffkreislaufes dann konkurrieren (Dannenmann et al., 2009; Fotelli et al., 2002, 2005; Geßler et al., 2005). Die erwartete Temperaturerhöhung wird vermutlich nicht in der Lage sein, das Temperaturoptimum (Gessler et al., 1998) der beteiligten Enzyme am Stickstofftransport innerhalb der mykorrhizierten Wurzelspitzen zu überschreiten (Rennenberg et al., 2009).

Das eigentliche Problem stellen wahrscheinlich die vermehrt auftretenden Hitze- und Trockenperioden mit stark absinkender Bodenfeuchte dar (Bolte, 2005). Die Bodenfeuchte ist eine der wichtigsten Größen im Stickstoffhaushalt des Bodens, da die Konzentrationen der einzelnen Stickstoffformen in der Bodenlösung und die Transportvorgänge im Boden von ihr abhängig sind (BassiriRad et al., 1999; Geßler et al., 2005). Die mikrobielle Aktivität nimmt mit steigender Feuchte bis zu einem Optimum zu, das in Kalkbuchenwäldern in Süddeutschland bei ca. 60 – 70 % Bodenfeuchte liegt (Dannenmann et al., 2006) und sinkt danach wieder ab. Durch den prognostizierten Klimawandel werden vermutlich häufiger Bodenfeuchtewerte unterhalb des Optimums auftreten (Dannenmann et al., 2009). Die Abnahme der mikrobiellen Aktivität mit abnehmender Bodenfeuchte ist durch die zunehmende Dehydrierung der Mikroorganismen zu erklären, die bei Fortdauer bis zu deren Tod führen kann (Borken and Matzner, 2009). Bei anschließender Wiederbewässerung ist häufig ein starker Anstieg von DON zu beobachten. Der Stickstoff wird aus den sich lösenden toten Mikroorganismen freigesetzt (Borken and Matzner, 2009). Zusätzlich können bisher überlebende Mikroorganismen absterben, da durch die Bewässerung sehr starker osmotischer Stress ausgelöst wird (Kieft, 1987). Stickstoffverluste über die Hydrosphere sind die Konsequenz.

Unter trockenen Bedingungen kommt den Mykorrhizapilzen eine besondere Bedeutung zu. Die Vorteile mykorrhizierter Wurzelspitzen sind die Oberflächen- und Reichweitenvergrößerung und der Zugriff der Hyphen auf den Feinporenbereich (Smith and Read, 2010). Es wurde beobachtet, dass unter trockenen Bedingungen das Wasserpotential der mykorrhizierten Pflanzen im Vergleich zu unmykorrhizierten Pflanzen verzögert absinkt (Beniwal et al., 2010; Pena and Polle, 2013). Bei adulten Bäumen im Bestand, die über ein tiefreichendes Wurzelsystem verfügen, könnte die Versorgung der Wirtspflanze mit Nährstoffen von entscheidenderer Bedeutung sein, als die Versorgung der Wirtspflanze mit Wasser (Lehto and Zwiazek, 2011). Ein Hinweis darauf ist, dass mykorrhizierte Buchensämlingen unter trockenen Bedingungen besser mit Stickstoff versorgt werden (Pena et al., 2013b). Unter Bedingungen mit ausreichender Wasserversorgung tritt dieser Effekt nicht auf (Pena and Polle, 2013). Geßler et al. (2005) haben allerdings im Feld unter trockenen Bedingungen eine verminderte Nitrataufnahme mykorrhizierter Wurzelspitzen bei gleichzeitig höheren Ammonifikations- und Nitrifikationsraten feststellen können. Sie vermuten daher, dass nicht die Bereitstellung von anorganischem Stickstoff durch die Bodenbakterien oder veränderte Stickstoff-Allokationsmuster zwischen Bakterien und

Wurzeln sondern u. A. die reduzierte Aufnahmekapazität für die Zuwachseinbußen verantwortlich sind.

Darüber hinaus ist auch das Konkurrenzverhalten der Naturverjüngung durch die Wasserverfügbarkeit gesteuert. So zeigt die Buchennaturverjüngung Nachteile im Wettbewerb um Stickstoff und Wasser, wenn sie mit schnellwachsenden Unterstand-Arten, z. B. *Rubus fruticosus*, konkurrieren muss (Fotelli et al., 2001, 2002). Ebenfalls zu starken Zuwachseinbußen der Buchennaturverjüngung führte die Konkurrenz um Wasser mit Grasarten (Coll et al., 2003).

Zusammenfassend könnten diese Effekte in Zukunft zu einer Reduktion des Zuwachses führen. In Buchenaltbeständen der Apenninen wurde dies bereits festgestellt (Piovesan et al., 2008). Nach dem sehr trockenen Sommer im Jahre 2003 wurde auch auf vielen Standorten Mitteleuropas eine starke Reduktion des Zuwachses beobachtet (Ciais et al., 2005). In Nordostspanien, wo Trockenheit für die Verbreitung der Buche limitierend ist, wurde seit 1975 eine starke Verringerung des Wachstums der Buche festgestellt (Buée et al., 2005; Jump et al., 2006). Rennenberg et al. (2004) folgerten daher, dass der einseitig geförderte Anbau der Buche mit einem hohen Risiko behaftet ist. Hanewinkel et al. (2012) prognostizieren, dass sich dass Verbreitungsgebiet der Buche bis zum Jahre 2070 - 2100 in Richtung Norden und Osten verschieben wird, falls die klimatischen Verhältnisse die Buche dort weiterhin begünstigen, während es im südlichen Verbreitungsgebiet trockener wird.

In Mitteleuropa wurde hingegen in vielen Beständen in den letzten Dekaden, vermutlich aufgrund der besseren N Versorgung (Aber et al., 1998) und der verlängerten Vegetationsperiode (Menzel et al., 2006) ein erhöhter Zuwachs festgestellt (Spiecker, 1999). Sehr starke Zuwächse wurden in einer bayernweiten Studie an den wärmsten Standorten der Studie erreicht (Felbermeier, 1994). Hinzu kommt, dass die Buche eine große genetische Variabilität zwischen ihren Beständen aufweist (Chmura and Rozkowski, 2002; Vornam et al., 2004; Von Wuehlisch et al., 1996) und an sehr unterschiedliche Standortverhältnisse angepasst ist (Kölling et al., 2005; Schraml and Rennenberg, 2002). Jedoch gibt es auch innerhalb von Beständen eine hohe genetische Variabilität (Comps et al., 2001; Konnert et al., 2000). So finden sich weit verbreitet bereits trockenheitsangepasste Buchen in der Naturverjüngung (Czajkowski and Bolte, 2006). Dadurch hat die Buche das Potential sich an ändernde Bedingungen anzupassen. Manthey et al. (2007) prognostizieren deshalb, dass sich zwar die ökologische Fitness an den Grenzen des Verbreitungsgebietes und auf flachgründigen Standorten verringert, aber die Buche vorherrschende Baumart in Mitteleuropa bleibt.

1.5 Ziele dieser Arbeit

Die Mykorrhizagesellschaften der Buche sind komplex aus vielen Pilzarten mit unterschiedlicher Abundanz zusammengesetzt und unterliegen einer hohen Dynamik (Buée et al., 2005; Pena et al., 2010). Um solche Mykorrhizagesellschaften untersuchen zu können, ist es entscheidend, die einzelnen Morphotypen mit Hilfe morphologischer und anatomischer Merkmale sicher ansprechen zu können, auch wenn die Identität der Arten im Nachhinein durch molekulare Analysen festgestellt wird. Für die Funktion der Pilze im Ökosystem sind ebenfalls die Morphologie und die Anatomie entscheidend. In Anbetracht von ca. 7000 bis 10000 ektomykorrhizabildenden Pilzen (Taylor and Alexander, 2005), die teilweise mit mehreren Wirtspflanzen Symbiosen eingehen können und somit mehrere Morphotypen ausbilden können, sind bisher nur sehr wenige Morphotypen (< 1000) exakt beschrieben und in entsprechenden Datenbanken (DEEMY, EDD) veröffentlicht worden. Im Rahmen der vorliegenden Arbeit wurde daher eine Erstbeschreibung eines Morphotypen, der auf den Probeflächen der nachfolgend genannten Versuche vorkommt, angefertigt.

Ektomykorrhizapilzen kolonisieren die Feinwurzeln von Buchen zu fast 100 % (Pena et al., 2010) und sind daher für die Stickstoffversorgung der Buchen von hoher Bedeutung. Durch ein Experiment unter kontrollierten Bedingungen ohne Stressapplikation soll untersucht werden, ob es nach warmen und trockenen Bedingungen, die eine Anpassung der Bakterien- und Mykorrhizagesellschaften mit sich bringen, eine langfristige Verminderung in der Stickstoffakkumulation in den Mykorrhizen und der Stickstoffaufnahme von Buchennaturverjüngung gibt. Vorausgehende Untersuchungen legen dies nahe (Fotelli et al., 2004; Geßler et al., 2005). Es wurde dafür Buchennaturverjüngung mit deren assoziierten Mykorrhiza- und Bakteriengesellschaften von zwei klimatisch unterschiedlichen Standorten für zwei Monate unter gleichen edaphischen und klimatischen Bedingungen kultiviert. Danach wurde mit markiertem Stickstoff der Verbleib des Stickstoffs in den verschiedenen Pools überprüft und mit den Mykorrhiza- und Bakteriengesellschaften in Beziehung gesetzt.

In einem Freilandversuch soll gezeigt werden, wie sich Mykorrhizagesellschaften in Bezug auf die Stickstoffakkumulation in einem intakten Buche-Mykorrhiza-Mikroben-Boden-Systemen verhalten. Insbesondere soll geklärt werden, welche Stickstoffformen in welchem Maße akkumuliert werden und ob sich die Präferenzen für die Stickstoffformen oder das Ausmaß der Akkumulation durch geänderte klimatische Bedingungen verändern. Dafür wurde in Tuttlingen (Baden-Württemberg) Buchennaturverjüngung mit umgebenden Boden in Stahlzylindern von einem NW Hang auf einen SW Hang und von einem NW auf einen NW Hang transferiert. Nach einem Jahr Inkubationszeit wurden die Mesokosmen nach Injizieren von ^{15}N markierten Stickstoffformen (Glutamin, Ammonium, Nitrat) in den Boden im Juni, August und September unter verschiedenen Bedingungen wie einer vorausgehende Trockenphase oder einem Starkregenereignis geerntet. Der SW-exponierte Standort wies eine höhere Bodentemperatur und eine geringere Bodenfeuchte auf als der NW-Standort und kann somit als Modellstandort für das zukünftig herrschende Klima gelten. Erhöhte Temperaturen, niedrigere Bodenfeuchte und Starkregenereignisse werden für die Zukunft in Mitteleuropa prognostiziert (IPCC, 2007).

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2 „Fagirhiza inflata“ + *Fagus sylvatica* L.

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2.1 Short description

This ectomycorrhiza has an orangish ochre colour, a monopodial pinnate to pyramidal ramification and is densely cottony covered by cystidia. These cystidia are at the polytomies three- to sixfold furcated and there characteristically inflated. Outer mantle layers are plectenchymatous and gelatinous, formed by irregularly shaped cells and covered by a hyphal net embedded in a gelatinous matrix. Middle mantle layers are between plectenchymatous and pseudoparenchymatous with irregularly shaped hyphae. The cells of the inner mantle layer are orientated in parallel. Mantle and cystidia show a dextrinoid reaction with Melzer’s reagent.

2.2 Characterization of “Faghiriza inflata”

2.2.1 Morphological characters (Fig. 2.1a)

Mycorrhizal system monopodial, pinnate to pyramidal, with one order of ramification, laying mostly between old leaves. - Main axes up to 15 mm long, 0.2 – 0.4 mm diam. - Unramified ends straight to bent, 0.5 – 1.5(3) mm long, 0.2 – 0.3 diam., cylindric, sometimes slightly inflated at middle parts, orange-ochre, at tips partly with some darker bands, older parts brownish grey; hydrophilic, of the short-distance exploration type - Surface of unramified ends at the tip smooth with some cystidia, in the middle and basal densely cottony and covered with soil particles. - Rhizomorphs not observed. – Sclerotia not observed.

2.2.2 Anatomical characters of mantle in plan views (Fig. 2.2)

Mantle with three distinct layers, hyphae colourless. Clamps lacking. - Outer mantle layers (Fig. 2 a) plectenchymatous, gelatinous, with irregularly shaped hyphae, sometimes star-like arranged, hyphal cells 10-30 µm long, 2-4.5 µm diam., walls 0.2-0.3 µm thick, covered by an irregularly shaped hyphal net from which ramified cystidia originate, hyphal net cells 20-50 µm long, 2-5 µm diam., walls 0.2-0.4 µm thick, matrix between net and outer mantle layer, many adhering soil particles. - Middle mantle layers (Fig. 2.2 b) a transition type between plectenchymatous and pseudoparenchymatous, irregularly shaped cells, sometimes with very big cells, hyphal cells 10-25(70) µm long, 1.5-3.5 µm diam., walls 0.25-0.5 µm thick. - Inner mantle layers (Figs. 2.2 c) transitional type between plectenchymatous and pseudoparenchymatous, hyphae orientated in parallel and slightly bent, irregularly shaped, hyphal cells 12-32 µm long, 1.5-2.25 µm diam., walls 0.2-0.25 µm thick. - Very tip similar to the other areas.

2.2.3 Anatomical characters of emanating elements (Figs. 2.1b, c)

Lacking are clamps, anastomoses not found. - Rhizomorphs lacking. - Emanating hyphae lacking. - Cystidia (Fig. 2.1c), originating in the hyphal net of the outer mantle layers, with polytomous ramifications with 3 - 6 branches, mostly characteristically inflated at the points of ramifications (Fig. 1b), main hypha (2.5)3.5(5) µm diam., (50)200(500) µm distance between polytomies, branches 1.5 - 3 µm diam., cell walls 0.2 - 0.5 µm thick, surface smooth, distal ends needle-like, colourless, septa rare. Chlamydospores not observed.

2.2.4 Colour reaction with different reagents

Mantle and Cystidia preparations: Melzer's reagent: dextrinoid

2.2.5 Autofluorescence

Not tested.

2.2.6 DNA-Analysis

DNA of some mycorrhized root tips was extracted using the innuPREP DNA Kit (Analytik Jena, Jena, Germany) as recommended by the manufacturer. The ITS region was amplified using the PCR primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). The obtained PCR product was purified with sodium acetate. Sequencing was performed by the sequencing service of the Büsgen-Institute, Department Forest Genetics and Forest Tree Breeding of the Georg August Univerity Göttingen using Big Dye Terminator 3.1 Cycles Sequencing Kit (applied Biosystems, Foster City, USA). The sequences were assembled with StadenPackage V4.10 and registered in the NCBI GenBank with the accession number JN701901. A BlastN search in GenBank showed that this fungus belongs to the genus Sebacina. The sequence of Fagirhiza inflata is identical with the sequence FR852335 (uncultured *Sebacina*, Identities 593/593 (100 %), Score 1104, E value 0.0, belonging to a related project near the location of Fagirhiza inflata). A sequence of *Sebacina incrustans* (UDB000118, Identities 563/633 (88 %), Score 644, E value 0.0) is the best hit with an identified fungus.

2.2.7 Reference specimen for *Fagus sylvatica* ectomycorrhiza

Germany, Baden-Württemberg, Tuttlingen, Forest near Möhringen, coord.: N47.975609, E8.753881, 15.5.11, ML1 in FEA (Göttingen, Germany); beech dominated forest, low mountain range, altitude 690 m a.s.l., NE-slope, rendzic leptosols; Identification of mycorrhiza as a member of Sebacina by DNA Analysis (see above) and morphological features (Agerer and Rambold 2004-2011); ectomycorrhiza exc., isol. and ident. M. Leberecht.

2.3 Discussion

Anatomical and morphological features like the characteristic form of cystidia and the lack of clamps and rhizomorphs suggest a classification of this ectomycorrhiza as a member of the genus *Sebacina* (Agerer and Rambold 2004-2011). Sequence analysis of the rDNA ITS region confirmed this affiliation.

Members of the genus *Sebacina* are known for their ability to form ecto-, orchid, ericoid, and jungermannioid mycorrhizae (Weiss et al. 2004). They produce ectomycorrhizae on angiosperms (Glen et al. 2002; Selosse et al. 2002; Avis et al. 2003; Urban et al. 2003; Richard et al. 2005; Tedersoo et al. 2006; Smith et al. 2007) and gymnosperms (Urban et al. 2003, 2008; Obase et al. 2009).
Five ectomycorrhizae from this genus have been described (Agerer and Rambold 2004-2011). Four of them do not have hyphae orientated in parallel in the inner the mantle layer. Some additional differences enable a differentiation of Fagirhiza inflata from the other sebacinoid ectomycorrhizae (s. below).
Sebacina incrustans (Pers.) Tul. & C.Tul on *Picea abies* (Urban et al. 2003) has shorter Cystidia (150 µm). The ramifications of the hyphae are often only Y-shaped. Polytomies with three branches are rare. Quercirhiza dendrohyphidiomorpha on *Quercus suber* (Azul et al. 2006) has hyphae with elbow-like structures. The cystidia or hyphae have no inflated ramification points and no polytomies with more than 3 branches. Pinirhiza multifurcata on *Pinus tabulaeformis* Carr. (Wei and Agerer 2011) has thicker walls (0.3-0.8 µm) of emanating hyphae. They are not so strongly inflated at the ramification points, and have only polytomies with 3 branches. Pinirhiza nondextrinoidea on *Pinus tabulaeformis* Carr. (Wei and Agerer 2011) has simple rhizomorphs and no cystidia. The ramification of the hyphae is Y-shaped and the ramification points are often not inflated. There is no dextrinoid reaction with Melzer's Reagent. The only known ectomycorrhiza of *Sebacina* with parallel hyphal arrangement in the inner mantle is Sebacinoid sp. on *Tilia* sp. (Urban et al. 2003) or, as a synonym, Tiliaerhiza sebacinoides (Agerer and Rambold 2004-2011).
High similarities of the sequence (AF509964, Identities 590/603 (97 %), Score 1070, E value 0.0) suggest a close relation of F. inflata and T. sebacinoides. Some morpho-anatomical differences identify F. inflata, however, as a separate ectomycorrhiza. The cells of the superficial net are larger in diam. with 3.5-7 µm in comparison to those of F. inflata with 2-5 µm. In the inner mantle the hyphae of T. sebacinoides are often ramified in Y-shape and have thinner cell walls (< 0.2 µm), but they are arranged in parallel like in F. inflata. The emanating hyphae have polytomies with only 3 - 4 branches and their cell walls are thicker (up to 1.3 µm), as compared to 3 – 6 branches and a cell wall thickness of 0.2 - 0.5 µm, respectively, in F. inflata.

2.4 Acknowledgments

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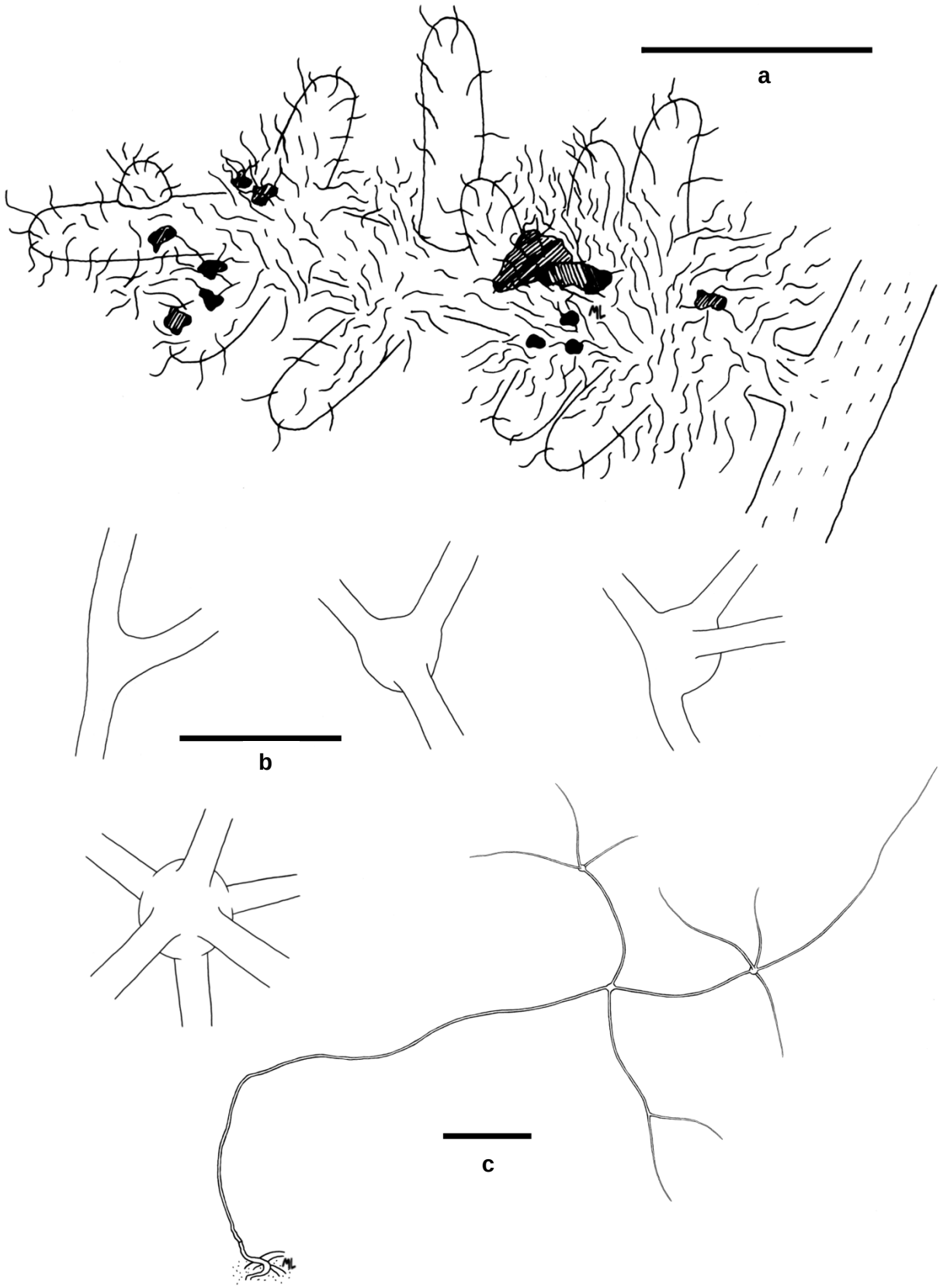

Figure 2.1: a) Habit of the mycorrhiza (bar 1 mm). b) Different ramification forms of the cystidia (bar 10 µm). c) Complete cystidia (bar 100 µm)

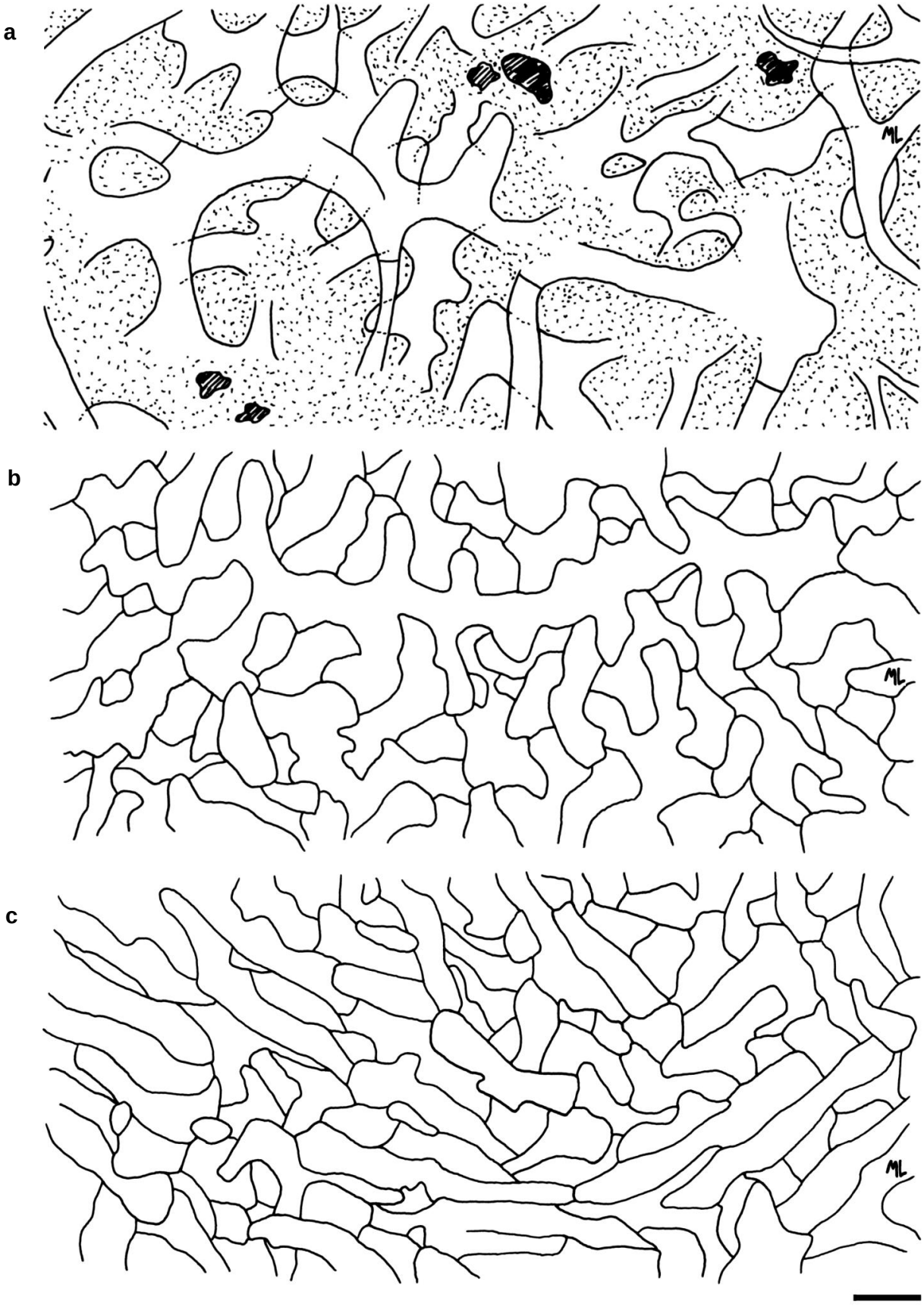

Figure 2.2: a) Structure of the outer mantle layers b) Structure of the middle mantle layers. c) Structure of the inner mantle layers (bar 5 µm).

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3 Dissecting the contributions of local ectomycorrhizal assemblages and microbial communities on nitrogen uptake of European beech (Fagus sylvatica)

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3.1 Abstract

The contributions of soil-mycorrhizosphere microbes, ectomycorhizal assemblages and tree origin on the nitrogen nutrition of beech (*Fagus sylvatica*) trees, a dominant forest species in Central Europe, were dissected. Young trees from natural regeneration of two genetically similar populations, one grown under dryer and warmer conditions (SW) and the other from cooler, moist climate (NE) were transplanted into a homogeneous substrate in the same environment and labelled with $^{13}CO_2$ and $^{15}NH_4^+$ to test the hypotheses that ectomycorrhizal assemblages from trees of SW and NE origin differ in fungal species composition and N acquisition and control N transfer to the trees, when competition by free microbes is low. Compared to systems with NE trees, those with SW trees exhibited the most rapid ^{15}N accumulation, higher nitrification activities and stronger $^{15}NO_3^-$ accumulation in soil microbial biomass. ^{15}N accumulation was similar in non-mycorrhizal root tips of SW and NE trees. A strong delay in ^{15}N enrichment was found in the ectomycorrhizal assemblage of NE trees, which were dominated by basidiomycota, whereas more rapid and higher accumulation of ^{15}N occurred in the acomycota-enriched assemblage of the SW trees. Because ^{15}N accumulation in fine roots and transfer to leaves were delayed and lower in NE compared to SW trees, our data support that ectomycorrhizal assemblages control N transfer to their host and demonstrate that fungal assemblages from dry conditions are more efficient in N acquisition when environmental constraints are relieved. These findings highlight the importance of adapted ectomycorhizal communities for forest nutrition in a changing climate.

3.2 Introduction

In Europe, forests are often confined to N-limited soils (Solberg et al. 2009), where ectomycorrhizal fungi (EMF) are important for tree nutrition (Read and Perez-Moreno 2003) The productivity of beech (*Fagus sylvatica* L.), a wide-spread, dominant forest species of great economic value and ecological importance in Central Europe (Ellenberg and Strutt 2009), is especially sensitive to N limitation under environmental stress (Rennenberg et al. 2009). Beech roots are colonized by a diverse flora of EMF taxa, which play critical roles in host nutrition. The composition of EMF assemblages is influenced by abiotic environmental factors such as drought as well as by host carbon allocation to roots (Shi et al. 2002; Buée et al. 2005; Druebert et al. 2009; Pena et al. 2010; Lang et al. 2011), but information whether different *in situ* EMF communities exhibit functional redundancy or diversity for host nutrition is sparse.

The control of EMF on N flux is complex because on the one hand decreased host carbon supply results in decreased N delivery by EMFs, whereas high N availability leads to enhanced C drain to the EMF (Corrêa et al. 2008; Albarracín et al. 2013; Näsholm et al. 2013). On the other hand, EMFs are instrumental to maintain host N supply under drought stress and for the access to organic N from degrading leaf litter (Pena et al. 2013b; Pena and Polle 2014). Because the latter process is much slower than the capture of soluble N by soil borne microbes (Pena et al. 2013b) free-living microbes are strong competitors for this resource (Dannenmann et al. 2009; Kaiser et al. 2010).

In recent years, it has been realized that bulk soil, rhizosphere soil and the mycorrhizosphere of roots are colonized by distinct microbial communities and that the composition of the root-associated fractions of both bacteria and fungi is influenced by the host genotype (Bradley et al. 2007; Bulgarelli et al. 2013; Danielsen et al. 2013). In rhizosphere soil, and also in the mycorrhizal mantle, a high density of bacteria is common (Mogge et al. 2000; Schloter et al. 2005). Besides other functions these bacteria catalyze the mineralization of organic matter and, thus, determine the nutrient supply of plants to a large extent. Because of their flexible genomes and short generation times bacterial communities have the potential to respond to environmental changes much faster than plants and EMF (Allen et al. 1995; Bossio and Scow 1995; Bossio et al. 1998; Pettersson and Bååth 2003). The activity and community composition of these microbes is strongly dependent on the quality and amount of root-derived carbohydrates (Dannenmann et al. 2009; Kaiser et al. 2010; Koranda et al. 2011; Rasche et al. 2011). Therefore, differences in belowground C allocation patterns, which are a result of the plant developmental stage as well as biotic and abiotic stressors (Meier and Leuschner 2008), may also affect bacterial and EMF assemblages and functions and, thereby, impact N availability for forest trees.

In the present study, we relieved environmental constraints to unravel the influence of ectomycorrhizal assemblages in relation to bacterial activities for N uptake. We used natural beech regeneration of similar population genetics (Bilela et al. 2012) originating from well characterized beech forests with north-east (NE) and south-west exposure (SW) from a mountainous area in Southern Germany (Tuttlingen) (Fotelli et al. 2004; Geßler et al. 2005). At the SW site, the water availability is reduced to an extent similar to that predicted by climate models for the coming decades and the temperature is slightly increased compared with the NE site (Geßler et al. 2001; Holst et al. 2010). Soil type and N availability are similar in the NE and SW forests, but wood production of the beech trees at the SW site is significantly lower than that at the NE site (Geßler et al. 2001, 2005). It was, therefore, suspected that N utilization was impaired by physiological constraints in the SW compared

with the NE trees. To dissect tree origin, bacterial and ectomycorrhizal contributions to tree N nutrition, we transplanted young beech trees into a fertilized peat-sand mixture and cultivated them under the same environmental conditions. We hypothesized that beech trees from SW exhibit lower N uptake than those from NE, as observed previously under field conditions (Fotelli et al. 2004; Geßler et al. 2005) because of lower N acquisition and translocation by the EMF assemblages at roots of trees from SW than NE. We further hypothesized that these differences were related to lower belowground carbon allocation of trees from SW than of NE origin. To test these hypotheses, a 48h-pulse of $^{13}CO_2$ was applied and the soil system was repeatedly and homogenously labelled with $^{15}NH_4^+$ for seven days to investigate the accumulation kinetics of new N in microbial, ectomycorrhizal and plant tissues. In addition, EMF and bacterial community structures were determined and carbon allocation was traced by monitoring the $\delta^{13}C$ signature in different plant tissues and soil respiration.

3.3 Materials and Methods

3.3.1 Plant origin and plant cultivation

Young beech (*Fagus sylvatica*) trees were collected in July 2010 in two 80 to 90-year-old beech dominated forests in the Swabian Jura near Tuttlingen (longitude 8°45'E, latitude 47°59'N, South West Germany) from the natural regeneration, which shows similar population genetics in these forests (Bilela et al. 2012). The forests are located on the North East (NE) and the South West (SW) exposure of a narrow valley (Krähenbachtal) with steep slopes (23°-30°) (Dannenmann et al. 2007). The mean long-term annual regional air temperature (1961-1990) is 6.6 °C and the mean annual precipitation is 810 mm (Geßler et al. 2001). Because of the higher irradiance on the SW site the soil temperature at 10 cm depth is 0.8 °C higher and the soil water potential generally more negative than on the NE site (Keitel et al. 2003). On both sites, the soil type is a shallow Rendzic Leptosol (sceletic) (International Union of Soil Sciences Working Group WRB 2007) developed on limestone and marls of the Jurassic Malm formation (Dannenmann et al. 2006). Soil pH (water) is 5.7 in the organic layer and 7.5 at 0.6 m depth (Geßler et al. 2001).
Forty-five trees of stem heights of about 0.5m were collected on each site. The trees were excavated with intact roots in their soil compartment and transported to a nursery (Forest Botanical Garden, University of Göttingen, longitude 9°57'E, latitude 51°33'N). The root systems were carefully cleaned under running water to remove adhering soil. The beech trees were planted separately in 5l containers (17 x 17 x 17 cm) in a homogenous mixture of coarse sand (0.7 – 1.2 mm diameter, Melo Schwimmbadtechnik, Göttingen, Germany; 4.5 parts,), fine sand (0.4-0.8 mm diameter, Melo Schwimmbadtechnik; 4.5 parts) and peat (Torfwerk Zubrägel, Vechta, Germany; 1 part). The sand was washed with tap water before use to remove small particles. To match stand light conditions the trees were shaded with a 65 % shading net (Herrmann Meyer KG, Rellingen, Germany) and placed outdoors. Each plant was watered daily with 50 ml tap water. Not all trees survived the transplantation, leaving 32 trees per site for the further treatments. After one month, the beech trees were transferred into a greenhouse with 50 % air humidity, an air temperature of 20 °C and long day conditions [16 h light achieved by additional irradiation with MT 400 DL/BH lamps (Eye Iwasaki Electric Co. Ltd., Tokyo, Japan), resulting in a photosynthetically active radiation of 150 µmol m^{-2} s^{-1} quantum flux density as determined by a photometer Li-185B equipped with a quantum sensor Li-190SB (LiCor INC., Lincoln, USA). This light intensity is typical for

natural regeneration in beech forests (Ritter et al. 2005). Trees were cultured under these conditions for one month and watered daily with 50 ml of a Hoagland-based nutrient solution (pH 5.7), which contained 0.4 mM NH_4Cl as sole nitrogen source in addition to 0.05 mM $NaSO_4$, 0.1 mM K_2SO_4, 0.06 mM $MgSO_4$, 0.13 mM $CaSO_4$, 0.03 mM KH_2PO_4, 0.005 mM $MnSO_4$, 0.005 $FeCl_3$, 5 µM H_3BO_3, 0.13 µM $NaMoO_4$, 0.18 µM $ZnSO_4$ and 0.16 µM $CuSO_4$ (adapted after (Dyckmans 2000)). Two days before experimental labeling, root collar diameters at soil level, and heights of all trees were measured.

3.3.2 ^{13}C and ^{15}N labeling

The beech-soil systems were labeled with $^{13}CO_2$ and $^{15}NH_4Cl$. $^{13}CO_2$ (1.5 % ^{13}C, Cambridge Isotope Laboratories, Andover, USA) fumigation started on the 4.10.12 at 8 am and ended after 48 h. During this time a CO_2 concentration of 1091 ± 193 ppm was recorded (UNOR 610 CO_2 analyzer, Maihak, Hamburg, Germany). For homogenous distribution, the ^{13}C labeled air in the greenhouse was intensively mixed by a fan (SF16R, SMC, Hong Kong, China).

$^{15}NH_4Cl$ (0.4 mmol, 99 % ^{15}N, Cambridge Isotope Laboratories, Andover, USA) was applied daily on seven consecutive days starting on 4.10.12 at 8 am. To achieve homogenous ^{15}N soil labeling, the nutrient solution was injected by syringes (1.5 ml) applying a total of 50.7 ml by 39 injections of 1.3 ml of ^{15}N-solution at depths of 0, 5 and 10 cm per container. This treatment corresponds to a daily amount of 0.337 mg ^{15}N in excess of the natural abundance. Eight trees per site were maintained in another greenhouse without ^{13}C and ^{15}N labeling as non-labeled controls.

3.3.3 Soil respiration

On day 2, 3, 4, 6 and 8 after the start of the experimental labeling, soil respiration and soil $^{13}CO_2$ evolution were determined using a static chamber approach (Wu et al. 2010). At 11am, a chamber (area: 120 cm^2, height 10 cm) was gently inserted 1 cm into the soil at 3 randomly chosen containers of the NE and the SW treatment, respectively. Two minutes, 15 min and 30 min after chamber placement, a gas sample of 20 ml was taken via gastight syringe sampling through a septum. The air sample was immediately transferred in a septum-capped pre-evacuated gastight 5 ml vial. For this purpose, the entire sample volume of 20 ml was flushed from the syringe through the vial by using a second cannula in the septum cap, which was removed at the end of the flushing procedure, leaving an overpressure of 25% in the sample vial. Within four days after sampling, sampled air in vials was analyzed for the CO_2 concentration and the $\delta^{13}C$ signature of CO_2 using GasBench II coupled to the isotope ratio mass spectrometer Delta Plus XP (Thermo Fisher Scientific, Bremen, Germany). We used a PoraBOND Q column (Agilent, Böblingen, Germany) at 33 °C and a sample loop with 250 µL volume. Samples were calibrated using three standard gasses (325 ppm CO_2 with $\delta^{13}C$ = -8.296 ‰; 340 ppm CO_2 with $\delta^{13}C$= -29.3 ‰; and 550 ppm CO_2 with $\delta^{13}C$= -14.677 ‰ in synthetic air) (Air Liquide, Kornwestheim, Germany). Soil respiration was calculated from the increase in CO_2 concentrations during the 30 min sampling interval (Wu et al. 2010)). Due to the low soil respiration rates, the CO_2 concentration increase was linear over the 30 minutes period. The increase in CO_2 concentration over time had to match a quality criterion of $R^2 > 0.9$; otherwise the respective soil respiration flux was discarded (in one case).

3.3.4 Harvest

Eight plant-soil containers per site were harvested 1, 3 and 7 days after the start of ^{15}N labeling. Eight control plant-soil systems per site were harvested at d = 0. Leaves and above-ground woody parts (branches and stem) were separated after harvest and weighed immediately. Five leaves were separately weighed, scanned with a CCD camera KP-C551 (Hitachi, Tokyo, Japan) and analyzed with ImageJ 1.47v (National Institute of Health, Bethesda, USA). Whole plant leaf area was calculated as: area of leaves/mass of leaves × mass of all leaves.

The roots were then carefully washed under running tap water. Coarse (> 2 mm diameter) and fine roots (< 2 mm diameter) were separated and weighed. One g of fine roots from unlabeled beech seedlings was shock-frozen in liquid N_2 for amino acid determination (see below). Fine root samples were wrapped in moist paper towels and stored in darkness in plastic bags at 4°C. Dry mass of fine roots was determined after ectomycorrhizal analysis (see below). All remaining plant parts were weighed immediately after harvest, dried and weighed after one week at 60°C.

The entire soil of each planting container was mixed and a representative sample of 500 g per container was used for further analyses. In addition to bulk soil, rhizosphere soil (RS, defined as soil attached to roots after slight shaking) and rhizosphere root complex (RRC, containing fine roots with tightly adhering soil, which could not be removed) were sampled as described previously (Gschwendtner et al. 2010). RS and RRC samples were immediately frozen on dry ice and stored at -80°C.

3.3.5 Soil analyses

Immediately after harvest 300 g of representative bulk soil was weighed and dried at 105°C for 24 hours. Soil water content (SWC [%]) was determined as:

$$SWC = \frac{sfw - sdw}{sdw} \times 100$$

with sdw being soil dry weight (g) and sfw soil fresh weight (g).

Further fresh soil aliquots of 60 g were either directly extracted with 0.5 M K_2SO_4 solution at a soil:solution ratio of 1:2 or after 24 hours of fumigation with chloroform (Dannenmann et al. 2009). All extracts were filtrated with 0.45 µm syringe filters (Schleicher & Schuell, Dassel, Germany) and immediately frozen. Aliquots of the soil extracts were used for spectrophotometric determination of ammonium (NH_4^+) and nitrate (NO_3^-) concentrations by a commercial laboratory (Dr. Janssen GmbH, Gillersheim, Germany) and for total dissolved N (TN) and total dissolved organic C (DOC) by auto-analyzers as described by (Dannenmann et al. 2009). Dissolved organic N (DON) was calculated as the difference between TN in extracts and inorganic N in extracts. Microbial biomass C and N was calculated from the difference in TN and DOC between extracts from fumigated and unfumigated soil, without application of correction factors (Dannenmann et al. 2009).

In addition to N concentrations of the soil microbial biomass, NH_4^+, NO_3^- and DON pools, their respective ^{15}N signatures were determined. The approach was based on diffusion of NH_4^+ via pH increase on acid filter traps prepared for isotope ratio mass spectrometry, after sequential conversion of all target-N compounds in soil extracts to NH_4^+ as described in detail in earlier studies (Guo et al. 2013b). Determination of ^{15}N enrichment in total extractable N of both unfumigated control soils and fumigated soils was based on alkaline persulfate oxidation of all N compounds to NO_3^- and subsequent NO_3^- reduction to NH_4^+ as

described by (Wu et al. 2011). Subsequent diffusion of NH_3 on acid filter traps enabled the quantification of ^{15}N enrichment in microbial biomass as calculated from the difference in N concentration and ^{15}N enrichment in TN between unfumigated control soils and fumigated soils (Guo et al. 2013b). No correction factors were applied in order to obtain estimates of ^{15}N uptake into active microbial biomass (Perakis and Hedin 2001).

3.3.6 Identification of ectomycorrhizal fungi

The washed fine roots were spread under a dissecting microscope (205 FA, Leica, Wetzlar, Germany) and remaining soil particles were removed using fine forceps. Randomly chosen 2 to 3 cm long root fragments were used for mycorrhizal analysis. Four hundred mycorrhizal root tips per plant were counted and classified as mycorrhizal, non mycorrhizal or dead root tips. If not enough root material was available to reach 400 mycorrhizal root tips, all available root tips were counted. Aliquots of mycorrhizal, non-mycorrhizal and dead root tips were collected of each plant and freeze-dried for ^{15}N and ^{13}C measurements.
The mycorrhizal root tips were assigned to morphotypes, based on morphological features like color, mantle structure, type of ramification, shape of unramified ends, emanating hyphae and rhizomorphes (Agerer 1987). All morphotypes were photographed with a DFC420 C camera (Leica, Wetzlar, Germany) at 10 - 40 x magnification and aliquots were stored at -20°C for species identification by internal transcribed spacer (ITS) sequencing.
The molecular identification of ectomycorrhizal fungi was conducted as previously described in (Druebert et al. 2009; Lang et al. 2011)). DNA of about 20 root tips assigned to one morphotype was extracted (innuPREP DNA Kit, Analytik Jena, Jena, Germany) as recommended by the manufacturer. The ITS region was amplified using the PCR primers ITS1F and ITS4 (Eurofins MWG Operon, Ebersberg, Germany) (White et al. 1990; Gardes and Bruns 1993). The obtained PCR products were purified with sodium acetate. Sequencing was performed by the Sequencing service of the Büsgen-Institute, Department Forest Genetics and Forest Tree Breeding of the Georg August University Göttingen using the Big Dye Terminator 3.1 Cycles Sequencing Kit (Applied Biosystems, Foster City, USA). The sequences were assembled with StadenPackage V4.10 and compared with UNITE (http://unite.ut.ee) and NCBI (http://www.ncbi.nlm.nih.gov) databases. Species names were accepted when 97 % identities and a score over 800 bits were achieved. All sequences have been deposited in NCBI Genbank with GenBank accession numbers KF498567-KF498582.

3.3.7 C and N measurements and isotope analysis of plant tissues and mycorrhizal root tips

Dry plant tissues were ground with a ball mill (Retsch, Düsseldorf, Germany). Mycorrhizal, non-mycorrhizal and dead root tips were processed without milling. Samples were weighed (Supermicro S4; Sartorius, Göttingen, Germany) into 5 x 9 mm tin capsules (IVA Analysetechnik, Meerbusch, Germany). For leaves 0.8 mg, for stem 5.0 mg, for coarse roots 3.0 mg, for fine roots 2.0 mg and for mycorrhizal, non-mycorrhizal and dead root tips 0.5-3 mg of tissue were used. ^{14}N, ^{15}N, ^{12}C, and ^{13}C isotope analyses were conducted at the service unit KOSI (Kompetenzzentrum für Stabile Isotope, University Göttingen, Germany) on a Delta Plus mass spectrometer (Finnigan MAT, Bremen, Germany; Interface: Conflo III, Finnigan MAT, Bremen, Germany; elemental analyzer: NA2500, CE Instruments, Rodano, Milano, Italy).

APE (^{15}N atom-% excess) was determined as:

$$^{15}N\ APE = atom\text{-}\%_{sample} - atom\text{-}\%_{natural\ abundance}$$

$$with \quad atom - \% = \frac{^{15}N}{^{14}N + ^{15}N} \times 100.$$

δ^{13}C values were determined as:

$$\delta^{13}C = \left(\frac{\frac{^{13}C_{Sample}}{^{12}C_{Sample}}}{\frac{^{13}C_{VPDB}}{^{12}C_{VPDB}}} \right) x\ 1000$$

with C_{VPDB} = Vienna Pee Dee Belemnite Standard.

APE (^{13}C atom-% excess) was determined as:

$$^{13}C\ APE = atom\text{-}\%_{sample} - atom\text{-}\%_{natural\ abundance}$$

$$with \quad atom - \% = \frac{^{13}C}{^{12}C + ^{13}C} \times 100.$$

3.3.8 Amino acid determination

Amino compounds were extracted from 96h-freeze-dried fine root samples according to the method of (Winter et al. 1992). Amino compounds were determined in 50 µl samples using a Waters Acquity UPLC-System (Waters Corp., Milford, MA, USA) with a modified standard protocol (using an AccQTag™ Ultra column 2.1 x 100 mm, 1.7 µl, 0.7 ml min^{-1} flow, column temperature 61°C) (Luo et al. 2009). Standard H (#NCI0180, Pierce Biotechnology, Inc., Rockford, IL, USA) was used as the analytical standard.

3.3.9 Extraction of bacterial DNA

DNA was extracted from 0.4 g of rhizosphere soil and 0.1 - 0.2 g of rhizosphere root complex, respectively, using the NucleoSpin Soil Kit (Macherey Nagel, Düren, Germany) and the Precellys 24 Instrument (Bertin Technologies, Montigny-le-Bretonneux, France). Quantity and quality of the extracted DNA were checked with a Nanodrop spectrophotometer (PeqLab, Erlangen, Germany) and by gel electrophoresis (Gschwendtner et al. 2010). The extracts were stored at -20°C until use.

3.3.10 Total bacterial community structure

To assess bacterial community structure, amplicons of the 16S rRNA gene were analyzed by terminal restriction fragment length polymorphism (TRFLP). The universal primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3', 6-Fam-labelled) and 1401r (5'-CGGTGTGTACAAGACCC-3') were used to amplify 1.4 kb fragments of the 16S rRNA gene (Schreiner et al. 2010). Each PCR assay (total volume 50 µl) contained 60 ng of template DNA, 0.3% bovine serum albumin (BSA) (Sigma Aldrich, Taufkirchen, Germany), 5% dimethyl sulfoxid (DMSO) (Sigma Aldrich), 200 µM desoxynucleotide (dNTP) (Fermentas, St.

Leon Rot, Germany), 2.5 mM $MgCl_2$, 2.5 U Taq Polymerase (Life Technologies, Darmstadt, Germany), 1x Taq buffer (Life Technologies) and 10 pmol of each primer. PCR was performed in a T3 thermocycler (Biometra, Göttingen, Germany) using the following conditions: 10 min at 94°C for initial denaturation, followed by 30 cycles of 1 min at 94°C, 1 min at 57°C and 1.5 min at 72°C and a final extension step for 10 min at 72°C. PCR products were analyzed by electrophoresis on 1.5% agarose gels and purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The enzymatic restriction was performed as described previously (Schreiner et al. 2010), using 200 ng of labelled PCR product, 5 U of MspI (Fermentas) and 1x reaction buffer (total volume 25 µl). The reaction mixtures were incubated for 6 hours at 37°C, followed by 20 min at 65°C for enzyme inactivation. Afterwards, the samples were purified using the MinElute Reaction Cleanup Kit (Qiagen). The TRFLP profiles were generated using an ABI Prism 3730 Genetic Analyzer. Sizes and relative abundances of terminal restriction fragments (TRFs) were analyzed using GeneMapper software and T-REX software (http://trex.biohpc.org/) with a binning range of 2 bp. Only TRFs with a signal above 1% of the sum of all peak heights were included in the analysis.

3.3.11 Abundance of mineralizers, nitrogen fixers, ammonia oxidizers and denitrifiers

Quantitative real-time PCR (RT-PCR) was performed using an ABI 7300 Cycler (Applied Biosystems, Foster City, USA) to assess the abundance of selected marker genes which were used as proxy for microbes involved in different steps of the nitrogen cycle with the following assay reagents: DMSO and BSA (Sigma Aldrich), primers listed in Table 3.1 (Metabion, Germany) and 2x Power SYBR Green master mix (Life Technologies, Darmstadt, Germany). The respective 25 µl reaction mixtures for quantification of the genes [*nifH* (nitrogenase), *amoA* AOA (ammonia monooxygenase in ammonia oxidizing archaea), *amoA* AOB (ammonia monooxygenase in ammonia oxidizing bacteria), *narG* (nitrat reductase), *nirS* (nitrite reductase), *nirK* (nitrite reductase), *nosZ* (nitrous-oxide reductase), *chiA* (chitinase), apr (protease)] were composed as follows: 12.5 µl SYBR Green master mix, 5 pmol of each primer (for *apr* gene: 10 pmol of each primer), 0.5 µl 3% BSA and 2 µl DNA template. For the amplification of *narG, nirK,* and *nirS* genes, 0.5 µl DMSO was added. Primer sources and measuring conditions are summarized in Table 3.1.
For quantification, serial dilutions (10^1 to 10^6 gene copies μl^{-1}) of plasmid DNA containing PCR products of the respective genes listed in Table 3.1 were used to calculate standard curves. The PCR detection limit was assessed to 10 gene copies according to manufacturer's instruction. To avoid PCR inhibition, the optimal dilution for each amplification assay was determined in advance by dilution series of randomly chosen DNA extracts. The RT-PCR assays were performed in 96-well plates (Life Technologies) for all target genes. All PCR runs started with a hot start at 95°C for 10 minutes. To confirm the specificity of the SYBR Green-quantified amplicons, a melting curve analysis and a 1.5% agarose gel were performed after each PCR run. The amplification efficiency was calculated as Eff = $[10^{(-1/slope)}-1]$ and resulted in the following average efficiencies (standard deviation less than 5% of mean): *nifH*, 87%, *amoA* AOA, 92%, *amoA* AOB, 86%, *narG*, 92%, *nirK*, 94%, *nirS*, 93%, *nosZ*, 86%, *chiA*, 92%, *apr*, 94%.

Table 3.1: Primer sets and thermal profiles used for the absolute quantification of the respective genes nifH: nitrogenase, amoA AOA: ammonia monooxygenase in ammonia oxidizing archaea, amoA AOB: ammonia monooxygenase in ammonia oxidizing bacteria, narG: nitrate reductase, nirS: nitrite reductase, nirK: nitrite reductase, nosZ: nitrous-oxide reductase, chiA: chitinase, apr: protease.

Target gene	Primer	References	Thermal profile	No. of cycles
nifH	nifH-f, nifH-r	Rösch et al. 2002	95°C-45s/55°C-45s/72°C-45s	40
amoA AOA	amo19F, CrenamoA16r48x	Leininger et al. 2006; Schauss et al. 2009	94°C-45s/ 55°C-45s/ 72°C-45s	40
amoA AOB	amoA1F, amoA2R	Rotthauwe et al. 1997	94°C-45s/ 59°C-45s/ 72°C-45s	40
narG	narG-f, narG-r	Bru et al. 2007	95°C-15s/ 63-58°C-30s/ 72°C-30s	5 *
			95°C-15s/ 58°C-30s/ 72°C-30s	40
nirS	cd3aF, R3cd	Michotey et al. 2000; Throbäck et al. 2004	95°C-45s/ 57°C-45s/ 72°C-45s	40
nirK	nirK876, nirK5R	Braker et al. 1998; Henry et al. 2004	95°C-15s/ 63-58°C-30s/ 72°C-30s	5 *
			95°C-15s/ 58°C-30s/ 72°C-30s	40
nosZ	nosZ2F, nosZ2R	Henry et al. 2006	95°C-15s/ 65-60°C-30s/ 72°C-30s	5 *
			95°C-15s/ 60°C-30s/ 72°C-30s	40
chiA	chiF2, chiR	Xiao et al. 2005	95°C-30s/60°C-30s/72°C-60s	40
Apr	FPapr1, RPapr2	Bach et al. 2001	95°C-20s/53°C-30s/72°C-60s	40

* Touchdown: -1°C per cycle

3.3.12 Data analysis

Data were analyzed with Origin Pro 8.5 (OriginLab Corporation, Northampton, USA) using Students´ t tests for normal distributed data sets. Normal distribution was tested with the Kolmogorov-Smirnov test. If data did not show normal distribution, they were log-transformed to meet the requirement of normality. General Linear Models (GLM) were applied to investigate the main factors (time, site) and their interactions (Statgraphics Centurion XVI Version 16.2.03 (Statpoint Technologies, Warrenton, USA). In tables and figures data are shown as means ± standard error (SE). Differences between means were considered significant at $P \leq 0.05$. Diversity indices and their comparisons were calculated with PAST 2.17c using a bootstrap of 200 (Hammer et al. 2001). Similarity indices were calculated as generalized Morisita-Horn index C_qN by comparing N communities on species information shared by at most q communities using the procedure developed by (Chao et al. 2008) and implemented in the program SPADE by Chao and Shen (2010) (http://chao.stat.nthu.edu.tw). EMF species abundances, and microbial abundances for RRC and RS from the TRFLP analysis, normalized to 10000, were used as input parameters and run with a bootstrap value of 200. Rarefaction curves were calculated using EstimateS Win 7.5.3 (http://purl.oclc.org/estimates).

3.4 Results

Characteristics of the plant-soil system of beech trees originating from two contrasting field sites after cultivation under common environmental conditions

Young beech trees from NE and SW were transplanted into a peat-sand-mixture, grown for two months in the same environment and then labeled in a greenhouse for 48h with $^{13}CO_2$ and for one week daily with ^{15}N (Fig. 3.1). During the labeling period SWC, NH_4^+, NO_3^-, DOC and DON soil concentrations showed no important temporal fluctuations and, therefore, mean values for the containers with beeches from NE or SW are shown (Table 3.2). Microbial biomass in bulk soil, determined as microbial N and microbial C, neither differed, but soil respiration, which is the result of microbial and root respiration, was higher in the NE than in the SW containers (Table 3.2). However, the $\delta^{13}CO_2$ signature of soil respiration was not significantly different between NE and SE beech containers, suggesting similar belowground utilization of newly acquired photosynthetates (Fig. 3.1).

Although the beech trees were selected on the basis of similar stem heights and diameters, differences in whole-plant biomass were observed: NE beech trees exhibited significantly greater whole-plant leaf area (+23%) and root biomass (+27%) than those from SW (Table 3.3). The differences in belowground biomass resulted in a significantly higher root-to-shoot ratio of NE compared to SW beech trees (Table 3.3).

The natural $\delta^{13}C$ signature of leaves is an integrative indicator for water availability (Keitel et al. 2006). Because no significant differences of the $\delta^{13}C$ signatures were detected at the start of the labeling (t = 0, Table 3.4), we have no evidence that the differences in plant biomass were the result of acute drought periods during their growth under field conditions. ^{13}C pulse labeling did not indicate differences in photosynthetic performance because the leaves from NE and SW beeches showed the same changes in response to the $^{13}CO_2$ labeling pulse at t = 8d (Table 3.4). However, carbon allocation of recent photosynthetate to fine roots was stronger in SW than in NE trees, evident from a higher $\delta^{13}C$ signature in SW than in NE fine roots at 8d (Table 3.4).

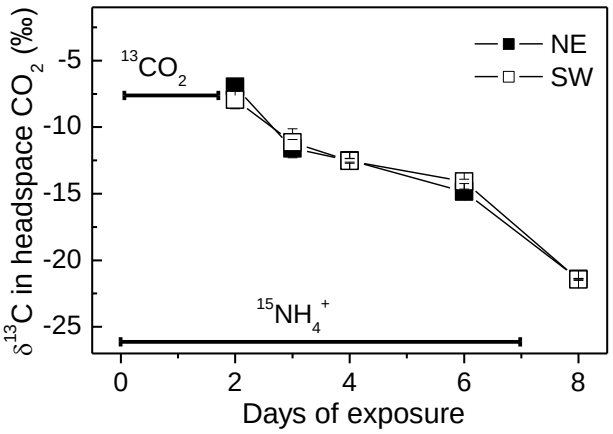

Figure 3.1: $\delta^{13}C$ signature of CO_2 in static chamber headspace after labeling for 48h with $^{13}CO_2$. The duration of the ^{13}C and ^{15}N labeling is indicated by bars. Data are means ± SE gained during static chamber soil respiration measurements in containers with beech trees from the SW (open) and NE (closed symbols) stands. Error bars are smaller than the symbols.

Table 3.2: Characteristics of the soil parameters two months after transplantation of young beech trees (*Fagus sylvatica*) originating from beech forests on slopes with south west (SW) and north east (NE) exposure in a low mountain range in southern Germany (Jura). Natural beech regeneration was transplanted into a sand-peat mixture, grown with regular fertilizer application for two months under identical conditions and labeled for one week with $^{15}NH_4^+$. Measurements of soil parameters were taken regularly during the experimental week. Data show means ± SE of all sampling dates. Significant differences between soils of beech from different sites are indicated by P-values < 0.05 (bold letters). The abbreviations refer to SDW: soil dry weight, DON: dissolved organic nitrogen, DOC: dissolved organic carbon, MBN nitrogen in microbial biomass, MBC: carbon in microbial biomass.

	NE	SW	P
Soil water content [% SDW]	7.95 ± 0.24	8.51 ± 0.25	0.11
Ammonium [mg N kg^{-1} SDW]	0.51 ± 0.07	0.44 ± 0.06	0.44
Nitrate [mg N kg^{-1} SDW]	0.22 ± 0.03	0.21 ± 0.03	0.71
DON [mg N kg^{-1} SDW]	2.17 ± 0.18	2.1 ± 0.13	0.76
DOC [mg N]	18.97 ± 1.34	16.8 ± 1	0.2
MBN [mg N kg^{-1} SDW]	3.42 ± 0.22	3.28 ± 0.16	0.61
MBC [mg C kg^{-1} SDW]	11.2 ± 1.13	11.5 ± 0.59	0.81
Soil respiration [mg CO_2-C m^{-2} h^{-1}]	11.53 ± 1.08	8.48 ± 0.22	0.03

Table 3.3: Characteristics of young beech trees (*Fagus sylvatica*) originating from beech forests on slopes with south west (SW) and north east (NE) exposure in a low mountain range in southern Germany (Jura). Trees from the natural regeneration were cultured for two months in a sand-peat mixture, fertilized regularly and labeled with ^{15}N for one week. Trees were harvested regularly within the experimental week (n = 8 per site and sampling date). Data show means ± SE of all sampling dates per site, for amino acids only at day 8. P-values of General Linear Models for the factor "Site" are shown, because the factor "Time" had no significant effect ($P_{time} > 0.05$). P-values < 0.05 are highlighted in bold letters.

	NE	SW	P_{site}
Stem height [cm]	53.84 ± 1.03	53.19 ± 1.15	0.66
Stem diameter [mm]	5.63 ± 0.1	5.45 ± 0.1	0.2
Leaf area [cm^2]	454 ± 19	369 ± 18	<0.001
Whole-plant dry mass [g plant-1]	11.58 ± 0.53	9.48 ± 0.38	0.01
Aboveground dry mass [g plant-1]	6.03 ± 0.32	5.43 ± 0.23	0.14
Belowground dry mass [g plant-1]	5.54 ± 0.25	4.35 ± 0.2	<0.001
Root/Shoot	0.94 ± 0.04	0.82 ± 0.04	0.02
Amino acids [µmol g-1 dry weight]	5.06 ± 0.44	5.79 ± 0.38	0.25
Mycorrhizal root tips [%]	38.08 ± 2.38	30.36 ± 2.55	0.03
Dry root tips [%]	6.57 ± 0.93	12.87 ± 1.94	<0.001
Non mycorrhizal root tips [%]	55.34 ± 2.51	56.77 ± 2.48	0.69

Table 3.4: $\delta^{13}C$ signatures in plant tissues and ectomycorrhizas of young beech trees (*Fagus sylvatica*) originating from beech forests on slopes with south west (SW) and north east (NE) exposure in a low mountain range in southern Germany (Jura). Trees from the natural regeneration were cultured for two months in a sand-peat mixture, fertilized regularly and exposed to $^{13}CO_2$ for two days. $\delta^{13}C$ was analyzed at day 0 and day 8 after the start of labeling (n = 8 per site and sampling date). Data show means ± SE. P-values for a multivariate ANOVA with site and time as fixed factors and for a one way ANOVA for the comparisons of tissues are shown in columns and rows, respectively. Different letters indicate significant differences (P < 0.05) of the tissues calculated post hoc with the HSD test. P-values < 0.05 are highlighted in bold letters. FR = fine roots, EMF = ectomycorrhizal fungi, NM = non-mycorrhizal roots

		$\delta^{13}C$ (‰)				
Site	Time	Leaf	FR	EMF	NM	$P_{(tissue)}$
NE	0	-31.3 ± 0.2a	-30.0 ± 0.3ab	-29.2 ± 0.5b	-30.7 ± 0.6a	**0.021**
NE	8	-30.3 ± 0.3a	-28.2 ± 0.3a	-27.0 ± 0.7a	-17.4 ± 1.9b	**<0.001**
SW	0	-32.0 ± 0.3a	-28.7 ± 0.6b	-29.9 ± 0.3b	-30.0 ± 0.6b	**0.002**
SW	8	-30.4 ± 0.2a	-27.3 ± 0.5b	-27.0 ± 0.9b	-16.3 ± 1.4c	**<0.001**
P_{Site}		0.123	**0.023**	0.571	0.482	
P_{Time}		<**0.001**	**0.002**	**0.001**	<**0.001**	
$I_{(Site\ xTime)}$		0.403	0.734	0.607	0.858	

Visual analysis of the root tips revealed an almost two-fold larger fraction of apparently dead root tips on SW than on NE beech trees (Table 3.3). The root tips had a distorted and shrunken appearance (Winkler et al. 2010) and exhibited only low ^{15}N enrichment (0.11 ± 0.02 APE compared with 4.3 ± 0.6 APE of vital root tips) underpinning their reduced physiological activity. Enhanced root mortality at SW was also typical for the young trees at their field sites (fraction of dead root tips at NE: 11 ± 2% and SW = 35 ± 7%, P_{site} = 0.004). After cultivation in the soil-peat mixture, the beech roots exhibited a relatively high percentage (55 %) of non-mycorrhizal roots (Table 3.3), whereas non-mycorrhizal roots were barely found on young trees directly after excavation from their native soils (fraction of EMF-colonized root tips at NE and SW: 99.3 ± 0.4%, P_{site} = 0.859). Therefore, the non-mycorrhizal roots must have developed during the culture in the sand-peat medium with regular fertilization. A fraction of about 30 to 38% of the total root tips were vital EMF-colonized root tips under the experimental conditions applied (Table 3.3).

Mycorrhizal and bacterial communities associated with roots of NE and SW beech trees
We identified a total of 41 different EMF based on morphological features (Table 3.5), half of which colonized about 80% to 90% of the mycorrhizal root tips and were identified by ITS sequencing (Table 3.5). *Cenococcum geophilum* was the most abundant species on both root tips of SW and NE trees, but occurred twice as frequent on roots of beeches from SW than on those from NE (Fig. 3.2, Table 3.5). Two further ascomycetes (*Peziza* sp.) were also more abundant on roots of SW compared to NE trees (Fig. 3.2). On roots of NE trees basidomycetes were generally more abundant than on SW trees, in particular *Lactarius rubrocinctus* and two other *Lactarius* species (La 1, La 2) as well as *Laccaria amethystina* (La) and a *Russula* species (Ru1, Fig. 3.2). Species rarefaction curves indicated higher species richness of EMF at SW than at NE trees (Fig. 3.3, Table 3.6), but the Shannon-

Wiener index and Evenness of the EMF assemblages at SW were lower than at NE (Table 3.6) because of the dominance of *C. geophilum* (Table 3.5).

Analysis of the mycorrhizosphere-root microbial community (RRC) of NE and SW trees revealed no significant differences for species richness, Shannon-Wiener index and Evenness, respectively (Table 3.6). The rhizosphere soil microbes (RS) showed a moderate increase in species richness at roots of NE compared to SW beech trees, but no differences for the Shannon Wiener index or Evenness (Table 3.6). The Morisita Horn index, which compares the similarities of species assemblages based on their richness and abundances, was higher than 0.9, demonstrating that the microbes in the RRC fraction from NE and SW were highly similar (1 = identical); the same was true for RS microbes of NE and SW roots (Fig. 3.4). A lower Morisita Horn index of the EM than of the microbes supports the assumption that the assemblages from NE and SW trees were more dissimilar among each other than the free microbial communities (Fig. 3.4). However, there were differences in microbial communities between RRC and RS, which were stronger for the SW than for the NE trees (Fig. 3.4).

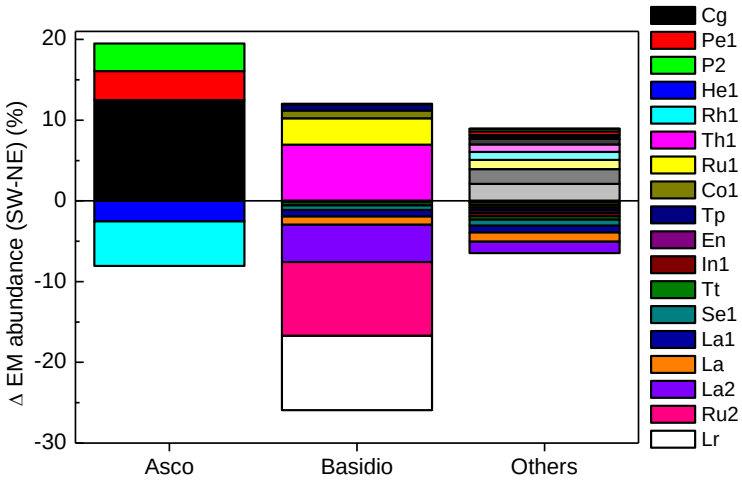

Figure 3.2: Differences in the abundance of ectomycorrhizal species on beech roots from the SW and the NE site. The fungal morphotypes were recorded on a total of 13093 and 10898 root tips from NE and SW trees, respectively. The data were normalized to 10.000 root tips. Differences were calculated as: relative abundance of species$_{(i)}$ at SW – relative abundance of species$_{(i)}$ at NE. Data were stacked according to ascomycota (Asco), basidiomycota (Basidio) and others. Others are rare morphotypes for which species information was not available. Abbreviations refer *to Cenococcum geophilum* (Cg), *Peziza* sp 1 and 2 (Pe1, Pe 2), uncultured Helotiales (He1), uncultured *Rhizoscyphus* (Rh1), uncultured Thelephoraceae (Th1) , *Russula* sp. 1 and 2 (Ru1, Ru2), *Cortinarius* sp. (Co1), *Tomentella punicea* (Tp), *Entoloma nidorosum* (En), *Inocybe* sp. (In1), *Thelephora terrestris* (Tt), *Sebacina* sp (Se1), *Lactarius* morphotype 1 and 2 (La1, La2*), Laccaria amethystina* (La), *Lactarius rubrocinctus* (Lr). Further information is given in Table 3.5.

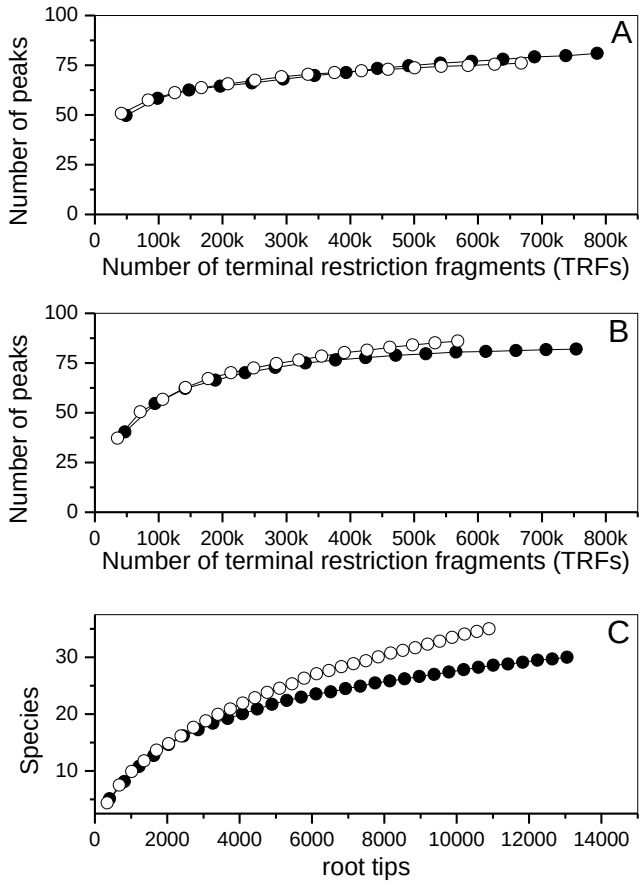

Figure 3.3: Saturation curves of rhizosphere soil (A), mycorhizosphere-root (B) associated bacteria and mycorrhizal (C) communities. Data of NE trees are shown in closed circles, data of SW trees in open circles. Species data all sampling dates were combined (n=16 per site for microbial communities, n=32 per site for mycorrhizal communities).

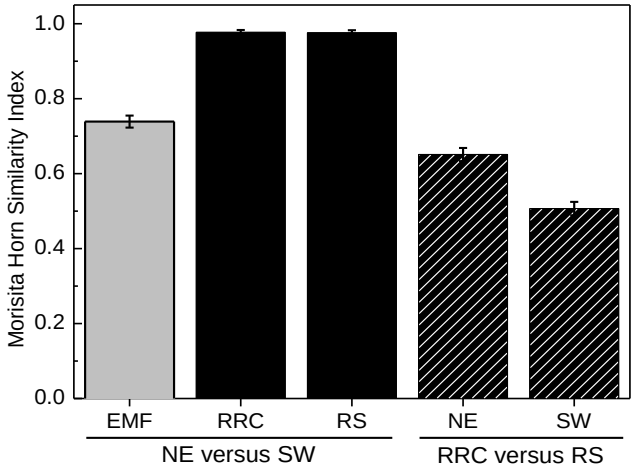

Figure 3.4: Morisita Horn Similarity Indices for pairwise comparisons of the microbial communities. The analyses are based on species richness and abundance data normalized to 10.000 individuals. Error bars indicate the calculated 95-% confidence intervals.

Table 3.5: Abundances (%) and species identities of ectomycorrhizal fungi on roots of *Fagus sylvatica* trees natural regeneration originating from beech forests on a northeast (NE) and south west (SW) slope on the Swabian Jura. A: Ascomycota, B: Basidiomycota. MT: number of morphotype(s), name: a species names was assigned when the identity of the best match in NCBI or UNITE was ≥ 97%. Accession number indicates the accession number under which the species were deposited in NCBI.

MT	NE (%)	SW (%)	Division	Name (Accession Number)	Best match (Acc. Num.)	Score	Identities (%)
1	13.90	26.38	A	*Cenococcum sp.* (KF498567)	Uncultured Cenococcum (EU668240)	872	99
9	11.35	2.14	B	*Lactarius rubrocinctus* (KF498568)	Lactarius rubrocinctus (JF908273)	1447	100
11	11.03	14.29	B	*Russula sp.* 1 (KF498569)	Russula subfoetens (UDB016206)	1128	96
22	9.57	0.43	B	*Russula sp.* 2 (KF498570)	Russula romellii (UDB011365)	1096	96
14	6.27	0.76	A	Uncultured *Rhizoscyphus* (KF498571)	Uncultured Rhizoscyphus (HQ212326)	1003	98
24	5.77	4.96	B	*Lactarius sp.* (KF498572)	Lactarius pterosporus (JQ446153)	563	98
16	5.73	4.73	B	*Laccaria amethystina* (KF498573)	Laccaria amethystina (UDB000039)	1298	99
21	5.35	2.80	A	Uncultured *Helotiales* (KF498574)	Uncultured Helotiales (GU174410)	999	98
15	5.01	0.38	B	L*actarius sp.**			
8	4.13	3.73	B	*Thelephora terrestris* (KF498575)	Thelephora terrestris (UDB003346)	1239	99
7	3.54	10.52	B	Uncultured *Thelephoraceae* (KF498576)	Uncultured Thelephoraceae (AJ893330)	910	96
30	3.29	3.12	B	*Inocybe sp.* (KF498577)	Uncultured Inocybe (HE687062)	831	94
25	2.83	2.26	B	*Sebacina sp.* (KF498578)	Uncultured Sebacina (JN701901)	1275	100
26	1.68	0.54					
13	1.51	5.11	A	*Peziza sp.* (KF498579)	Uncultured Peziza (FR852092)	860	97
34	1.41	0.00					
39	1.30	0.56					
3	1.15	0.31					
20	0.71	0.30					
5	0.62	1.40	B	*Tomentella punicea* (KF498580)	Tomentella punicea (UDB000950)	1082	99
2	0.58	0.27					
17	0.53	0.40					
28	0.44	0.00					
18	0.38	0.43	B	*Entoloma nidorosum* (KF498581)	Entoloma nidorosum (UDB008239)	1616	99
40	0.34	0.00					
4	0.32	2.15					
36	0.31	0.00					
33	0.29	0.18					
29	0.29	0.00					
12	0.19	0.21					
32	0.18	0.65					
6	0.00	3.41	A	Uncultured *Pezizales* (KF498582)	Uncultured Pezizales (EU668248)	896	98
37	0.00	2.12					
23	0.00	1.12					
35	0.00	1.00					
19	0.00	0.96	B	*Cortinarius sp.**			
27	0.00	0.92					
10	0.00	0.71					
38	0.00	0.46					
31	0.00	0.29					

*Identification by morphotyping after (Agerer 1987-2006).

Table 3.6: Diversity indices for ectomycorrhizas, mycorrhizosphere-root associated (RRC) and rhizosphere soil bacteria (RS) of young beech trees (*Fagus sylvatica*) originating from beech forests on slopes with south west (SW) and north east (NE) exposure in a low mountain range in southern Germany (Jura). Natural beech regeneration was transplanted into a sand-peat mixture and grown with regular fertilizer application for two months under identical conditions and harvested after 0, 1, 4 and 8 days (ectomycorrhiza) or after 0 and 8 days after the start of labeling (n = 8 per site and sampling date). Data of all sampling dates were pooled to reach species saturation. $P_{(perm)}$ values were calculated by permutation with 1000 random matrices. Data for RS and RRC were calculated after normalization of the peak abundances identified by RFLP analyses to 10000 arbitrary units and those for ectomycorrhizas based on species and morphotype data normalized to 10.000 root tips per site.

Diversity	Ectomycorrhiza			RRC			RS		
index	NE	SW	$P_{(perm)}$	NE	SW	$P_{(perm)}$	NE	SW	$P_{(perm)}$
Species richness	32	36	0.001	86	91	0.261	85	89	0.027
Shannon Wiener index	2.92	2.8	0.001	3	3.09	0.18	3.28	3.28	0.362
Evenness	0.58	0.46	0.001	0.23	0.24	0.748	0.29	0.34	0.115

Table 3.7: ^{15}N and N content and mean concentrations in young beech trees (*Fagus sylvatica*) originating from beech forests on slopes with south west (SW) and north east (NE) exposure in a low mountain range in southern Germany (Jura), ^{15}N and N content and mean concentrations in soil microbial biomass and the ^{15}N recovery rates of trees, microbes and total plant soil systems. Natural beech regeneration was transplanted into a sand-peat mixture, grown with regular fertilizer application for two months under identical conditions and labeled for one week with ^{15}N. ^{15}N excess was determined at day 8 (n = 8) in all plant tissues, soil microbes, soil NH_4, NO_3 and DON after subtraction of the natural ^{15}N abundance. ^{15}N and N concentrations are weighed means for all plant tissues and compartments of the plant soil system, respectively.

Site	^{15}N APE	N	^{15}N APE	N	^{15}N $APE_{microbes}$
	(µg $plant^{-1}$)	(mg $plant^{-1}$)	(µg g^{-1} dw)	(mg g^{-1}dw)	(ng g^{-1} sdw)
NE	262 ± 131	83 ± 13	23.7 ± 4.9	7.21 ± 0.57	71.9 ± 29.1
SW	337 ± 135	72 ± 10	31.9 ± 4.6	6.76 ± 0.33	87.9 ± 59.0
P	0.28	0.066	0.245	0.077	0.511

	Recovery (% ^{15}N APE)		
	(trees)	(microbes)	(total system)
NE	11.1 ± 5.6	17.7 ± 7.6	43.0 ± 13.7
SW	14.3 ± 5.7	23.8 ± 11.9	55.3 ± 17.9
P	0.277	0.283	0.149

Beeches from SW and NE show differences in ^{15}N-accumulation related to differences in microbial-mycorrhizal N processing

During the 7d labeling period with $^{15}NH_4^+$, a significant time-dependent accumulation of ^{15}N was found in all soil and plant fractions analyzed (P_{time} in the GLM for all data in Fig. 3.5 < 0.001). Overall, we recovered 49% and 59% of the applied ^{15}N in the NE and SW plant-soil systems, respectively (Table 3.7).

As expected, the strongest ^{15}N enrichment appeared in NH_4^+, which was used for labelling of the soil solution (Fig. 3.5a), but NO_3^- and microbial biomass also showed strong ^{15}N enrichments indicating microbial uptake and nitrification (Fig. 3.5b, d). In contrast, the ^{15}N enrichment in DON was about an order of magnitude lower than in NO_3^- or soil microbes (Fig. 3.5c). Notably, the ^{15}N enrichment was higher in inorganic N compounds and in microbial N in the containers with beeches from SW than in those with beeches from NE (Fig. 3.5a, b, d). The greatest difference was found for NO_3^-, where the ^{15}N enrichment was about twice higher in containers with SW trees than in those with NE trees after one week of labeling (Fig. 3.5b).

We also found stronger ^{15}N enrichment in fine roots and leaves of the SW than of the NE beech trees (Fig. 3.5e, f). Because N acquired by the plant is taken up by the active zone of the non-mycorrhizal root tips or the ectomycorrhizas, we measured the ^{15}N enrichments in these tissues (Fig. 3.5g, h). Interestingly, the ^{15}N enrichment in non-mycorrhizal root tips was very strong and exceeded that of ^{15}N in the microbial fraction (P= 0.004), but without any significant differences between the NE and SW trees (Fig. 3.5g). The non-mycorrhizal root tips of both, NE and SW trees, also showed strong ^{13}C enrichment at 8d, suggesting a high C demand of these tissues (Table 3.4).

In contrast to non-mycorrhizal roots tips, the ectomycorrhizal root tips showed striking, significant differences in ^{15}N enrichment between SW and NE trees. The ectomycorrhizas of SW trees accumulated ^{15}N slightly delayed compared to microbial ^{15}N, but eventually reached a similar enrichment, whereas ^{15}N enrichment in NE ectomycorrhizas was much slower and lagged behind ^{15}N uptake into microbes (Fig 3.5d, h). To find out whether the differences in N uptake were related to differences in carbon allocation to the ectomycorrhizas of SW and NE beech trees, respectively, the ^{13}C signatures of the colonized root tips were measured, but significant differences were not observed at d8 (Table 3.4). The $\delta^{13}C$ signatures of the ectomycorrhizas were similar to those of fine roots (Table 3.4).

The differences in N processing and plant N uptake were also linked to the relative abundance of microbes involved in N turnover in the RRC. Here, overall higher gene copy numbers for N-mineralizers, N-fixers, nitrifiers, and denitrifiers were found indicating a generally faster N–turnover in the RRC of SW than of NE trees (P = 0.013). Most pronounced differences were observed in the organic nitrogen cycle for microbes involved in protein and chitin degradation (based on increased abundance of the *chiA* and *apr* gene). In the inorganic nitrogen cycle mainly ammonia oxidizing bacteria and nitrite oxidizers harboring *nirS* took benefit from the high loads of inorganic N in the RRC of SW trees (Fig. 3.6a). In the RS fraction no significant differences in the abundance of microbes involved in the different transformation steps of the nitrogen cycle were found between NE and SW trees (Fig. 3.6b).

To find out whether the observed differences in N cycling affected whole-plant N uptake, the ^{15}N content was determined in all plant fractions at d8. However, the total amounts of ^{15}N taken up did not differ between SW and NE trees (Table 3.7), probably because of the short duration of this study and also because of the slightly higher biomass of the NE than of the

SW beeches. Significant differences at P <0.05 were neither found for the N content nor the N concentrations based on whole plant biomass (Table 3.7). The majority of the newly taken up N was localized in fine roots (88 ± 2 %) regardless the origin of the trees (data not shown). The total amino acid concentrations in the fine roots, which play a role in the regulation of plant N uptake (Geßler et al. 2004b), neither varied significantly between NE and SW trees (Table 3.3).

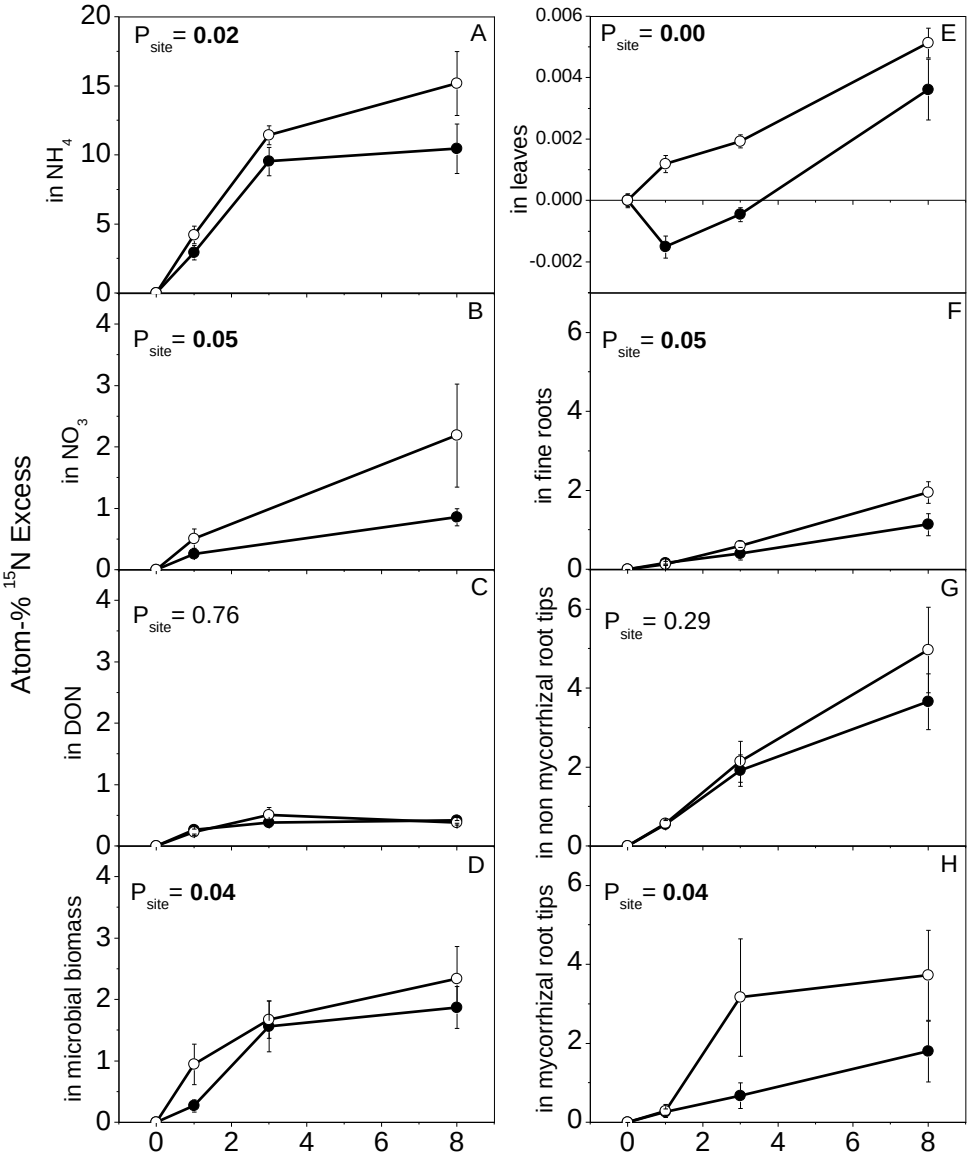

Figure 3.5: ^{15}N accumulation kinetics in soil N compounds, microbial biomass, ectomycorrhizas, and plant tissues. Natural beech regeneration was transplanted into a sand-peat mixture, grown with regular fertilizer application for two months under identical conditions and labeled for 8d with $^{15}NH_4^+$. Data are means (n = 8, ± SE) in atom-% excess (APE) for ammonia (a), nitrate (b), DON (dissolved organic nitrogen, c) and microbial biomass (d) in soil as well as for leaves (e), fine roots (f), non mycorrhizal root tips (g), and mycorrhizal root tips (h). Note different scales. NE: closed circles, SW: open cicles, Data were compared by General Linear Models with “Time” and “Site” as fixed factors. P-values for the factor “Site” are indicated in the figure. All P values for the factor “Time” were significant at P < 0.05.

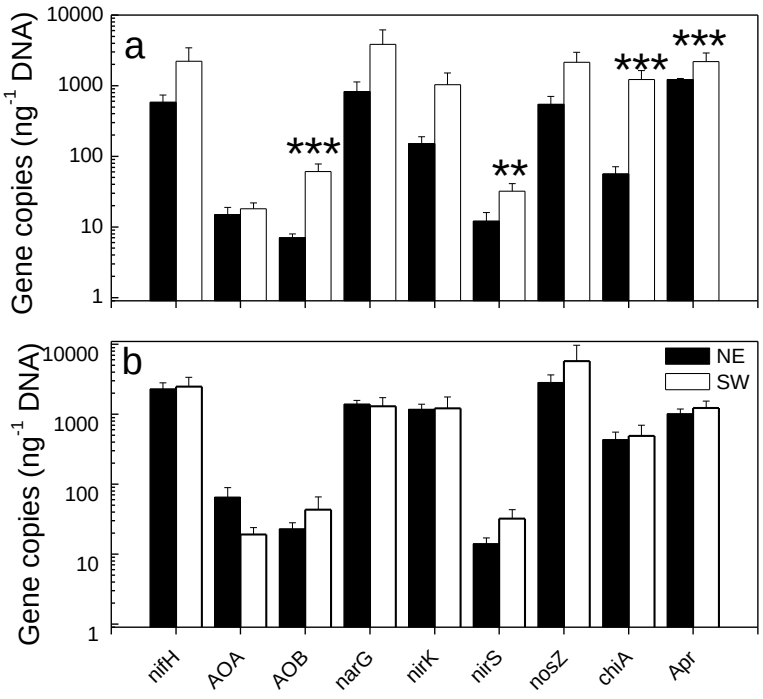

Figure 3.6: Relative abundances of genes coding for important steps of the nitrogen cycle (gene copies ng^{-1} DNA) in rhizosphere root complex (a) and rhizosphere soil (b). *nifH*: nitrogenase, *amoA* AOA: ammonia monooxygenase in ammonia oxidizing archaea, *amoB* AOB: am monia monooxygenase in ammonia oxidizing bacteria, *narG*: nitrate reductase, *nirS*: iron dependent nitrite reductase, *nirK*: copper dependent nitrite reductase, *nosZ*: nitrous-oxide reductase, *chiA*: chitinase, *apr*: alkaline protease. Eight biological replicates of two time points were combined. Data indicate mean values (n = 16, ± SE) for NE (black bars) and SW (white bars). Stars indicate significant differences at P < 0.001*** and P< 0.01**.

3.5 Discussion

3.5.1 The difference in N acquisition by trees of different origin is not due to plant-inherent features

In this study, we disentangled the influence of the origin of the plants from that of their associated soil-mycorrhizophere microbial community on N nutrition of young beech trees. Previous genetic and physiological studies with beeches from sites differing in water availability revealed significant differences in tree performance suggesting that adaptation to the environmental conditions may have occurred (Jump et al. 2006; Rose et al. 2009; Pluess and Weber 2012; Weber et al. 2013). However, population analysis with neutral genetic markers in the natural beech regeneration at the SW and NE sites, from where the present plants were collected, did not show pronounced differentiation between the tree origins (Bilela et al. 2012). At the physiological level, the amino acid concentrations in roots can modify uptake of inorganic nitrogen (Geßler et al. 2004b), but here, no significant differences for the different tree origins were observed. Still, SW and NE trees exhibited significant differences in ^{15}N accumulation when micro-scalic soil heterogeneity, differences in N supply

or climatic constraints were relieved. In contrast to our expectation that N uptake by the SW beeches would be hampered in comparison with the NE beeches as under field conditions (Fotelli et al. 2004; Geßler et al. 2005), higher N influx into the fine roots of the SW trees was found in this study. This result indicates that environmental constraints on the trees or their associated micro-organisms limited N uptake of the SW trees under field conditions.

Plant carbon allocation to the root-rhizosphere system has been identified as an important control for plant N provision (Dannenmann et al. 2009; Näsholm et al. 2013). Higher carbon consumption as indicated by higher respiration occurred, however, in soil from NE than in that from SW trees. This might have been due to the larger root system of NE trees, but not to soil microbes because their biomass in containers with NE trees was similar to those with SW trees. Furthermore, in our study soil respiration was probably dominated by the roots, because soil microbial biomass was one to two orders of magnitude smaller than in forest soil under field conditions (Dannenmann et al. 2006, 2007). The gross soil respiration was apparently fueled by stored carbon because the signature of ^{13}C coming from new photosynthetate was unaffected. Overall, we have no evidence that higher ^{15}N uptake instantaneously required higher photosynthetic C allocation to root tips, but the slightly higher ^{13}C signature in fine roots of SW trees suggests that increased N nutrition may lead to C trade-offs in the long run.

We can exclude that the increased N uptake into the fine roots was due to more efficient N uptake systems of the trees because no differences in ^{15}N enrichment were found for the non-mycorrhizal root tips of NE and SW trees. Assuming that the NO_3^- and NH_4^+ levels found per kg of dry soil were completely dissolved in the soil solution, the N concentrations were 290 to 360 µM for NH_4^+ and 39-44 µM for NO_3^- in the substrate of NE and SW trees. The NH_4^+ concentrations are in the optimal to excess range for non-mycorrhizal beech roots (Stoelken et al. 2010) and in the saturation range of mycorhizal roots of field grown beeches (Geßler et al. 2005), whereas NO_3^- is rather in the limiting range. Because of the relatively high fraction of non-mycorrhizal root tips, which exhibited the highest uptake rate of all fractions regardless the plant origin, the overall influx into the roots might have been higher than under field conditions, where we found that the roots of the natural regeneration almost 100% mycorrhizal. Because the NE trees had a larger root system, the increased ^{15}N acquisition of the fine roots of the SW trees is also unlikely to be the result of a higher root surface.

It is notable that non-mycorrhizal root tips exhibited the highest $\delta^{13}C$ signature, suggesting that they are strong sinks for energy. Preferential C allocation to reward more cooperative symbionts of plants compared with less efficient ones has been demonstrated for arbuscular mycorrhizas (Bever et al. 2009; Kiers et al. 2011). Our results suggest that this mechanism may also work for the non-mycorrhizal root tips, which were the most efficient organs for N uptake under the present unstressed conditions and often superior to mycorrhizal plants (Pena et al. 2013a). However, this will not hold under field conditions because the N uptake capacity of non-mycorrhizal roots tips is highly vulnerable to environmental fluctuations, such as drought or shading, whereas N flux through ectomycorrhizal root tips is maintained under stress (Pena and Polle 2014). In conclusion, our findings indicate that non-mycorrhizal root tips contributed significantly to N flux, but do not support that plant-inherent features including non-mycorrhizal roots were responsible for differences in N acquisition of the beeches from SW and NE.

3.5.2 Mycorrhizal and bacterial contributions to beech N supply

The acceleration of N uptake into the roots of the SW compared to the NE beeches is remarkable because this finding suggests that N delivery by mycorrhizal and rhizosphere processes were enhanced and/or that the competition by soil microbes was suppressed for the SW trees. Under field conditions, microbes are significant competitors for N (Dannenmann et al. 2009; Koranda et al. 2011; Guo et al. 2013b) and acquire soluble N faster than EMF (Pena et al. 2013b). Our data support that, in fact, soil microbes accumulated ^{15}N faster than EMF or roots, but this was a short-term effect overcome within few days. The competition was neither relevant under N mass balance considerations due to an extremely low microbial activity in our soil system compared to field conditions (here: 3 mg microbial N kg^{-1} sdw *versus* 100 to 300 mg microbial N kg^{-1} sdw in Tuttlingen forest soil, (Dannenmann et al. 2006, 2009). The finding that about 20 % of the added ^{15}N was recovered in soil microbial biomass and about 13% in the beech trees further supports that microbial competition for N was relieved in our system because under field conditions N uptake by microbial biomass is more than a magnitude larger than by beech natural regeneration (Guo et al. 2013b).

Under our conditions, the SW trees may have benefited from the higher relative abundances of microbes involved in protein and chitin degradation (Apr, chiA) and the potentially higher mobilization rates of NH_4^+ in the RRC. Obviously, the high availability of NH_4^+ reduces competition between trees and microbes for N to a larger extent in the RRC of SW trees because in addition to the observed higher ^{15}N accumulation in the fine roots also higher abundances of N consuming microbes like AOB were found compared to NE trees. The activities of Apr and chiA also imply higher degradation of organic matter in the RRC of SW trees than at those from NE. This is surprising because the substrate was mainly composed of sand. However, the SW trees contained more dead roots, which may have fostered enhanced decomposing activities. The relative increase of denitrifiers as indicated by the higher abundance of *nirS* might be a direct consequence of the increased turnover of organic matter in the RRC of SW trees and consequently reduced availabilities of oxygen, which in combination with increased NO_3^- concentrations (due to higher abundance of AOB) stimulate this process. Under long term conditions degradation of root litter is also important for beech N supply (Guo et al. 2013a), but this source is unlikely to influence N nutrition within the time scale of this study.

Although our data support functional differences of bacteria, the similarity of the RS and RRC community structures between NE and SW beech associated assemblages suggest, that the unifying substrate and climatic conditions stratified the RS and RRC assemblages (Fierer and Jackson 2006). This was in sharp contrast to the EMF community. Because ectomycorrhizal root tips have a lifespan from months up to several years (Zhou and Hogetsu 2002; Guidot et al. 2004; Treseder 2004), the observed dissimilarity of the fungal assemblages reflects differences of the SW and NE sites. The SW assemblage exhibited higher EMF species richness and faster and stronger N accumulation than the NE assemblage. These superior features corresponded to higher N accumulation in the fine roots of SW trees and higher transport to the leaves and therefore support that the EMF assemblages control N delivery to the host. Apparently, the structures of divergent EMF assemblages can play decisive roles in the observed variations in beech N uptake under field conditions (Geßler et al. 2004a). The finding that divergent *in situ* EMF assemblages exhibit functional diversity is an important result, because variation in EMF species composition and

abundance has often been interpreted to reflect the adaptation of the fungi to fluctuations of the external conditions, but the consequences for the host were unknown.
In dry ecosystems, ascomycetes are generally more abundant on roots of trees than under moist conditions (Danielson and Pruden 1989; Gehring et al. 1998; Nilsen et al. 1998). Here, ascomycota were also relatively more prevalent in the EMF assemblage of SW roots than the basidiomycota, which dominated on NE roots. The most abundant species of the present study, *C. geophilum* is well known for its drought tolerance (Jany et al. 2003; Pietro et al. 2007). It maintains N uptake under unfavorable environmental conditions, when other fungal species show a pronounced decline in N acquisition (Pena and Polle 2014) and persists under limited supply with photosynthetic assimilates (Pena et al. 2010). However, in other studies in which the functions of distinct taxa for N acquisition were characterized, *C. geophilum* was not the most efficient accumulator of N (Pena et al. 2013b; Pena and Polle 2014) and, thus, may result in trade-offs for tree nutrition in a fluctuating environment.

In conclusion, among the different components of the experimental soil-mycorrhizosphere-plant system, the composition of the EMF assemblage was crucial for host N provision, whereas evidence for physiological differences as the result genetic adaptation or long lasting acclimation due to plant origin was not observed. The differences in soil and mycorrhizosphere microbial communities and activities were mainly driven by the plants suggesting slightly stronger effects of SW than of NE trees on microbial N cycling. The ability of soil microbes to prevent short-term plant ^{15}N uptake, despite saturating N concentrations, underlines that improved mechanistic knowledge of microbial interactions with EMF is required to understand their impact on tree nutrition in fluctuating environments. Our findings highlight that the associated EMF assemblages of beech from contrasting environments exhibit important differences in N processing in the soil-plant system. To counteract the predicted negative consequences of climate change for forest productivity (Hanewinkel et al. 2013), the identification of drought tolerant beech provenances for the establishment of resistant forests has been suggested (Bolte et al. 2007; Weber et al. 2013). Our results suggest that this strategy will be insufficient when not combined with the application of adapted microbial communities, in particular EMF assemblages, because these communities control tree N supply, and therefore are crucial for forest productivity in N limited ecosystems.

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4 Climate change impairs nitrogen cycling in European beech forests

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4.1 Introducing Abstract

The vulnerability of European beech forest ecosystems to reduced soil water availability in a changing climate has been associated with large-scale loss of ecological services and economical value (Geßler et al. 2007; Meier and Leuschner 2010; Hanewinkel et al. 2013a). However, the mechanisms of climate change impacts on European beech forests are largely unknown. It has been proposed that reduced soil water availability may promote nitrogen (N) limitation of European beech due to impaired microbial N cycling in soil (Rennenberg et al. 2009), but this hypothesis has not yet been tested (Kreuzwieser and Gessler 2010). Here we present data on the influence of climate change on N transformation in the plant-soil interface *in situ*, using intact beech seedling-soil-microbe systems covering plant as well as soil microbial N turnover processes. We show that nitrate (NO_3^-) is the dominant N source for beech natural regeneration. Reduced soil water content resulted in a persistent decline of ammonia oxidizing bacteria and therefore, in a massive attenuation of gross nitrification and NO_3^- availability in the soil. Consequently, NO_3^- and total N uptake by beech seedlings were strongly reduced. Already after two growing seasons under simulated climate change conditions, this resulted in a strongly reduced performance of beech seedlings, but also in reduced soil microbial biomass. These findings support a dramatic decline by 78% of the

distribution of beech forests on calcareous soils in Europe until 2080 predicted by statistical modelling. Therefore, the present results question the sustainability of this widespread ecosystem on marginal soils already in the near future.

4.2 Results and Discussion

4.2.1 European beech forests on calcareous soil are endangered by climate change

European beech (*Fagus sylvatica* L.) dominates the natural forest vegetation in moist to moderately dry areas of the sub-mountainous altitude range in Central Europe (Ellenberg and Leuschner 1996). One third of the potential beech forest area is spread on calcareous soil (Fig. 4.1) that is highly susceptible to water deprivation. For future forestry in Central Europe, it has even been suggested to replace spruce by beech (Tarp et al. 2000; Moosmayer 2002). However, the apparent drought sensitivity of beech is a current matter of concern and debate (Leuschner et al. 2001; Rennenberg et al. 2006, 2009; Geßler et al. 2007; Kreuzwieser and Gessler 2010), due to observations of increased heat waves and drought periods in wide regions of Central Europe (Coumou et al. 2013). This trend is expected to continue and intensify in the coming decades (Seneviratne et al. 2006; Smiatek et al. 2009). Based on statistical species distribution models driven by climatic predictors (Hanewinkel et al. 2013 a, b), we computed the distribution range of beech forests on calcareous soil in Europe and found drastic reductions by almost 80% under an SRES A2 scenario (IPCC 2000) until the year 2080 (Fig. 4.1). However, the physiological processes behind this dramatic biome shift on calcareous soil are rarely understood.

Two different mechanisms have been proposed to explain the sensitivity of beech to increased temperature and drought (Geßler et al. 2007; Rennenberg et al. 2009; Kreuzwieser and Gessler 2010): (1) physiological limitations such as xylem embolism, restricted nutrient uptake capacity and reduced growth; and (2) impaired provision of bioavailable N by soil microbes. The latter mechanism may be of particular importance in calcareous soil, because Rendzic Leptosols derived from limestone are poor in bioavailable N (Dannenmann et al. 2006, 2009). Furthermore, these soils are characterized by a shallow profile, high gravel content, heavy texture, and low water and nutrient retention capacity. Hence, N is frequently limiting growth of this forest type (Dannenmann et al. 2009; Rennenberg et al. 2009; Simon et al. 2011; Weber et al. 2013).

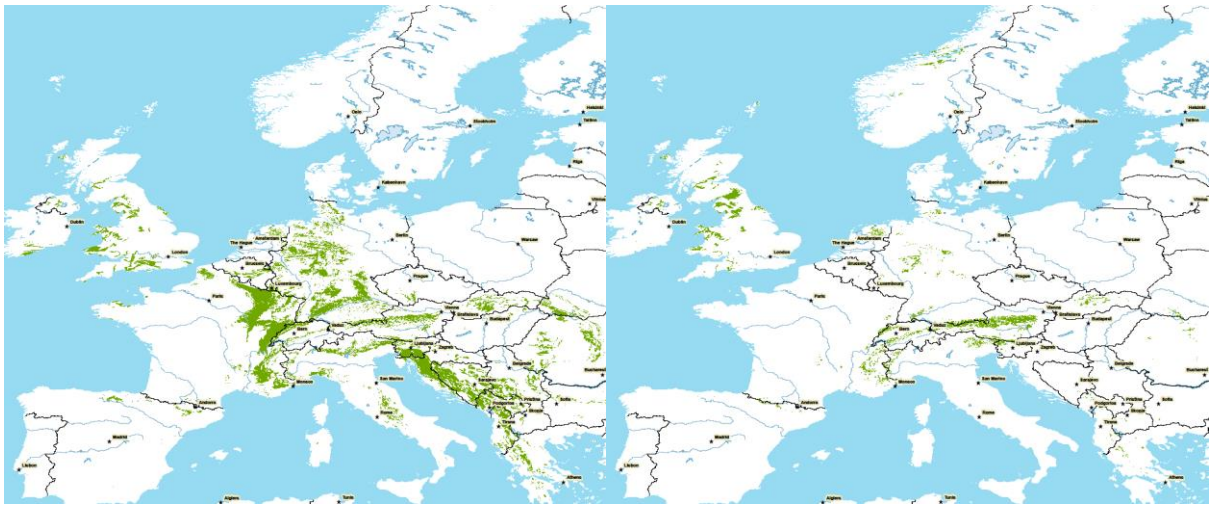

Figure 4.1: Modelled potential distribution of beech forests on calcareous soils in Europe (green colour) under current climatic conditions (left panel). These forests would cover an area of 31.4 million ha, i. e. approximately one third of the potential beech forests in Europe (104.0 million ha). For the SRES A2 scenario (IPCC 2000), we computed a potential distribution of 7.2 million ha in the year 2080 (right panel), i. e. a reduction to 22% of the current distribution.

Here we present an isotope-based experimental approach to simultaneously quantify all major N turnover processes in undisturbed beech seedling-soil-microbe systems, thereby maintaining plant-soil microbe interaction and –competition for N. By translocation of intact soil mesocosms containing natural beech regeneration across a narrow valley from the northwest (NW) the southwest (SW) aspect, we combined this approach with a space-for-time climate change experiment (Fig. 4.2). This treatment increased soil temperature on average by 1 °C (Fig. 4.3) and persistently decreased soil water availability over the entire growing season (Figs. 4.4, 4.5). The NW exposure corresponds to a model climate for present day conditions of many beech forests in Central Europe, while the SW exposure is considered a model for climatic conditions expected for the coming decades (Geßler et al. 2004). Additionally, a roof system accelerated drought during a 39 days period at SW aspect (Fig. 4.4). Supporting measurements included microbial N cycle gene abundances, mycorrhizal colonization and N metabolite levels in fine roots as well as plant biomass and long-term isotope recovery in beech seedlings.

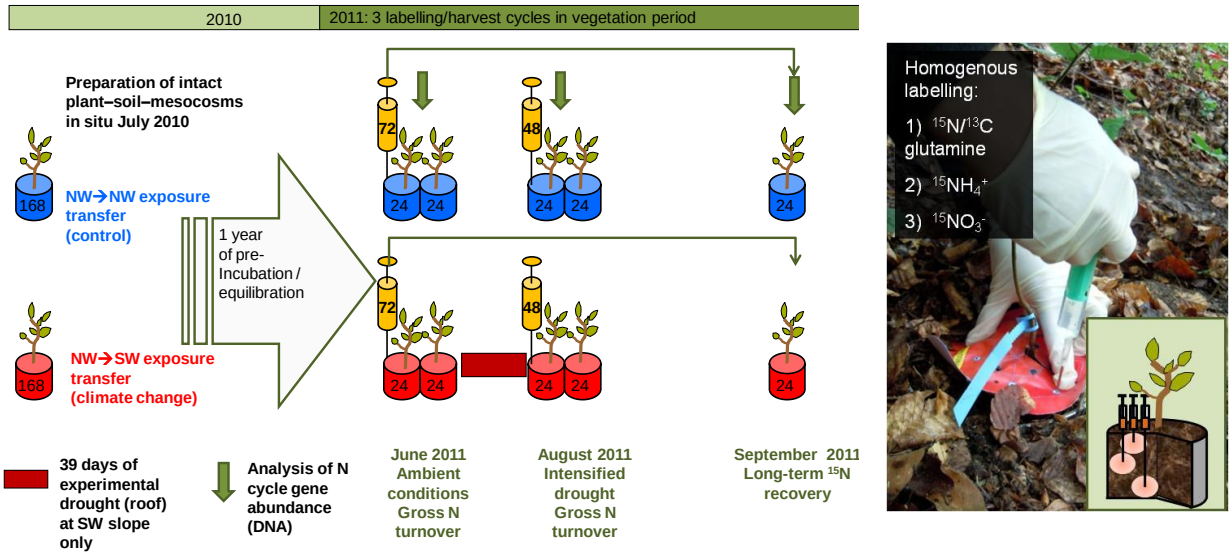

Figure 4.2: Experimental design. The figure illustrates coring of beech-soil-mesocosms by use of stainless steel cylinders (16.4 cm inner diameter, 15 cm height) with subsequent pre-incubation for one year either under cool-moist microclimate at the coring site (NW exposure, control) or warm-dry microclimate (SW-exposure, climate change). After pre-incubation and equilibration, homogenous and reproducible labelling of the intact plant-soil-microbe systems (40 injections per beech-soil-mesocosm) with $^{15}N/^{13}C$-enriched glutamine, ^{15}N-ammonium (NH_4^+) or ^{15}N-nitrate (NO_3^-) and subsequent double harvests (6 and 48 hours after labelling) were conducted twice. Analysis of total N and ^{15}N enrichment in soil, microbial, mycorrhizal and plant N pools facilitated determination of simultaneously occurring gross N turnover rates in the plant-soil-microbe system in June (comparison of ambient NW vs. SW climatic conditions) and August (ambient NW conditions vs. roof-intensified drought at SW) via ^{15}N tracing and pool dilution calculations, while plant-soil microbe interaction and competition for N persisted throughout the experimental incubation period. A final harvest of mesocosms labelled in June allowed investigating long-term isotope recovery in September. All three harvest dates were accompanied by determination of supporting soil and plant parameters such as microbial N cycle gene abundance, mycorrhization and ^{15}N signature of mycorrhizal root tips, and plant metabolites. Generally, the replication was n=8 for every harvesting date, exposure treatment, and labelling treatment. Parallel harvests of unlabelled beech-soil-mesocosms (n=4 to 8) were conducted in order to quantify ^{15}N natural abundance and background N concentrations (not shown in the graph).

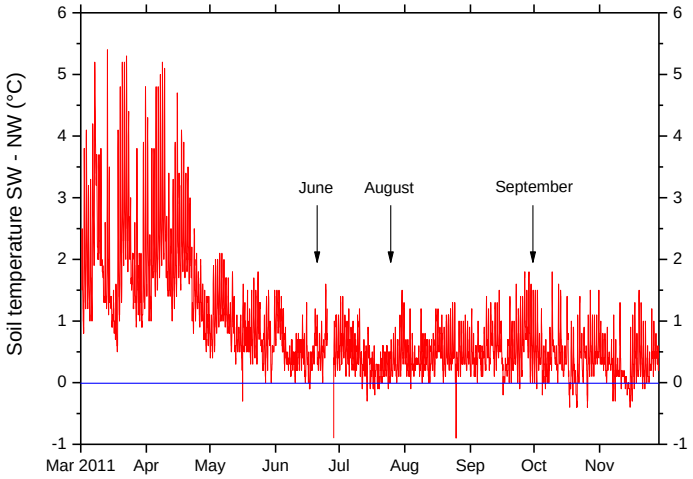

Figure 4.3: Soil temperature differences (5 cm depth) between beech-soil-mesocosms incubated at SW exposure (warm-dry microclimate, climate change treatment) and at NW exposure (cool-moist microclimate, control treatment). Data represent mean values of five temperature probes per treatment directly installed horizontally in soil of transferred beech-soil-mesocosms. Arrows indicate the three harvest campaigns. The period between the harvests in June and September equals the roof period of 39 days (see Fig. 4.2).

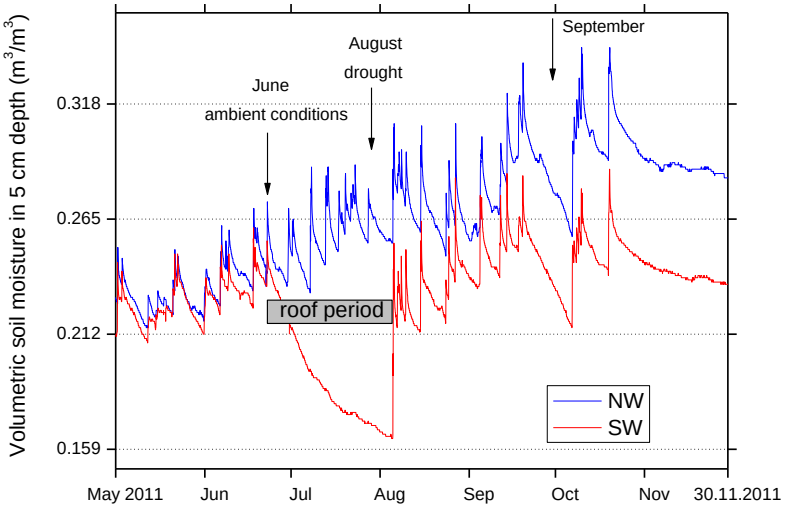

Figure 4.4: Dynamics of volumetric soil moisture in 5 cm depth (mean values of n=5 measurements) in intact beech-soil-mesocosms of the control treatment (NW exposure, cool-moist microclimate) and climate change treatment (SW exposure, warm-dry microclimate) in the growing season 2011, i. e. 1 year after implementation of treatments by transferring beech seedling-soil-mesocosms within NW exposure or to SW exposure in summer 2010. Arrows indicate harvest campaigns (see Fig. 4.2).

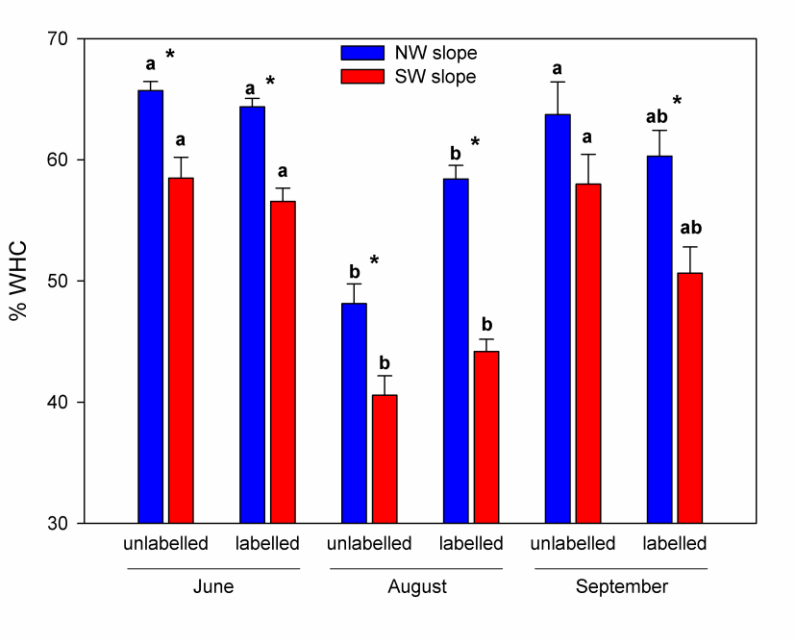

Figure 4.5: Gravimetric soil moisture related to water holding capacity (WHC) as determined from harvested labelled (n=48) and unlabelled (n=4 to 8) beech-soil-mesocosms during harvests in June (ambient conditions at both exposures), August (intensified drought at SW exposure due to roof) and September (final harvest). Asterisks indicate significant differences ($p<0.05$) between NW and SW exposure at the respective harvest. Different indices indicate significant differences between different harvesting dates and labelled and unlabelled beech-soil-mesocosms.

4.2.2 Climate change decelerates beech N nutrition as a consequence of impaired nitrification

Ammonification, nitrification and microbial immobilization of inorganic N were approximately one order of magnitude larger than plant uptake (Fig. 4.6). Microbial biomass N was several times larger than the plant N pool (Fig. 4.6). Extractable soil ammonium (NH_4^+) and NO_3^- pools were in the magnitude of daily rates of gross inorganic N production and consumption so that microbial turnover largely controlled soil inorganic N availability. Nitrate was the dominant N source for beech seedlings in June, followed by NH_4^+, whereas organic N supplied as glutamine was hardly recovered (Fig. 4.6). Significantly larger N uptake in June than in August (Fig. 4.6) confirmed an earlier study investigating N uptake capacities of adult beech and beech seedlings. This study showed that the onset of the growing season is a key period for N acquisition by beech seedlings as part of a competition avoidance strategy with adult beech, which preferentially take up N later in the season (Simon et al. 2011). Such studies on N uptake capacity also indicate that glutamine is of high importance for N nutrition of beech (Simon et al. 2010, 2011; Stoelken et al. 2010). Here, the application of double-labelled ^{13}C-carbon (^{13}C)/^{15}N glutamine homogeneously applied to the intact beech seedling-soil systems showed that ^{15}N but not ^{13}C was retrieved in mycorrhizal and plant tissues (Fig. 4.6, Tables 4.1, 4.2). This suggests that either uptake of intact glutamine was not significant under such realistic field conditions or glutamine derived C was already subjected to respiration in the mycorrhizal mantle (Näsholm et al. 2009). These findings show the limitation of uptake capacity studies to explain actual *in situ* uptake of organic N in the presence of microbial competition.

The N cycle in soil of the NW treatment was characterized by high nitrification rates (Fig. 4.6). In contrast, transfer to SW exposure resulted in a five-fold decline of gross nitrification and soil NO_3^- concentrations already in June (Fig. 4.6). Consequently, plant NO_3^- uptake was also reduced by a factor of five (Fig. 4.6), although mycorrhizal colonization of vital roots remained unaltered (Table 4.3). This suggests a tight link between soil water availability, gross nitrification, soil NO_3^- availability and NO_3^- uptake of beech seedlings. In conjunction with decreased NH_4^+ uptake, this resulted in a significantly diminished plant N pool (Fig. 4.6). From June to August, plant biomass N increased significantly at NW but only marginally at SW under drought conditions (Fig. 4.6). Plant N uptake calculated from short-term $^{15}NH_4^+$ and $^{15}NO_3^-$ tracing in June and August corresponded well with long-term N uptake calculated from the increment between June and August, with both approaches clearly showing a severely reduced N uptake for SW (Table 4.4). In August, rates of N turnover and plant uptake were generally less than in June both at NW and SW with similar but less pronounced climate change treatment effects on N cycling as observed in June (Fig. 4.6).

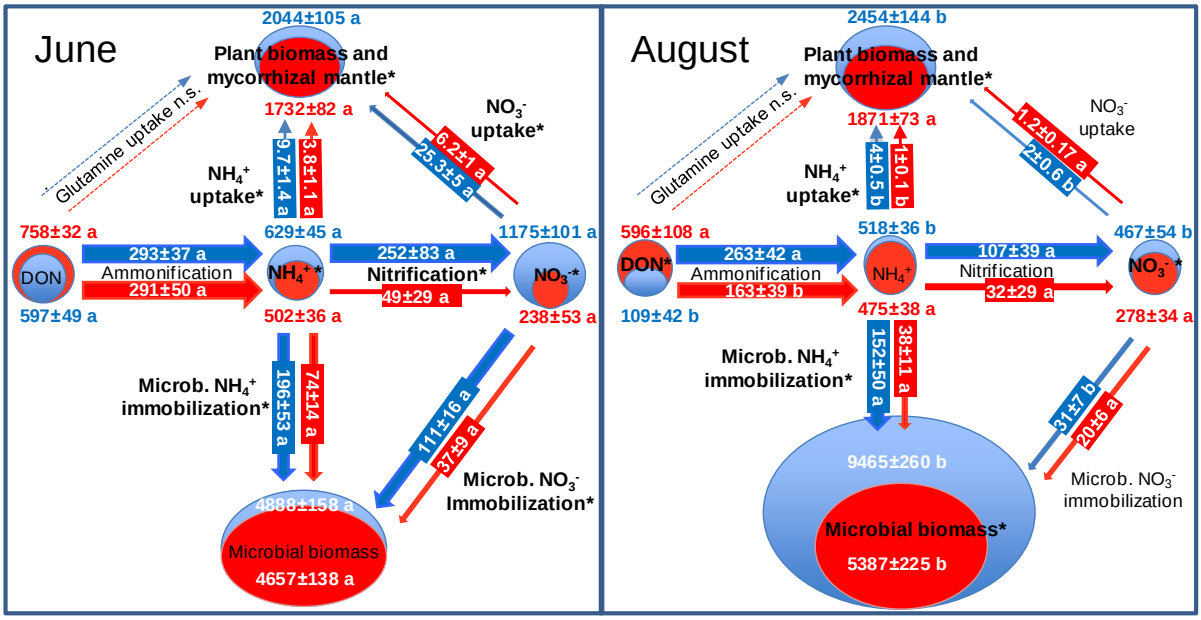

Figure 4.6: Gross N turnover rates (mg N m^{-2} day^{-1}) (n=8) and N pool sizes (mg N m^{-2}) (N= 48) in intact beech seedling-soil-mesocosms. Blue: NW exposure (control treatment); Red: SW exposure (climate change treatment). The June harvest represents the onset of the growing season after full development of leaves, while the August harvest was conducted in the middle of the growing season after 39 days of rainfall exclusion at SW. Gross rates of N turnover were calculated based on ^{15}N tracing and pool dilution approaches following homogenous labelling of the intact soil with double-labelled $^{15}N/^{13}C$-glutamine, $^{15}NH_4^+$, or $^{15}NO_3^-$. Thickness of process arrows and nitrogen pool signatures is representative for respective turnover rates and pool sizes. Processes and pools significantly affected by the climate change treatment are indicated by bold letters with asterisks. Different indices show significant differences between June and August for a given treatment/exposure.

Table 4.1: ^{13}C recovery (mg ^{13}C excess, and % of ^{13}C excess applied via glutamine) in plant (sum of fine roots, coarse roots, stem, leaves) for the single harvesting dates (month_time after glutamine labelling). No significant differences were observed between NW and SW.

	NW			SW		
	Mean [mg ^{13}C excess]	SD	% of applied ^{13}C excess	Mean [mg ^{13}C excess]	SD	% of applied ^{13}C excess
June_6h	0.0072	0.0078	0.4280	-0.0028	0.0031	-0.1657
June_48h	-0.0006	0.0032	-0.0347	-0.0127	0.0082	-0.7547
August_6h	0.0003	0.0056	0.0194	-0.0007	0.0031	-0.0419
August_48h	0.0002	0.0048	0.0108	0.0005	0.0043	0.0322
September_3months	0.0248	0.0086	1.4719	0.0117	0.0112	0.6929

Table 4.2: ^{13}C enrichment (atom%) in excess of natural abundance in mycorrhizal root tips harvested in June, August and September. No significant ^{13}C excess enrichment was observed in mycorrhiza within 48 hours after ^{13}C glutamine labelling.

Harvest date_time after ^{13}C labelling	NW		SW		Two-Way-ANOVA		
	mean	SE	mean	SE		p	F
June_6h	-0.0004	0.0001	-0.0006	0.0003	Exposure	0.924	0.009
June_48h	-0.0002	0.0002	-0.0014	0.0003	Time	0.000	6.178
August_6h	0.0005	0.0006	0.0000	0.0005	Interactions	0.003	4.602
August_48h	-0.0010	0.0003	-0.0007	0.0004			
September_3 months	-0.0003	0.0005	0.0012	0.0002			

Table 4.3: Percentage of vital root tips colonized with mycorrhizal fungi. No significant differences in mycorrhizal colonization rates were observed between the control treatment (NW exposure) and the climate change treatment (SW exposure).

	NW		SW		Two-Way-ANOVA		
	mean	SE	mean	SE		p	F
June	99.0	0.5	99.2	0.4	Slope	0.56	0.34
August	99.1	0.5	98.8	0.4	Time	0.08	2.51
September	99.7	0.1	99.9	0.1	Interactions	0.83	0.18

Table 4.4: Total plant N uptake (mg N m^{-2} day^{-1}) calculated (1) from the net increment of the mean plant N pool between June and August (n=48 each), divided by the time span of 41 days between these harvesting dates, (2) from the sum of NH_4^+ and NO_3^- uptake rates calculated from short-term (6h) $^{15}NH_4^+$ and $^{15}NO_3^-$ tracing into plant biomass in June and August. Nitrogen uptake was always significantly smaller at SW exposure than at NW exposure ($p<0.05$) for all approaches shown in the table.

	N increment June-August (42 days) (mg N m^{-2} day^{-1})	Short-term $^{15}NH_4^+$ and $^{15}NO_3^-$ tracing June (mg N m^{-2} day^{-1})	Short-term $^{15}NH_4^+$ and $^{15}NO_3^-$ tracing August (mg N m^{-2} day^{-1})
NW	10	35 ± 7	6 ± 1.1
SW	3.4	9 ± 1.6	2.2 ± 0.31

The observed effects of the climate change treatment on N processes (Table 4.5) in the plant-soil interface were confirmed by a range of supporting data. Concurrent analyses of microbial communities involved in selected processes of the N cycle revealed a pronounced reduction of ammonia oxidizing bacteria (AOB) in bulk soil in June and September and in the rhizosphere in August (Fig. 4.8, Table 4.6). Gross nitrification rates were strongly positively correlated with the abundance of AOB in soil (Fig. 4.8). Therefore, the still significantly reduced number of gene copies of AOB at the end of the growing season in September (Fig. 4.8) suggests that the climate-change triggered mechanism of impaired nitrification with associated consequences for NO_3^- availability and uptake by beech is persistent. In contrast, effects on other functional groups of microbes involved in N turnover were not or not consistently observed (Table 4.6).

Reduced N uptake by beech seedlings of the climate change treatment is supported by persistently higher ^{15}N enrichment in mycorrhizal root tips grown in NW exposure than in SW exposure (Table 4.7). Furthermore, long-term ^{15}N recovery in beech seedlings as determined in September, i. e. three months after ^{15}N labelling was persistently smaller at SW than at NW regardless of the ^{15}N source provided (Fig. 4.9). Moreover, levels of N-bearing metabolites in fine roots of beech seedlings of the climate change treatment were reduced (Fig. 4.7). Finally, above- and belowground biomass of beech seedlings were persistently smaller in SW than in NW beech-soil-mesocosms (Table 4.8).

Ammonification and soil NH_4^+ levels were only marginally reduced in the climate change treatment (Fig. 4.6). This indicates that lower soil moisture accounted for attenuated nitrification through detraction of AOB metabolism and limitation of N substrate diffusion (Norton and Stark 2011). Soil moisture is the major environmental driver of *in situ* nitrification in Rendzic Leptosols with maximum nitrification at 65 % of maximal water holding capacity (Dannenmann et al. 2006), i. e. the soil water levels frequently found at NW exposure (Fig. 4.7). A strong reduction of gross nitrification rates when soil moisture falls below this optimum is in line with our general understanding of nitrification (Norton and Stark 2011) as well as with earlier observations for Rendzic Leptosols in beech stands (Dannenmann et al. 2006). The strong sensitivity of the AOB community to climatic change conditions may be related to the fact that these microorganisms obligatorily depend on ammonia oxidation without metabolic alternatives. Thus, NO_3^--dominated N nutrition of beech seedlings

represents a major obstacle for beech performance under reduced soil water levels in marginal soil in a changing climate. This may also apply for adult beech growing on Rendzic Leptosols, which exhibit decreased maximum NO_3^- uptake capacity and basal stem area with declining soil water availability (Geßler et al. 2005).

Dynamic vegetation models so far focus on light and water as determinants of stand development and species competition in a changing climate. Our work shows that N dynamics is a critical component to be included in such models. Our data facilitate testing whether dynamic vegetation models coupled to process-oriented biogeochemical ecosystem models (Haas et al. 2013) can consider effects of climate change on beech performance more comprehensively, taking also into account N availability as mediated by soil microbes.

Table 4.5: Results of two-way ANOVA analysis testing the factors exposure (NW vs. SW), time (June/August/September) and the interaction of exposure and time on gross rates of N turnover in the plant-soil-microbe system and corresponding N pools. Numbers represent P (upper number, significant at P<0.05) and Eta squared (lower number) as a measure of effect size

	Exposure	Time	Exposure x Time
Ammonification	0.223	0.064	0.24
	0.055	0.121	0.051
Nitrification	0.01	0.119	0.213
	0.215	0.085	0.055
Micr. NH_4 inmob.	0.004	0.301	0.906
	0.26	0.038	0.001
Micr. NO_3 immob.	<0.001	<0.001	0.006
	0.367	0.433	0.243
Plant NH_4 uptake	<0.001	0.002	0.633
	0.433	0.291	0.008
Plant NO_3 uptake	0.001	<0.001	0.002
	0.335	0.494	0.29
DON pool	<0.001	<0.001	0.003
	0.069	0.115	0.049
NH_4 pool	0.034	<0.001	0.506
	0.024	0.205	0.007
NO_3 pool	<0.001	<0.001	<0.001
	0.309	0.404	0.293
Plant pool	<0.001	<0.001	0.517
	0.065	0.061	0.005
Micr. Biomass pool	0.0067	<0.001	0.022
	0.014	0.125	0.032

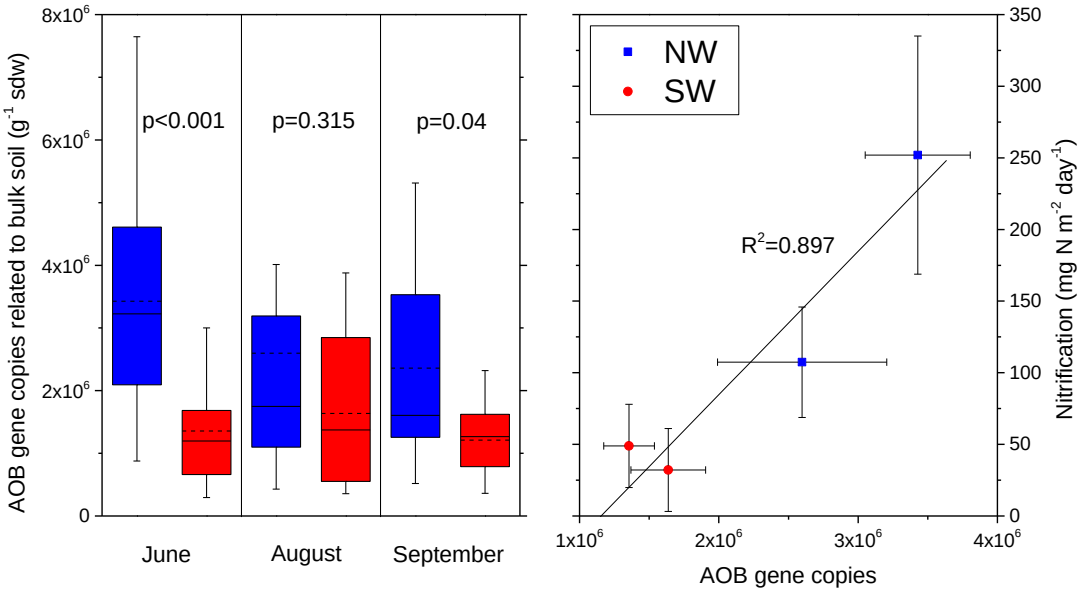

Figure 4.7: Ammonia oxidizing bacteria and nitrification rates. Abundance of genes encoding for ammonia monooxygenase of ammonia oxidizing bacteria in bulk soil (AOB, left panel) and relationships between AOB gene abundance and gross rates of nitrification (right panel). Blue colour: control treatment (NW exposure). Red colour: climate change treatment (SW exposure).

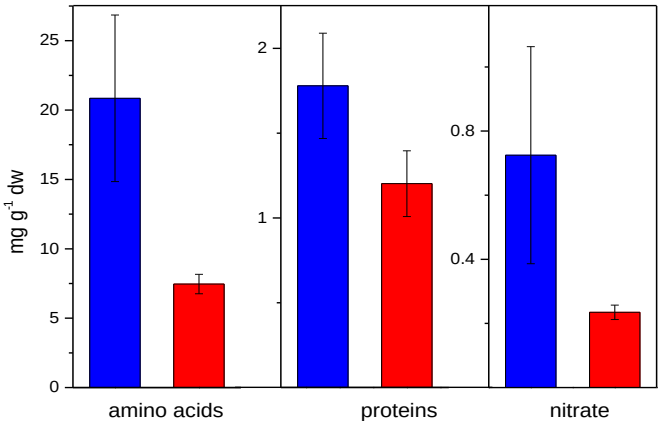

Figure 4.8: Metabolites (total amino acids, total soluble proteins, NO_3^-) extracted from fine roots of beech seedlings in June. Blue colour represents the control treatment (NW exposure), red colour represents the climate change treatment (SW exposure). Error bars denote standard errors of the mean. n= 4 per time and treatment. Amino acid and NO_3^- metabolite levels were significantly lower in trees of the climate change treatment.

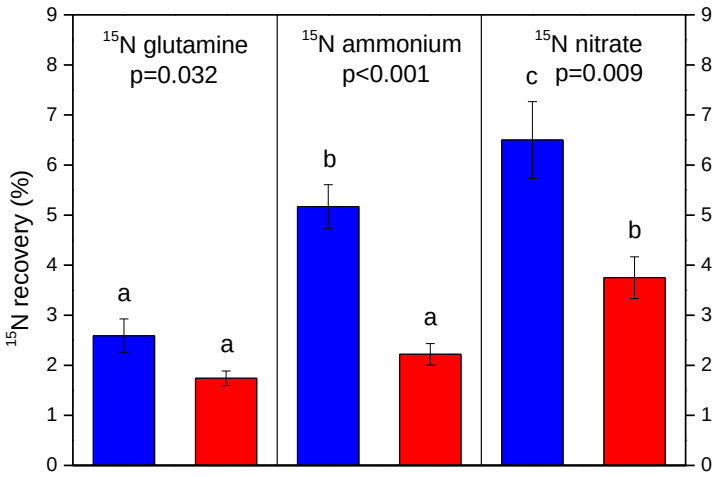

Figure 4.9: Long-term ^{15}N recovery (n=8) in beech seedlings. ^{15}N recovery (sum of fine roots, coarse roots, stem and leaves) was determined at the end of the growing season in September three months after ^{15}N labelling with glutamine, NH_4^+ or NO_3^-. Data indicate recovered % of isotopic excess, i. e. after subtracting ^{15}N natural abundance. Blue: NW exposure (control treatment); red: SW exposure (climate change treatment). ^{15}N recovery was highest after nitrate labelling both for SW and NW as indicated by different indices. The climate change treatment always reduced ^{15}N recovery, as indicated by $p<0.05$.

Table 4.6: Abundance of microbes involved in selected steps of the N cycle based on the quantification of marker genes. Nitrogen fixing microorganisms based on the nitrogenase gene *nifH*, archaeal and bacterial nitrifiers based on the ammonia monooxygenase gene *amoA*, denitrifiers based on the nitrite reductase genes *nirK* and *nirS,* as well as on the nitrous oxide reductase gene *nosZ*, and mineralising microorganisms based on the chitinase gene *chiA* and the metalloprotease gene *apr*. Significantly more gene copies in soil at NW exposure compared to SW exposure are highlighted in dark blue, while dark red is indicating significantly larger values at SW exposure. SD: standard deviation of the mean.

		Bulk soil				Rhizosphere			
		NW	SD	SW	SD	NW	SD	SW	SD
nifH	June	$4.1x10^7$	$1.2x10^7$	$3.9x10^7$	$2.1x10^7$	$2.6x10^8$	$1.3x10^8$	$2.5x10^8$	$1.4x10^8$
	August	$3.4x10^7$	$1.4x10^7$	$4.1x10^7$	$2.0x10^7$	$3.1x10^8$	$1.3x10^8$	$3.2x10^8$	$3.6x10^8$
	September	$2.7x10^7$	$1.1x10^7$	$4.0x10^7$	$1.9x10^7$	$2.8x10^8$	$1.1x10^8$	$1.7x10^8$	$1.3x10^8$
AOA	June	$1.5x10^8$	$1.4x10^8$	$1.2x10^8$	$1.1x10^8$	$6.1x10^8$	$6.9x10^8$	$8.9x10^8$	$8.9x10^8$
	August	$1.3x10^8$	$1.5x10^8$	$1.5x10^8$	$1.8x10^8$	$9.8x10^8$	$5.3x10^8$	$1.0x10^9$	$1.1x10^9$
	September	$5.8x10^7$	$4.0x10^7$	$2.5x10^8$	$2.6x10^8$	$2.1x10^8$	$1.1x10^8$	$5.4x10^8$	$6.3x10^8$
AOB	June	$3.4x10^6$	$1.8x10^6$	$1.4x10^6$	$8.7x10^5$	$3.0x10^7$	$2.3x10^7$	$1.9x10^7$	$1.4x10^7$
	August	$2.6x10^6$	$2.7x10^6$	$1.6x10^6$	$1.1x10^6$	$2.7x10^7$	$1.1x10^7$	$1.4x10^7$	$1.7x10^7$
	September	$2.4x10^6$	$1.6x10^6$	$1.2x10^6$	$5.8x10^5$	$6.4x10^6$	$2.4x10^6$	$9.3x10^6$	$9.4x10^6$
nirK	June	$2.1x10^8$	$7.3x10^7$	$1.7x10^8$	$5.3x10^7$	$1.9x10^9$	$8.3x10^8$	$2.0x10^9$	$5.9x10^8$
	August	$1.7x10^8$	$5.7x10^7$	$2.1x10^8$	$9.2x10^7$	$1.9x10^9$	$6.5x10^8$	$1.9x10^9$	$9.4x10^8$
	September	$1.1x10^8$	$4.0x10^7$	$1.9x10^8$	$7.9x10^7$	$8.2x10^8$	$4.3x10^8$	$9.9x10^8$	$4.2x10^8$
nirS	June	$9.4x10^6$	$3.7x10^6$	$6.2x10^6$	$1.8x10^6$	$8.1x10^7$	$8.3x10^8$	$7.7x10^7$	$3.8x10^7$
	August	$7.9x10^6$	$2.7x10^6$	$8.6x10^6$	$3.9x10^6$	$9.8x10^7$	$5.6x10^7$	$6.6x10^7$	$5.2x10^7$
	September	$5.3x10^6$	$1.7x10^6$	$8.7x10^6$	$3.7x10^6$	$3.4x10^7$	$1.5x10^7$	$3.1x10^7$	$2.0x10^7$
nosZ	June	$1.5x10^8$	$4.2x10^7$	$1.2x10^8$	$5.3x10^7$	$8.9x10^8$	$5.1x10^8$	$1.0x10^9$	$4.9x10^8$
	August	$1.1x10^8$	$4.5x10^7$	$1.4x10^8$	$8.0x10^7$	$7.6x10^8$	$2.5x10^8$	$6.0x10^8$	$3.1x10^8$
	September	$1.0x10^8$	$3.4x10^7$	$9.4x10^7$	$2.4x10^7$	$4.2x10^8$	$1.4x10^8$	$6.8x10^8$	$4.0x10^8$
chiA	June	$2.3x10^8$	$9.9x10^7$	$1.7x10^8$	$6.8x10^7$	$1.1x10^9$	$6.4x10^8$	$7.8x10^8$	$3.2x10^8$
	August	$1.6x10^8$	$6.7x10^7$	$1.8x10^8$	$8.4x10^7$	$8.5x10^8$	$4.9x10^8$	$1.3x10^9$	$1.5x10^9$
	September	$1.4x10^8$	$5.6x10^7$	$1.8x10^8$	$6.8x10^7$	$5.6x10^8$	$3.9x10^8$	$7.2x10^8$	$3.1x10^8$
apr	June	$5.9x10^8$	$1.7x10^8$	$4.8x10^8$	$1.6x10^8$	$4.6x10^9$	$1.8x10^9$	$6.1x10^9$	$2.0x10^9$
	August	$4.9x10^8$	$1.5x10^8$	$6.2x10^8$	$3.3x10^8$	$3.8x10^9$	$1.2x10^9$	$4.7x10^9$	$1.8x10^9$
	September	$3.9x10^8$	$1.3x10^8$	$5.3x10^8$	$2.3x10^8$	$2.1x10^9$	$8.1x10^8$	$4.3x10^9$	$2.3x10^9$

Table 4.7: ^{15}N enrichment (atom%) in excess of natural abundance in mycorrhizal root tips harvested in June and August (6 and 48 hours after isotope labelling each) and in September (3 months after isotope labelling). Significantly higher ^{15}N enrichments at NW compared to SW is indicated by dark blue colour.

	NW		SW		Two-Way-ANOVA		
N form	mean	SE	mean	SE		p	F
Glutamine							
June_6h	0.0166	0.0022	0.0018	0.0003	Exposure	0.000	258
June_48h	0.0575	0.0166	0.0048	0.0030	Time	0.000	274.9
August_6h	0.0266	0.0113	0.0052	0.0014	Interactions	0.000	147.1
August_48h	0.0491	0.0157	0.0259	0.0111			
September_3 months	0.0690	0.0076	0.0610	0.0085			
Ammonium							
June_6h	0.1586	0.0432	0.2157	0.0634	Exposure	0.049	4.1
June_48h	0.4825	0.0847	0.3964	0.0624	Time	0.000	11.6
August_6h	0.2371	0.0668	0.0833	0.0437	Interactions	0.534	0.8
August_48h	0.5961	0.1149	0.4223	0.1737			
September_3 months	0.7681	0.1160	0.5523	0.0894			
Nitrate							
June_6h	0.0648	0.0252	0.0551	0.0136	Exposure	0.005	8.8
June_48h	0.2155	0.0546	0.1027	0.0327	Time	0.010	3.7
August_6h	0.0960	0.0264	0.0339	0.0085	Interactions	0.269	1.3
August_48h	0.2387	0.0790	0.0621	0.0161			
September_3 months	0.1871	0.0445	0.1615	0.0448			

Table 4.8: Aboveground and belowground dry plant biomass (mg) of beech seedlings for the three harvest dates. n=48 (June and August); n=24 (September). Significantly larger biomass at NW compared to SW tested for single harvest dates is indicated by dark blue colour.

		June	August	September
aboveground	NW	2325±134	2590±155	1919±93
	SW	2031±106	2065±90	1762±98
belowground	NW	1893±98	2421±155	2789±126
	SW	1546±7	1576±60	1955±96

4.2.3 Implications for future forest management and ecosystem services

Impaired microbial provision of bioavailable N may be a stressor for beech in addition to plant physiological limitations under reduced soil water availability, enhancing the drought sensitivity of beech under predicted climatic changes. The nutritional deficiency could be further exacerbated by enhanced N leaching after prolonged drought due to retarded stabilization of microbial N in organo-mineral associations (Bimüller et al. 2014).

By highlighting the relationships between drought, N cycling and beech N nutrition, our work provides pointers to developing mitigation options to increase forest resilience and adaptation potential in a changing climate. Nutritional limitations could be counteracted by fertilization to increase levels of bioavailable N in soil. However, trade-offs such as undesired rapid hydrological NO_3^- leaching (Bimüller et al. 2014) in the joint acquifers of limestone karst systems would need to be quantified. The associated risk for nitrous oxide losses may remain small due to high nitrous oxide reductase activity in such soils, converting nitrous oxide into harmless dinitrogen (Dannenmann et al. 2008). Another option is silvicultural reduction of stand density. This treatment increased soil water availability via increased throughfall and reduced competition for water in coniferous stands (Kohler et al. 2010). A further alternative is mixing beech stands with deep-rooting trees such as oak, which appears to improve water balance of soil and beech via hydraulic lift of water from deeper aquifers (Pretzsch et al. 2013), and thus may increase the resilience of forest stands to climate change conditions. In view of the long time spans needed to convert forests, there is an urgent need to consider alternative, more drought-robust tree species with higher N use efficiency for these ecosystems to maintain sustainable forest development and ecological services in Central European forests.

4.3 Acknowledgements

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4.4 Material and Methods

4.4.1 Maps intersection potential distribution of beech on calcareous sites

The map (Fig. 4.1) is an intersect of a species distribution model for European beech (Hanewinkel et al. 2013b) based on maps of geology for Europe. For the species distribution model we used presence/absence information for European beech derived from the 'Data on Crown Condition of the systematic grid (16 x 16 km)' (Level I) from the 'International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests' (ICPF) (Fischer et al. 2010) as response variable. This response was modelled using derivations of precipitation and temperature from the WorldClim- database as described in Hijmans et al. (2005). For the initial model we used nineteen bioclimatic variables included in the database and an additional calculated set of six bioclimatic variables consisting of the number of days per year with mean temperature above 5 degree Celsius, the yearly heat sum above 5 degree Celsius, mean temperature and precipitation sum in summer (defined as the months May to September) and annual and summer drought index according to O'Neill et al. (2007). For the projection under future conditions, we used output from the global circulation model HADCM3 (Gordon et al. 2000) driven by the SRES scenario A2 (IPCC 2000) until the year 2080, which was calibrated and statistically downscaled to 30-arc-second tiles using the WorldClim data for 'current' conditions.

As a statistical model formulation we fitted a Generalized Linear Model (GLM) with logit link functions, (i.e. logistic regressions). We used second-order polynomials of the described bioclimatic variables on the link scales of the GLM and reduced the number of the predictors using the sum of the adjusted deviances as a statistical measure. We calibrated the model by a stepwise selection checking for changes in the Bayesian Information Criterion (Schwarz 1978). The final five bioclimatic variables that entered the model in linear and quadratic form were: yearly sum of degree days above 5°C, iso-thermality, drought index after O'Neill et al. (2007) over the year, sum of precipitation in the warmest quarter of the year and the precipitation of the most humid month. As the threshold value for presence or absence we used Cohen's Kappa (Cohen 1960).

A detailed description of the modelling approach, the choice and selection of the predictors and the database of the model used for this investigation can be found in Hanewinkel et al. (2013b). For a general overview on species distribution models for major tree species in Europe see also Hanewinkel et al. (2013).

Using the described model we produced two maps depicting the potential distribution of European beech under current climate (1950-2000) and for scenario A2 until the year 2080 and intersected these maps with maps of the geology of Europe (Asch 2003). In these European maps with a scale of 1: 5'000'000 we selected all pixels that were assigned to formations including calcareous, limestone or other basic substrates. For Germany where no detailed information on the substrate was displayed in the European map, we used the 1:1'000'000 map for the Geology of Germany (BGR 1993). For areas in Europe such as Poland, Estonia, Lithuania and Latvia that did not display information on Geological map of Europe as well, we examined whether European beech plays a significant role in the species distribution. As this was not the case for both time periods we excluded these parts of

Europe from the analysis. The intersected maps were created using standard overlay functions.

4.4.2 Description of experimental study site

The field study was conducted in a 90 years-old beech forest (Dannenmann et al. 2009) located at an altitude of 800 m above sea level in the “Schwäbische Alb”, a low mountain range in Southern Germany (8°45´E; 47°59´N). Mean annual air temperature is approximately 6.5°C and the average annual rainfall amounts to 854 mm (1961-1990). Atmospheric N input at the study site is comparably small with less than 10 kg N ha^{-1} $year^{-1}$ (Dannenmann et al. 2008). The present experiment was conducted at approximately 1 km distance to the long-term ecological beech forest research site of the Tuttlingen research station (“NE” or “N” site) (Mayer et al. 2002; Geßler et al. 2004; Dannenmann et al. 2006, 2008, 2009; Simon et al. 2011; Guo et al. 2013). The clay-rich soil is classified as Rendzic Leptosol (Skeletic) according to the International Union of Soil Sciences Working Group WRB (2007) derived from horizontally bedded limestone and marls. Soil profiles are shallow followed by weathered parent rock containing > 45 % gravel and stones. Due to nutrient poor soils and low atmospheric N input, soil N cycling is characterized by competitive partitioning of N between beech and associated mycorrhiza vs. free living soil microorganisms (Dannenmann et al. 2009; Simon et al. 2011).

4.4.3 Experimental design

The climate change treatment was established using a space for time approach and combined with isotope-based process studies (Fig. 4.2). We used exposure-induced model ecosystems located on opposing slopes of a narrow valley, a cool-moist (NW aspect, representing “current climate conditions”) and a warm-dry local climate (SW aspect, representing “future climate conditions”) with a distance of less than 1 km. Microclimate at SW exposure is characterized by increased daily maximum of air and topsoil temperatures and thus reduced water availability (Geßler et al. 2004). Therefore, the SW aspect is considered to be a model ecosystem with local climatic patterns equalling the climatic conditions predicted for coming decades (Geßler et al. 2005). After full development of leaves, photosynthetic active radiation levels at the forest floor are mainly regulated by the canopy rather than by slope exposure (Mayer et al. 2002). After transfer of intact beech seedling-soil-microbe mesocosms either within NW or to SW in summer 2010, they were left undisturbed for an equilibration period of one year. Subsequently short-term-isotope-tracing-based process studies were conducted via homogenous ^{15}N labelling followed by destructive harvests to simultaneously quantify all relevant gross N turnover processes in the plant-soil-microbe system in June and August 2011. Long-term (>3 month) isotope recovery in the plant-soil-N pools was investigated in a third, final harvest in September 2011 (Fig. 4.2). At all three harvest dates, supporting soil, microbial, mycorrhizal and plant parameters as well as gene abundance of N cycle genes in soil were determined as described in the following sections.

4.4.4 Transfer of intact beech-soil-microbe systems to simulate climate change

The experiment was implemented in July 2010 by transferring intact beech-soil-mesocosms within the NW slope (control, “present climate conditions”) and to the SW slope (climate change treatment, “future climate conditions”). Transfer was conducted within one week in absence of precipitation. The simulated climate change conditions at the SW slope were

intensified through a temporal (June 27, 2011 - August 05, 2011) rain sheltering roof approximately 1 m above ground level to allow free air flow (Fig. 4.2, Fig. 4.4).

Identification of coring spots for beech-soil-mesocosms was based on two steps. First, a suitable sampling area of 50 * 50 m with identical slope (18 °) and similar soil profile was identified. In this area typical for the entire NW slope, finer grained soil (Ah horizon) was only found in the uppermost 15-20 cm (i. e. the main rooting zone of beech natural regeneration). There was a sharp transition between this almost gravel-free clay-rich finer grained soil and the deeper gravel-dominated periglacial layers which were not suitable for coring. Subsequently, beech natural regeneration trees of 2.2-2.5 mm stem diameter and 30-40 cm height were selected and marked in this area. Custom-made stainless steel cylinders (height 150 mm, outer diameter 168 mm, sharp edge at bottom, wall thickness 2 mm, open at bottom and top) were manually driven into the soil with the selected beech seedling growing in the centre. The litter layer was preserved and contained in the cylinders during coring. A custom-made extension tool fitting to the stainless steel cylinders facilitated coring under avoidance of damage to the aboveground part of the plant. Pre-tests showed that the entire root system of beech seedlings of this size fitted well to the embraced volume of the used steel cylinders. After coring, the cylinders containing an intact beech-soil-mesocosm were carefully excavated under preservation of an even ending at the bottom. Only beech-soil-mesocosms without visible damage or cut of the beech roots were used. Excavated beech-soil-mesocosms were immediately reburied vertically either within NW or after transfer to SW. The incubation areas were in fenced zones of 400 m^2 either in immediate vicinity to the sampling area (NW) or at the SW slope in 1 km distance characterized by the same inclination. Both NW and SW incubation areas showed closed canopy and only beech trees were present at a distance of 50 m. While reburying beech-soil-mesocosms, extreme care was taken to create a realistic transition between the bottom of the soil cores and the ambient soil. For this purpose, beech-soil-mesocosms were placed vertically in holes with a 5 cm thick horizontal bed made of homogenized NW soil and subsequently gently hammered down 2 cm at the new position. Finally, continuous contact between the litter layer on the transferred beech-soil-mesocosms and the surrounding litter layer at the new position was established. After transfer, all reburied intact beech-soil-mesocosms at both sites were irrigated with 500 ml of water over a period of two hours to avoid drying or death of enclosed beech seedlings following transfer. The amount of water corresponded to a precipitation event of 23.7 l m^{-2}, i. e. a typical summer convective rainfall event. The survival rate of transferred beech seedlings was 97 %. After transfer, the beech-soil-mesocosms were pre-incubated in situ for almost an entire year to allow adaptation to the new environmental surrounding and exposure to the climate conditions at NW and SW, respectively.

Additionally, 10 further beech-soil-mesocosms were transferred in special stainless steel cylinders (5 within the NW slope, 5 to the SW slope) to monitor soil temperature and soil moisture in 5 cm depth (n = 5 each slope) using horizontally installed combined soil moisture/temperature probes (DECAGON EC-5, Decagon Devices, Inc., Pullman, USA) with an hourly temporal resolution.

4.4.5 Isotope labelling

In order to facilitate the simultaneous quantification of gross rates of all major N turnover processes in the beech-soil-microbe system, the intact soil-root-mesocosms were labelled with either $^{15}N/^{13}C$-enriched glutamine, $^{15}NH_4^+$ or $^{15}NO_3^-$. To enable homogenous distribution of isotopes in the root-soil system, we applied an isotope injection method developed for large soil cores[20] and optimized and adapted it for the Tuttlingen soil. For this purpose, we conducted pre-experiments in March 2011 using Brilliant Blue FCF colour dye instead of ^{15}N enriched label solution. Various injection patterns and depths, numbers of injections per mesocosm and solution volumes per single injection were tested to comply with the opposing requirements (1) homogenous 3-dimensional distribution of label solution in the soil-root-system, (2) minimization of injected liquid to minimize label artefacts such as stimulation of N turnover, and (3) minimal leakage of label solution at the bottom of the beech-soil-mesocosms. In this pre-experiment, mesocosms were harvested one day after dye application by removing soil stepwise from bottom to top in layers of 2 cm. The distribution of the colour dye in the soil was monitored visually. Outflow at the bottom of the labelled beech-soil-mesocosms was examined by storing the beech-soil-mesocosms on white paper sheets. To inject dye – and later ^{15}N – solution - we used custom-made stainless steel side port cannulas with lasered depth check marks. In this labelling optimization experiment the following setup facilitated homogenous colour dye distribution with only minimal leakage: 16 single amounts of 3 ml solution each were injected into the soil-mesocosms to a depth of 1 and 3 cm each, and another 8 injections of 3 ml each to a depth of 6 cm. Additionally, 10 ml of ^{15}N-labelled solution were homogenously spread on top of the soil surface. Hence, the total amount of added label solution was 130 ml, applied to on average 2.3 kg dry soil contained in the beech-soil-mesocosms. Paper calibres indicating injection patterns and depths were constructed to fit onto the stainless steel cylinders in order to ensure reproducible labelling. Before ^{15}N-labelling, the litter layer was removed from the top of the soil surface and later replaced. An earlier study showed that the litter layer is of very minor significance for N nutrition of beech natural regeneration (Guo et al. 2013).

Labelling of beech-soil-microbe systems took place on June 22, 2011 (72 beech-soil-mesocosms at NW and SW each), to (1) compare gross N turnover between ambient conditions at SW and NW exposure via short-term ^{15}N tracing based on full destructive harvests six hours and 48 hours after labelling, and (2) to investigate long-term ^{15}N recovery at the end of growing season. A second labelling campaign took place August 2 (48 beech-soil-mesocosms at NW and SW each), followed by the same short-term isotope tracing approaches six and 48 hours after labelling. The August labelling/harvest cycle allowed to compare gross N turnover between ambient conditions at NW and roof-intensified drought (39 days) conditions at SW. Labelling was conducted simultaneously at NW and SW by a well-trained team of 10 persons starting at 5:00 am and took 3 hours (June) and 2 hours (August).

The label solution always contained all three N compounds used in this experiment, i. e. NH_4^+, NO_3^- and glutamine, and depending on the labelling treatment, either NH_4^+, NO_3^- or glutamine was isotopically enriched (50 atom % ^{15}N, and additionally 50 atom% ^{13}C for glutamine). All C and N atoms of glutamine used for labelling were isotopically enriched. Glutamine was chosen as an organic N compound because earlier studies showed high glutamine uptake capacities of beech seedlings and adult beech trees at this site

(Dannenmann et al. 2009; Simon et al. 2011). The amount of N added via label solution equalled to 3.5 mg NH_4^+-N kg^{-1} soil dry weight (sdw), 3.5 mg NO_3^--N kg^{-1} sdw, and 0.7 mg glutamine-N kg^{-1} sdw. Soil NH_4^+ background concentrations in unlabeled soil were hardly different between NW and SW. However, soil background soil NO_3^-concentrations were generally much smaller at SW than at NW. Therefore, the ratio of added NO_3^--N via label solution to background soil NO_3^- was larger for SW than for NW. In June, N added via label solution equalled to 76% and 80% of ambient NH_4^+ concentrations and 35% and 78% of ambient NO_3^- concentrations at NW and SW, respectively. In August, this equalled 84% and 114% of ambient soil NH_4^+ concentrations. However, NO_3^- levels were extremely low in particular at SW, so that added label equalled to 211% and 762% of ambient NO_3^- concentrations at NW and SW, respectively.

Hence, several-fold reduced soil NO_3^- levels were observed both in unlabelled mesocosms and in labelled mesocosms of the climate change treatment. The amount of added ^{15}N excess was 1.7 mg N kg^{-1} sdw for the NH_4^+ and NO_3^- labelling variants and 0.34 mg N kg^{-1} sdw for the glutamine labelling variants. Mean total ^{15}N isotope recovery six hours after labelling in soil and plant was 73±6% across all exposures, harvesting dates and labelling treatments.

4.4.6 Beech-soil-mesocosm harvest and sample preparation

Eight intact beech-soil-mesocosms were harvested for each of the three N-compound labelling treatments and each of the two slopes (NW exposure, control treatment and SW exposure, climate change treatment). Such harvesting of 48 beech-soil-mesocosms took place for the first labelling/harvest cycle on June 22, 2011 (6 hours after labelling) and June 24, 2011 (48 hours after the labelling). The same approach was repeated on August 2 (6 hours after labelling) and August 4 (48 hours after labelling) in order to compare NW conditions with intensified drought at SW. A final harvest of beech-soil-mesocosms was conducted on September 27, 2011, i. e. > 3 months after isotope labelling to investigate long-term ^{15}N recovery). The harvest times were chosen based on earlier experience with isotope dynamics in this soil and correspond well to time spans generally recommended to investigate soil N turnover and N uptake (Näsholm et al. 2009). The harvests on June 22-24, August 2-4 and September 27 were accompanied by additional harvests of unlabelled beech-soil-mesocosms to analyze ambient background inorganic N concentrations and isotopic natural abundance of investigated pools with 4-8 replicates per exposure.

For harvesting, beech-soil-mesocosms were excavated under maintenance of an even ending at the bottom and entirely harvested within two hours after excavation. The beech seedling was cut and further processed as described below. The remaining soil/root system was carefully separated by hand into soil, gravel, dead coarse organic material and living fine and coarse roots. Additionally, mycorrhizal root tips and a subsample of rhizosphere soil (defined as soil adhering to root after vigorous shaking) were sampled and further processed as described below.

The soil contained in each mesocosm was immediately homogenized by manual mixing for 10 minutes to assure full mixing to a homogeneous sample. All soil extraction steps for analysis of N compounds and their ^{15}N enrichment in soil were immediately conducted during harvest in the field lab with field fresh soil (see below). A subsample of ca. 100 g soil was air

dried. For nucleic acid analysis, other subsamples of bulk soil as well as the rhizosphere soil were immediately frozen at -80°C.

Fresh weight of the entire soil contained in the beech-soil-mesocsoms and the weight of the stainless steel cylinders were recorded. Gravimetric soil water content was determined with a subsample of approximately 400-500g of soil by drying at 105 °C until constant weight.

During each harvest, beech seedlings were carefully removed from mesocosms and separated into leaves, stems, coarse (>2 mm diameter) and fine roots (<2 mm diameter). Remaining adhering small soil was carefully washed from the roots and dried. The fresh weight of each plant tissue was determined. Samples were dried at 60°C until constant weight. After determination of the fresh weight, fine root samples were separated into two parts for subsequent analyses: one part for ectomycorrhizal analyses was wrapped in wet tissue and stored in plastic bags at 4°C until return to the laboratory, the second part was dried at 60°C for 2 days for later EA-IRMS analyses. For biochemical analyses of N metabolites in fine roots, additional samples were taken from unlabelled beech-soil-mesocosms on June 22 and shock-frozen in liquid N until sample analyses to avoid degradation of the metabolites.

4.4.7 Total organic carbon (C), total C and N and $\delta^{15}N$ in bulk soil

Soil samples were immediately frozen and freeze-dried after harvest. After removing roots and gravel using tweezers, bulk soil samples were finely ground. Determination of total soil C, N and $\delta^{15}N$ was performed in duplicate for every sample with an isotope ratio mass spectrometer (Delta V, Thermo Electron Corporation, Dreieich, Germany) coupled to an elemental analyzer (Euro EA, Eurovector, Milan, Italy) at the Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Soil Ecology in Neuherberg. The ^{15}N atom% excess enrichment was calculated by subtracting natural abundance values gained by the unlabelled mesocosms from the values obtained of the labelled soil mesocosms.

4.4.8 Analysis of extractable soil N pools

A representative subsample of 100 g of homogenized soil out of every harvested beech-soil-mesocosm was immediately extracted during the harvests in the field with 0.5 M K_2SO_4 at a soil:solution ratio of 1:1.5. Addition of K_2SO_4 solution was followed by 1 hour of shaking at 240 rotations per minute. After shaking, extracts were vacuum filtered using pumps and glass fibre filters (Dannenmann et al. 2009). Subsamples of the extract were transferred into different tubes and immediately frozen in the field for later analysis of (1) total organic C and total N concentrations, (2) NH_4^+ and NO_3^- concentrations, (3) ^{15}N enrichment in NH_4^+, NO_3^- and dissolved organic N (DON), and (4) ^{15}N enrichment in total dissolved (organic + mineral) N.

A second soil subsample was not immediately extracted, but first underwent a chloroform-fumigation over 24 hours as described in detail in an earlier publication (Dannenmann et al. 2006) and was extracted afterwards as described above with subsequent freezing of the extract for later analysis of (1) total N (mineral + organic) and (2) $\delta^{15}N$ in total N.

Ammonium and NO_3^- concentrations in extracts were analyzed colourimetrically by a commercial laboratory (Dr. Janssen, Gillersheim, Germany) (Dannenmann et al. 2009). Total organic C (TOC) and total N (TN) in extracts were quantified using an Infrared TOC analyzer with a coupled chemoluminescence-based total N module (DIMATEC GmbH, Germany) (Dannenmann et al. 2009). Dissolved organic N was calculated as the difference between total N and mineral N in extracts.

The ^{15}N enrichment in soil NH_4^+, NO_3^- and DON was quantified by sequential diffusion steps, based on conversion of the target compounds into NH_4^+, pH increase to induce volatilization as NH_3, and subsequent trapping of NH_3 on acid traps prepared for isotope ratio mass spectrometry (IRMS) at the Center of Stable Isotopes of KIT-IMK-IFU as described in detail in earlier publications (Wu et al. 2011; Guo et al. 2013).

Microbial biomass N and ^{15}N were quantified following the chloroform-fumigation extraction approach as described in detail in earlier publications (Wu et al. 2011; Guo et al. 2013). No conversion factors (k_{EN}) were used to render estimates of rather the active part of microbial biomass and newly immobilized N (Guo et al. 2013).

Total non-extractable soil N and the respective ^{15}N recovery were calculated as the difference between total soil N and all extractable N compounds (i. e. NH_4^+, NO_3^-, DON and microbial biomass N).

4.4.9 Analysis of ectomycorrhizal colonization and stable isotope analysis of ectomycorrhizal root tips

During harvest in the field laboratory, precleaned fine root samples (see 2.4) were placed in tap water filled petri dishes under a dissecting microscope (Stemi SV 11; Zeiss, Jena Germany) and were thoroughly cleaned from adhering soil particles using fine forceps.

Back in the laboratory randomly chosen, 2-3 cm long parts of the root system were used for examination under a dissecting microscope (205 FA; Leica, Wetzlar, Germany) and processed according to the method of Pena et al. (Pena et al. 2010). In each sample, 300 vital root tips were counted recording simultaneously the number of dead root tips. Occasionally, samples contained less than 300 living root tips. The root tips were classified as mycorrhizal, non mycorrhizal and dry root tips.

Samples of ectomycorrhizal root tips were collected for isotope measurements. For determination of C and N content as well as ^{15}N and ^{13}C enrichment, root tips were dried for three days at 60 °C. 1-5 mg of root tip material was weighted in 5 x 9 mm tin capsules (IVA Analysetechnik, Meerbusch, Germany) with a micro balance (Supermicro S4; Sartorius, Göttingen, Germany). Measurements were conducted at the service unit KOSI (Kompetenzzentrum für Stabile Isotope, University Göttingen, Germany) using an isotope ratio mass spectrometer (IRMS Delta Plus, Finnigan MAT, Bremen, Germany) coupled via interface (Conflo III, Finnigan MAT, Bremen, Germany) to an elemental analyzer (NA1110, CE-Instruments, Rodano, Milano, Italy). Acet anilide was used as standard and IAEA N1 (δ^{15}Nair 0.4 ‰) und N_2 (δ^{15}Nair 20.3 ‰) for N calibrations and IAEA 600 (δ^{13}CVPDB -27.7 ‰) for C calibrations.

4.4.10 Total N and C and $\delta^{15}N$ and $\delta^{13}C$ in beech tissues

To determine total N, as well $\delta^{15}N$ and $\delta^{13}C$ notation in plant tissues, oven-dried (48h, 60°C) samples were ground to a fine powder using a ball mill (Retsch MM 100, Retsch GmbH, Haan, Germany) for leaves and fine roots, and liquid N for stems and coarse roots. Aliquots of 1.0-2.5 mg for each tissue were weighed into tin capsules (IVA Analysentechnik, Meerbusch, Germany). Samples were analysed using an elemental analyser (Vario EL, elementar Analysensysteme GmbH, Hanau, Germany) coupled to an isotope ratio mass spectrometer (Delta Plus, Thermo Finnigen MAT GmbH, Bremen, Germany). Working standards (glutamic acid), calibrated against primary standards USGS 40 (glutamic acid δ^{13}CPDB=-26.39) and USGS 41 (δ^{13}CPDB=37.63) for $\delta^{13}C$ and USGS 41 ($\delta^{15}N_{air}$=47.600) for $\delta^{15}N$, were analyzed after every twelfth sample to enable correction of drift of isotopic analyses over time if required.

4.4.11 Metabolites: quantification total soluble protein, total amino acids and NO_3^- in the fine roots of beech seedlings

For biochemical analyses of N metabolites in the fine roots of beech seedlings, only fine roots from unlabelled samples were used from three time points (see above). Total amino acids in the fine roots were extracted (Winter et al. 1992) from frozen homogenous fine root material (~ 50mg) in 1 mL methanol:chloroform (3.5:1.5, v:v) and 0.2 mL buffer (pH 7.0) containing 20 mM Hepes, 5 mM EGTA and 10 mM NaF. Total amino acid concentration was quantified photometrically (Beckman Coulter Inc., Fullerton, CA, USA) at 570 nm as described by Li et al. (2005) using the colour reaction with ninhydrin reagent. Glutamine was used as a standard (Sigma, Hamburg, Germany). Total soluble proteins were extracted from (~ 50mg) frozen and ground fine root material in 1 mL buffer (1 mM EDTA, 50 mM Tris-HCl (pH 8.0), 1 mM phenylmethylsulfonyl fluoride (PMSF), 15% glycerol (v:v), 5 mM dithiothreitol (DTT) and 0.1% Triton-X 100) as described in detail earlier (Dannenmann et al. 2009). Concentrations of total soluble protein were quantified photometrically at 595 nm using Bradford reagent (Ameresco Inc., Solon, Ohio, USA) in a UV-DU650 spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA). Bovine serum albumine (BSA A-6918, Sigma Aldrich Chemie GmbH, Taufkirchen, Germany) was used as standard. Nitrate levels were extracted from c. 50 mg tissue in 0.1 g washed polyvinylpyrrolidone (PVP Sigma-Aldrich Inc., Steinheim, Germany) (Dannenmann et al. 2009). Nitrate concentration was determined using an ion chromatograph (DX 120, Dionex, Idstein, Germany) combined with an autosampler (AS 3500, Thermo Separation Products, Piscataway, USA) and equipped with the PeakNet software package (version 4.3, Dionex, Idstein, Germany). Nitrate, phosphate, and sulphate were used as standards.

4.4.12 Nucleic acid extraction from bulk soil and rhizosphere

DNA was extracted from 0.4 g bulk soil and 0.1 g rhizosphere soil, respectively, using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Heidelberg, Germany) and the Precellys 24 Instrument (Bertin Technologies, Montigny-le-Bretonneux, France). Quantity and quality of the extracted DNA were checked with a spectrophotometer (Nanodrop, PeqLab, Erlangen, Germany) and gel electrophoresis. The extracts were stored at -80°C until real-time PCR analysis.

4.4.13 Quantitative real-time PCR assay to quantify key marker genes for N turnover processes

Quantitative real-time PCR (qPCR) was performed using an ABI 7300 Cycler (Life Technologies, Darmstadt, Germany) with the following assay reagents: dimethyl sulfoxide (DMSO) and bovine serum albumin (BSA) (Sigma Aldrich, Taufkirchen, Germany), primers listed in Table 4.9 (Metabion, Martinsried, Germany) and 2x Power SYBR Green master mix (Life Technologies, Darmstadt, Germany). The respective reaction mixtures (total volume 25 µl) for quantification of the genes (Table S9) consisted of: 12.5 µl SYBR Green master mix, 5 pmol of each primer (for *apr* gene: 10 pmol of each primer), 0.5 µl 3% BSA and 2 µl DNA template. For the amplification of *nirS*, and *nirK* genes, 0.5 µl DMSO was added.

Table 4.9: Primer sets and thermal profiles used for the absolute quantification of the respective genes

Target gene	Source of standard	Primer	References	Thermal profile	No. of cycles
nifH	*Azospirillum irakense*	nifH-f, nifH-r	Rösch et al. 2002	95°C-45s/55°C-45s/72°C-45s	40
AOA	*Nitrosomonas europaea*	amo19F, CrenamoA16r48x	Leininger et al. 2006; Schauss et al. 2009	94°C-45s/ 55°C-45s/ 72°C-45s	40
AOB	*Fosmid clone 54d9*	amoA1F, amoA2R	Rotthauwe et al. 1997	94°C-45s/ 59°C-45s/ 72°C-45s	40
nirS	*Pseudomonas stutzeri*	cd3aF, R3cd	Michotey et al. 2000; Throbäck et al. 2004	95°C-45s/ 57°C-45s/ 72°C-45s	40
nirK	*Azospirillum irakense*	nirK876, nirK5R	Braker et al. 1998; Henry et al. 2004	95°C-15s/ 63-58°C-30s/ 72°C-30s	5 *
				95°C-15s/ 58°C-30s/ 72°C-30s	40
nosZ	*Pseudomonas stutzeri*	nosZ2F, nosZ2R	Henry et al. 2006	95°C-15s/ 65-60°C-30s/ 72°C-30s	5 *
				95°C-15s/ 60°C-30s/ 72°C-30s	40
chiA	*Streptomyces griseus*	chiF2, chiR	Xiao et al. 2005	95°C-30s/60°C-30s/72°C-60s	40
apr	*Pseudomonas aeruginosa*	FPapr1, RPapr2	Bach et al. 2001	95°C-20s/53°C-30s/72°C-60s	40

* Touchdown: -1°C per cycle

For quantification, standard curves were calculated using serial dilutions (10^1 to 10^6 gene copies μl^{-1}) of plasmid DNA containing PCR products of the respective genes (Table S9). According to manufacturer's instruction, the PCR detection limit was assessed to 10 gene copies. In advance, the optimal dilution for each amplification assay was determined by dilution series of randomly chosen DNA extracts (data not shown), in order to prevent PCR inhibition. The qPCR assays were performed in 96-well plates (Life Technologies, Darmstadt, Germany) for all target genes (Table 4.9). All PCR runs began with a hot start at 95°C for 10 minutes. After each run, the specificity of the SYBR Green-quantified amplicons was checked by melting curve analysis and gel electrophoresis. The amplification efficiency was calculated from the formula Eff = $[10^{(-1/slope)}-1]$ and resulted in the following average efficiencies (standard deviation less than 5% of mean) for the different genes: *nifH*, 88%, AOA, 89%, AOB, 97%, *nirS*, 90%, *nirK*, 94%, *nosZ*, 86%, *chiA*, 90%, *apr*, 90%.

4.4.14 Calculation of N pools, isotope recovery and gross rates of N turnover

Measurements of N pool size in soil and plant as well as isotopic information of plant, soil organic, inorganic, microbial and mycorrhizal N pools was used to calculate gross rates of N turnover and isotope tracer recovery.

Calculation of dry soil weight

$$sdw\ (g) = sfw * \frac{D}{W}$$

sdw : soil dry weight (g)
sfw : soil fresh weight in the mini lysimeter (g)
D/W : quotient between dry soil after drying at 105°C and field fresh wet soil

Total extractable soil N pool sizes

Ammonium-N pool ($^{14+15}NH_4^+$) [µg N/g sdw]

$$[NH_4^+] = ([concNH_4^+] - BV) * \left(\frac{VK_2SO_4 + sfw - sdw}{sdw}\right)$$

$concNH_4^+$: concentration of NH_4^+ in soil extract (mg N l^{-1})
BV = blind value $concNH_4^+$ -N in K_2SO_4
VK_2SO_4 = volume 0.5M K_2SO_4 (ml)

Nitrate pool ($^{14+15}NO_3^-$) [µg N/g sdw]

$$[NO_3^-] = ([concNO_3^-] - BV) * \left(\frac{VK_2SO_4 + sfw - asdw}{asdw}\right)$$

Dissolved organic N pool ($^{14+15}$DON) [mg N/kg sdw] calculated as TN_b-DIN

$$[14 + 15DON] = ([TN_b - DIN] - BV) * \left(\frac{VK_2SO_4 + sfw - asdw}{asdw}\right)$$

TNb: total chemically bound N in soil extracts (mg l^{-1})
DIN: dissolved inorganic N in soil extracts (mg l^{-1})

Microbial biomass nitrogen (MBN) [mg N kg^{-1} sdw]; $(X)_{fum}$= data from chloroform fumigated soil

$$[MBN] = \left([TN_b] - BV * \left(\frac{VK_2SO_4 + sfw - asdw}{asdw}\right)\right)_{fum} - \left([TN_b] - BW * \left(\frac{VK_2SO_4 + sfw - asdw}{asdw}\right)\right)$$

Plant pool (P) [mg N $mesocosm^{-1}$]

$$[P] = ([P])_{stem} + ([P])_{leaves} + ([P])_{\substack{coarse \\ roots}} + ([P])_{\substack{fine \\ roots}}$$

$$[P] = \frac{pdw * \%N}{100}$$

pdw=total dry weight of plant tissue (mg)

Information on soil and plant N pools was transferred to the unit mg N m^{-2}, considering the dry mass of soil contained in a beech-soil-mesocosm and/or the surface area.

^{15}N excess amount

NH_4^+, NO_3^-, DON [mg N kg^{-1} sdw]

$$[^*X] = [X] * \left(\frac{[^{\%}X]_t - ([^{\%}X]_t)_{nat\ abund}}{100} \right)$$

$[^*X]$= ^{15}N excess amount (mg N kg^{-1} sdw) with $X = NH_4^+, NO_3^-$ or DON
[X]: N concentration in pool X (mg N kg^{-1} sdw)
$[^{\%}X]$: Atom% ^{15}N enrichment of pool X
Nat abund: natural abundance ^{15}N atom% excess (unlabelled soil).

^{15}N excess amount in microbial biomass (MBN) [mg N kg^{-1} sdw]

$$[\ ^*MBN] = \frac{\left([^{\%}MBN] * [TN_b]\right)_{fum} - [^{\%}MBN] * [TN_b]}{([TN_b])_{fum} - [TN_b]}$$

^{15}N excess amount in plant biomass [mg N lysimeter^{-1}]

$$[\ ^*P]_t = \left(([P]_t)_{stem} * \left([^{\%}P]_t - \left([^{\%}P]_t\right)_{\substack{nat \\ abund}}\right)_{stem}\right) + \left(([P]_t)_{leaves} * \left([^{\%}P]_t - \left([^{\%}P]_t\right)_{\substack{nat \\ abund}}\right)_{leaves}\right) + \left(([P]_t)_{\substack{coarse \\ roots}} * \left([^{\%}P]_t - \left([^{\%}P]_t\right)_{\substack{nat \\ abund}}\right)_{\substack{coarse \\ roots}}\right) + \left(([P]_t)_{\substack{fine \\ roots}} * \left([^{\%}P]_t - \left([^{\%}P]_t\right)_{\substack{nat \\ abund}}\right)_{\substack{fine \\ roots}}\right)$$

The recovery of ^{15}N excess added by labelling was calculated by dividing the ^{15}N excess amount detected in the investigated pools by the original amount of ^{15}N excess added through the labelling solution.

Calculation of gross rates of nitrogen turnover

Gross rates of ammonification and nitrification were calculated following the ^{15}N pool dilution equations given by Kirkham and Bartholomew (Kirkham and Bartholomew 1954).

Ammonification [mg N kg^{-1} sdw day^{-1}]= $\frac{[NH_4^+]_6 - [NH_4^+]_{48}}{1.75\ days} * ln\left(\frac{[\ ^*NH_4^+]_6 * [NH_4^+]_{48}}{[\ ^*NH_4^+]_{48} * [NH_4^+]_6}\right) \% \ ln\frac{[NH_4^+]_6}{[NH_4^+]_{48}}$

Subscript [ ]: Measurement time (0 = before labelling; 6 = 6 hours after ^{15}N application; 48 = 48 hours after ^{15}N application)

Nitrification [mg N kg^{-1} sdw day^{-1}]= $\frac{[NO_3^-]_6 - [NO_3^-]_{48}}{1.75\ days} * ln\left(\frac{[\ ^*NO_3^-]_6 * [NO_3^-]_{48}}{[\ ^*NO_3^-]_{48} * [NO_3^-]_6}\right) \% \ ln\frac{[NO_3^-]_6}{[NO_3^-]_{48}}$

Plant N uptake and microbial immobilization of inorganic N was calculated based on short-term tracing (6 hours) of labelled substances ($^{15}NH_4^+$, $^{15}NO_3^-$) into plant biomass (Stark 2000). The short period was chosen to minimize bias due to unquantified tracer outflow from the sink pools and depletion of tracer in the source pool (Näsholm et al. 2009, Stark 2000).

Plant NH_4^+ *uptake [mg N lysimeter*$^{-1}$ *day*$^{-1}$*]*$= \frac{[\,^*P]_6}{Average\left([^{APE}NH_4^+]_0;[^{APE}NH_4^+]_6\right)*0.25\ days}$

[APE]: Atom% excess of pool X

$[^{APE}NH_4^+]_0 = {}^{15}NH_4^+$ *- N atomic % excess immediately after* ^{15}*N labelling*
$[^{APE}NH_4^+]_6 = {}^{15}NH_4^+$ *- N atomic % excess six hours after labelling.*

Plant NO_3^- *uptake [mg N lysimeter*$^{-1}$*]*$= \frac{[\,^*P]_6}{Average\left([^{APE}NO_3^-]_0;[^{APE}NO_3^-]_6\right)*0.25\ days}$

Microbial NH_4^+ *inmobilization [mg N kg*$^{-1}$ *sdw day*$^{-1}$*]*$= \frac{[MB]_6*[\,^*MB]_6}{Average\left([^{APE}NH_4^+]_0;[^{APE}NH_4^+]_6\right)*0.25\ days}$

Microbial NO_3^- *immobilization [mg N kg*$^{-1}$ *sdw day*$^{-1}$*]*$= \frac{[MB]_6*[\,^*MB]_6}{Average\left([^{APE}NO_3^-]_0;[^{APE}NO_3^-]_6\right)*0.25\ days}$

All N turnover rates were transferred to the unit mg N m^{-2} day^{-1}, considering total dry soil contained in the beech-soil-mesocosm and the surface area. Plant uptake rates were transferred to the same unit by considering the surface area of the beech-soil-mesocosm.

4.4.15 Statistics

Experimental data is generally shown as mean values with standard errors (SE) of the mean, if not otherwise stated. Data were log-transformed if necessary to meet the requirement of normality and subsequently analyzed using two-way ANOVA with the factors exposure (NW versus SW exposure, i. e. control and climate change treatments), time and their interaction. Depending on the parameters investigated, the factor time has different levels with independent observations, since samples were taken from different beech-seedling-soil mesocosms. There were two levels for gross rates of N turnover (June, September), three levels for N pools and microbial N cycle gene abundance (June, August, September) and five levels for isotopic composition of C and N pools (June_6 hours after labelling; June_48 hours after isotope labelling; August_6hours after isotope labelling; August_48 hours after isotope labelling and September_3months after isotope labelling). Differences between the levels of the time factor within each treatment level were further tested by applying the Tukey post-hoc test. For plant metabolites in fine roots, single comparisons between SW and NW were performed with non-parametric u-tests due to smaller number of replicates.

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5 Synopsis

In dieser Arbeit wurde gezeigt, dass die Zukunft der Buchennaturverjüngung und damit die Zukunft der Buchenwälder maßgeblich von der Versorgungssituation mit Stickstoff abhängt. Die sinkende Bodenfeuchte und die steigenden Temperaturen, die für die Zukunft prognostiziert werden, können sich über die Beeinträchtigung der Ektomykorrhiza- und Bakteriengesellschaften auf den Stickstoffkreislauf in Buchenwäldern auswirken. Von den Änderungen sind aufgrund der schlechten Wasser- und Stickstoffversorgung vorrangig die flachgründigen Kalkstandorte (Dannenmann et al. 2006, 2009) betroffen, die ca. ein Drittel aller Buchenwälder in Mitteleuropa ausmachen (Dannenmann et. al. unveröffentlicht). Unter Bedingungen, wie sie auf solchen Standorten vorherrschen, reagiert die Buche besonders sensitiv auf Trockenstress (Rennenberg et al. 2009). Diese Arbeit untersucht die Prozesse zwischen Buchen, Ektomykorrhizen und Bodenbakterien, die auf den genannten Standorten zu Wachstumseinschränkungen führen. Sowohl Ektomykorrhizen als auch Bodenbakterien spielen dabei eine entscheidende Rolle und konkurrieren um den verfügbaren Stickstoff. Die Rolle der Ektomykorrhizen wurde in dieser Arbeit, ausgehend von der Morphologie und Anatomie einer einzelnen Ektomykorrhizaart, sowohl in einem kontrollierten System unter Ausschluss der Konkurrenz durch Bodenbakterien und ohne umweltbedingte Einschränkungen als auch unter Freilandbedingungen in Stress- und Konkurrenzsituationen untersucht.

5.1 Morphologie und Anatomie als Basis für die Analysen von Ektomykorrhizagesellschaften und deren Funktionen

Funktionelle Eigenschaften der Ektomykorrhizapilze sind entscheidend, wie sich die einzelnen Pilze im Ökosystem, auch in Hinblick auf die Stickstoffversorgung ihrer Wirtspflanzen, verhalten. So hängt z.B. der Zugriff der Pilze auf unterschiedliche Stickstoffquellen maßgeblich von der Ausprägung des extramatrikalen Myzels und damit vom Explorationstyp ab (Pena et al. 2013). Informationen über solche funktionellen Merkmale können aber in vielen Fällen nicht ermittelt werden, da ein Großteil der Ektomykorrhizen, die mittels ITS-Sequenzierung bestimmt werden können, noch nicht wissenschaftlich beschrieben wurden. Damit fehlt die Verknüpfung von ITS-Sequenzen und Strukturen. Der Einsatz von Next Generation Sequencing, bei dem sehr große Mengen an Sequenzen erzeugt werden, verstärkt das Missverhältnis zwischen vorhandenen Sequenzen und vorhandenem morphologischen und anatomischen Wissen auf drastische Weise.

Die Grundlage vieler funktioneller Analysen von Ektomykorrhizapilzen ist, noch vor der ITS-Sequenzierung der Pilze, das Morphotyping, also das Zuordnen von mykorrhizierten Wurzelspitzen zu Morphotypen, die anhand morphologischer Merkmale unterscheidbar sind. Um dieses Verfahren wiederholbar anwenden zu können, sind Kenntnisse über die Morphologie der einzelnen Mykorrhizapilze zwingend notwendig und sollten durch Informationen zur Anatomie ergänzt werden (Anatomotyping). Werden nicht alle Möglichkeiten zur Bestimmung der Pilze ausgenutzt, ist die Gefahr von Fehlbestimmungen sehr hoch. Auf morphologischer Ebene stehen nur 7 Merkmale (Agerer 1987-2006) zur Verfügung, von denen ein sehr wesentliches, nämlich die Farbe, stark von der Lichtquelle,

dem Alter und dem Lagerungszustand der Mykorrhiza abhängt. Werden anatomische Merkmale wie z.B. Mantelaufbau, Hyphenanatomie und Cystidenformen zusätzlich herangezogen, erhöht sich die Zahl der Merkmale um ein Vielfaches und die Bestimmungen werden sicherer. Nach der anschließende Sequenzierung der DNA der Morphotypen und dem Abgleich mit Datenbanken wie NCBI oder UNITE wird der beste Treffer für die Benennung des betreffenden Morphotyps verwendet, wenn Qualitätskriterien wie z.B. die weitverbreiteten 97 % Identität (Brock et al. 2008) erfüllt werden. Laut Nilsson et al. (2005, 2006) sind allerding bis zu 20 % der Artnamen in den Datenbanken (GenBank, EMBL, DDBJ) inkorrekt. So kann es zu Fehlbestimmungen kommen. Diese Fehlbestimmungen können erheblich vermindert werden, wenn die durch ITS-Sequenzierung bestimmten Pilze unter morphologischen und anatomischen Gesichtspunkten überprüft werden.

Wissenschaftliche Beschreibungen von Ektomykorrhizen geben Hinweise auf die Funktion der Pilze und helfen bei der Erfassung grundlegender Daten zu den Mykorrhizagesellschaften sowie bei der Überprüfung von Sequenzierungsergebnissen. Damit diese Vorteile genutzt werden können, ist eine weitgehende Erfassung und Beschreibung der vorkommenden Morphotypen notwendig. In diesen Bereich liegt allerdings ein sehr großes Defizit vor. Gerade in Hinblick auf die Anwendung des Next-Generation-Sequencings ist es wichtig, den Sequenzen Strukturen oder sogar Artnamen zuordnen zu können (Glenn 2011; Shokralla et al. 2012; Bates et al. 2013). Am Ausbau der morphologischen und anatomischen Datenbanken und die Verknüpfung mit den Gendatenbanken sollte deshalb dringend gearbeitet werden. Dazu wurde hier mit der Beschreibung einer Mykorrhiza ein Beitrag geleistet.

5.2 Ektomykorrhizapilze in der Stickstoffversorgungskette von Buchen

Um den Einfluss der Mykorrhizapilze auf den Stickstoffkreislauf näher zu beleuchten, fanden zwei Versuche statt. Im ersten Versuch wurden genetisch ähnliche Buchen von einem NO- und einem SW-Hang mit spezifischen Mykorrhizagesellschaften in homogenem Boden unter guter Wasser- und N-Versorgung kultiviert. Unter diesen Bedingungen war die Anzahl an nicht mykorrhizierten Spitzen besonders hoch, so dass ein Teil des Stickstoffs über diese Wurzelspitzen aufgenommen wurde. Der zweite Versuch fand unter Freilandbedingungen auf einem NW- und einem SW-Hang unter Verwendung von weitgehend ungestörten Mesokosmen statt. Auf beiden Hängen stammten die Buchen vom NW Hang. Im Vergleich zu dem kontrollierten System war vor allem die mikrobielle Biomasse um eine Größenordnung erhöht, was zu einer starken Konkurrenz um den Stickstoff zwischen Mikroben und Mykorrhizen führte. Da die Wurzelspitzen nahezu vollständig mykorrhiziert waren, konnte der Stickstoff nur über die Ektomykorrhizen aufgenommen werden. Auf dem SW-Hang herrschten im Vergleich zum NW-Hang eine reduzierte Bodenfeuchte und eine erhöhte Temperatur. Die Bedingungen auf dem SW-Hang entsprachen in etwa den Bedingungen des prognostizierten Klimawandels.

Es stellte sich heraus, dass unter den kontrollierten Bedingungen durch die geringe mikrobielle Biomasse im Substrat die Konkurrenz um den Stickstoff zwischen Ektomykorrhizapilzen und Bakterien erheblich vermindert wurde. Die Ektomykorrhizapilze kontrollierten unter diesen Bedingungen den Stickstofftransport in die Pflanze und waren

somit entscheidend für die Stickstoffversorgung der Pflanze. Die beiden unterschiedlichen Mykorrhiza-Gesellschaften sorgten für eine unterschiedliche N-Versorgung der Buchen. Ein wesentlicher Unterschied zwischen den beiden Mykorrhizagesellschaften war das Verhältnis von Basidiomyceten zu Ascomyceten. Während an den NO-Buchen die Basidiomyceten dominierten, war an den SW-Buchen eine deutliche Steigerung des Ascomycetenanteils zu beobachten. Dies deckt sich mit der Beobachtung, dass in trockenen Ökosystemen Ascomyceten abundanter sind als in feuchteren Ökosystemen. Unter nicht limitierenden Umweltbedingungen ist die Mykorrhizagesellschaft der SW-Buchen also in der Lage, mehr N zu akkumulieren als die Mykorrhizagesellschaften der NO-Buchen. Es gab keine Hinweise, dass es physiologische Differenzen aufgrund von genetischer Anpassung oder langfristiger Anpassung gab, die die Unterschiede in der N Aufnahme verursacht haben.

Unter Freilandbedingungen akkumulierten die Mykorrhizen auf der NW-Seite unabhängig von der Stickstoffquelle mehr N als die Mykorrhizen auf der SW-Seite. Wegen der Inkubationszeit von einem Jahr unter den klimatischen Bedingungen auf der SW-Seite ist davon auszugehen, dass eine Anpassung der Mykorrhizapilze stattfand (Shi et al. 2002). Diese Anpassung war allerdings in Hinblick auf die Stickstoffakkumulation in den Mykorrhizen und die Stickstoffversorgung des Wirtes nicht wirksam. Sie wurde vermutlich durch die hohe Konkurrenzkraft und die geringe N Bereitstellung der Bakterien überdeckt. Unter Freilandbedingungen kontrollieren die Bakterien die Bereitstellung von anorganischem N und damit die Stickstoffversorgung der Buche. Unter den klimatischen Bedingungen auf dem SW Hang nimmt die Abundanz der nitrifizierenden Bakterien ab, was zu einem massiver Einbruch von der Bruttonitratproduktion und der Nitratverfügbarkeit führt. Die reduzierte N-Aufnahme der Buchen und Mykorrhizapilze auf dem SW Hang im Vergleich zum NW Hang spiegelte sich in der reduzierten Biomasse der Buchennaturverjüngung auf dem SW Hang wieder. Der gleiche Effekt ist auch anhand der Biomasse der adulten Buchen zu erkennen (Geßler et al. 2005).

Im Freiland war Nitrat die dominante N-Quelle für die Buchennaturverjüngung, gefolgt von Ammonium und Glutamin. Die mykorrhizierten Spitzen nahmen dagegen Ammonium bevorzugt auf. Dies stimmt mit zuvor durchgeführten Aufnahmeexperimenten überein (Geßler et al. 1998, 2005). Das Ammonium wird also von den mykorrhizierten Spitzen stark aufgenommen, verbleibt aber überwiegend dort und wird im Vergleich zu Nitrat in geringerem Umfang an die nachfolgenden Pflanzenteile weitergegeben. Auch unter den kontrollierten Bedingungen wurde von den Mykorrhizen viel Ammonium aufgenommen, aber im Vergleich zu Nitrat unter Freilandbedingungen ebenfalls nur in geringem Maße an die Pflanze weitergegeben. In früheren Publikation (Dannenmann et al. 2009; Näsholm et al. 2009; Simon et al. 2010, 2011; Stoelken et al. 2010) wurde vermutet, dass organische Stickstoffquellen eine bedeutende Rolle für die Stickstoffversorgung der Pflanzen spielen könnten. Im Experiment unter Freilandbedingungen bestätigte sich dies für die Buchennaturverjüngung aber nicht, da insbesondere nach 6 und 48 h Einwirkzeit des Labels im Vergleich zu den anorganischen Stickstoffformen nur sehr geringe Mengen an ^{15}N und ^{13}C aus dem Glutamin in den Mykorrhizen und den Buchen wiedergefunden wurden. Vermutlich liegt das an der Anwesenheit von konkurrenzstarken Bakterien, die in den Aufnahmeexperimenten von Simon et al. (2011), die an freigelegten Wurzeln in einer Nährlösung durchgeführt wurden und hohe Aufnahmeraten von Glutamin ergaben, keine Rolle spielten. Dies zeigt, dass nicht ohne weiteres von Ergebnissen aus solchen

Aufnahmeexperimenten auf die Zustände unter Freilandbedingungen, bzw. auf die Wirksamkeit für die Pflanzenernährung geschlossen werden kann.

Die Ergebnisse der beiden Experimente legen nahe, dass es für den Erhalt von produktiven Buchenwäldern nicht ausreicht, nach trockenheitstoleranten Buchen-Herkünften zu suchen. Vielmehr müsste mindestens im gleichen Umfang nach Ektomykorrhizen und Bakterien gesucht werden, die im Falle der Ektomykorrhizen unter zukünftigen Klimabedingungen konkurrenzstark Stickstoff akquirieren und diesen an die Pflanzen weitergeben und im Falle der Bakterien Nitrat in ausreichenden Mengen unter den künftigen Klimabedingungen bereitstellen können, um die Stickstoffversorgung der Buchen zu gewährleisten. Diese Arbeit liefert grundlegenden Vorstellungen zu den Prozessen der N-Versorgung von Buchen auf N-limitierten Böden im Zusammenspiel mit Ektomykorrhizen und Bodenbakterien unter Klimawandelbedingungen. Damit eröffnen sich neue Ansätze, den negativen Folgen des prognostizierten Klimawandel für die Buchenwälder zu begegnen.

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Abgrenzung der Beiträge der Autoren

„Fagirhiza inflata“ + *Fagus sylvatica* L.
Descriptions of Ectomycorrhizae 13: 37 – 41 (2012)
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[1]Erhebung und Auswertung der anatomischen/morphologischen Daten, Zeichnungen und Manuskript
[2]Korrektur des Manuskripts

Dissecting the contributions of local ectomycorrhizal assemblages and microbial communities on nitrogen uptake of European beech (*Fagus sylvatica*)
(submitted to Applied and Environmental Microbiology)

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[1]Planung des Experiments
[2]Durchführung des Experiments
[3]Erhebung und [4]Auswertung der Mykorrhiza- und Pflanzendaten
[5]Erhebung und [6]Auswertung der Bakteriendaten
[7]Erhebung und [8]Auswertung der Bodendaten
[9]Erhebung und [10]Auswertung der Aminosäuredaten
[11]Zusammenführen der Daten und zusammenfassende Auswertung
[12]Manuskript (mit Beteiligung aller Autoren)

Climate change impairs nitrogen cycling in European beech forests
(submitted to Nature Geoscience)
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[1]Planung des Experiments
[2]Durchführung des Experiments
[3]Erhebung und [4]Auswertung der Mykorrhizadaten
[5]Erhebung und [6]Auswertung der Bakteriendaten
[7]Erhebung und [8]Auswertung der Bodendaten
[9]Erhebung und [10]Auswertung der Pflanzendaten
[11]Modellierung und Verbreitungskarten
[12]Zusammenführen der Daten und zusammenfassende Auswertung
[13]Manuskript (mit Beteiligung aller Autoren)

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