DIVERSITY AND FUNCTION OF ROOT-ASSOCIATED FUNGAL COMMUNITIES IN RELATION TO NITROGEN NUTRITION IN TEMPERATE FORESTS

Dissertation

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List of abbreviations

15Am	whole plant ^{15}N content after labeling with $^{15}NH_4^+$
15Am_R_S	relative aboveground allocation of plant ^{15}N after labeling with ammonium
15Ni	whole plant ^{15}N content after labeling with $^{15}NO_3^-$
15Ni_R_S	relative aboveground allocation of plant ^{15}N after lableling with nitrate
A	Schwäbische Alb
AGI	arabidopsis genome initiative
AIC	Akaike's information criterion
ANOSIM	Analyses of similarity
ANOVA	analysis of variance
BM	whole plant biomass
BTE	beech transplantation experiment
C	carbon
Cege	*Cenococcum geophilum*
DEG	differentially expressed genes
DFG	German Research Foundation
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide
EC	enzyme commission
EEA	European Environment Agency
E_H	species evenness
EM	ectomycorrhiza
EMF	ectomycorrhizal fungi
et al.	and others (et alii)
Fasyl	*Fagus sylvatica* L.
g	gram
g	relative centrifugal force
GLM	general linear model
GO	gene ontology
H	Hainich-Dün
H'	Shannon diversity index
Hein	*Hebeloma incarnatulum*
IPCC	Intergovernmental Panel on Climate Change

ITS	internal transcribed spacer
KEGG	Kyoto Encyclopedia of Genes and Genomes
KOG	eucaryotic orthologous groups of protein
l	litre
m	metre
M	molar
min	minute
MMN	modified Melin-Norkrans
n	number of replicates
N	whole plant nitrogen
NMDS	non-metric multidimentaional scaling
NoMy	non-mycorrhizal root tips
O	original Hanich-Dün forest soil
°C	degree Celsius
OD	optical density
OTU	operational taxonomic unit
p	probability of error
PAT	pathotroph
PCA	principal component analysis
PCR	polymerase chain reaction
pH	negative log of the activity of the hydrogen ion
PPFD	photosynthetic photon flux density
PS	photosynthesis
R_S	root-to-shoot ratio
RAF	root-associated fungal or fungi
RIN	RNA integrity number
RNA	ribonucleic acid
RNA Seq	ribonucleic acid sequencing
RT	root tip
S	Schorfheide-Chorin
SAP	saprotroph
sec	second
SEM	standard error of means
SYM	symbiotroph
T	the original Hainich-Dün forest soil which was sterilized and amended

	with soil bacteria
Tosu	*Tomentella sublilacima*
Turu	*Tuber rufum*
VIF	variance inflation factor

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Summary

Ectomycorrhizal fungi form symbiotic association with roots of a variety of plant species. They provide plants with nutrients in exchange of photosynthetic carbohydrates. In natural ecosystems, plants form associations not only with ectomycorrhizal fungi but also with a variety of other microbes. Interest in root-associated fungal communities is increasing because they may play an important role in plant nutrition and fitness. Nitrogen (N) is an essential nutrient for plant growth and development but often a limiting factor in forest ecosystems. To date, little is known on the relationship of root-associated fungi with their environmental conditions and their potential function for tree N nutrition. Therefore, this study was designed to investigate environmental drivers and functions of root-associated fungal communities in relation to N.

Here, beech (*Fagus sylvatica* L.) was selected as a host plant to investigate the contribution of ectomycorrhizal fungi to plant N supply. Furthermore, this study used the infrastructure of the Biodiversity Exploratories which includes 150 forest plots (100x100 m) across three biogeographic regions of 422 to 1300 km^2, Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin located along a transect about 700 km across Germany. The Biodiversity Exploratories provide a well-organized platform for large-scale and long-term research on functional biodiversity in response to land use and geographical regions. A beech transplantation experiment, where young beeches of provenances from different Exploratory plots were grown side-by-side in the three research regions, was also used.

The objectives of this study were (1) to assess the impact of ectomycorrhizal diversity and community composition on inorganic N nutrition and performance of beech provenances, (2) to obtain insights into molecular mechanisms of ectomycorrhizal fungi-beech interactions, (3) to investigate root-associated fungal communities and their relationships with soil and root N and carbon in temperate forests.

(1) To assess the impact of ectomycorrhizal community composition and soil treatment on inorganic N nutrition and beech performance, I hypothesized: (i) the performance and N nutrition of beech provenances differ in a common environment; (ii) the performance and N nutrition of beech provenances differ with different ectomycorrhizal community compositions; and (iii) beech provenances from the three biogeographical areas prefer the N form that is prevalent in their natural environment. To test these

hypotheses, a greenhouse experiment was set up with three beech provenances grown from beech nuts. Diversity of ectomycorrhizal fungi was manipulated by planting beech seedlings in two soil types: original forest Hainich-Dün soil and Hainich-Dün soil which was sterilized and reinoculated with bacteria. After two years of exposure, I measured beech performance, identified EMF communities and investigated beech N nutrition by tracing ^{15}N labelled NH_4^+ and NO_3^-. The effects of soil and provenance and their interaction were analysed using general linear models. Analysis of similarity of ectomycorrhizal communities between soil treatments and among provenances were applied. Canonical correspondence analysis was conducted to explore the relationships among plant biomass, nitrogen and ectomycorrhizal communities. In contrast to my expectation, genetically determined effects on growth, nitrogen content and ^{15}N uptake were not detected. Improved performance and long-term N nutrition were observed for the beech provenances grown in original soil colonized by higher ectomycorrhizal diversity than for those in treated soil. However, increased ectomycorrhizal fungi diversity did not affect beech ^{15}N uptake. Different beech provenances preferred NO_3^- over NH_4^+. Overall, the results suggest that beech performance and N nutrition are controlled by soil microbes, in particular, by the ectomycorrhizal community composition.

(2) To obtain insights into molecular mechanisms of ectomycorrhizal fungi-beech interaction, beech and fungal transcriptomes were characterized, and the response of fungal and beech gene transcript levels to differences in environmental conditions were investigated. Young beech plants from the provenance “Schorfheide-Chorin” were planted in the three biogeographical regions of the Exploratories. After two years of growth under field conditions, beech roots were harvested and used to study transcriptomes. After quality filtering, cleaned sequences were mapped against transcriptomes of beech and ectomycorrhizal fungi. Transcripts with significantly decreased or increased abundance among the three regions were detected. The patterns of transcript abundance among the three regions were visualized by principal component analysis. Gene ontology term analysis was applied to detect possible functions of differentially expressed genes. The transcriptome of Schorfheide-Chorin was different from those from the other two regions. Most differentially expressed genes and enriched gene ontology terms were detected between Hainich-Dün and Schorfheide-Chorin. Beech genes (111) and ectomycorrhizal genes (374), which were related to N uptake and assimilation, were identified. The detected beech genes were

involved in nitrate uptake and assimilation, whereas fungal genes contributed to ammonium uptake and assimilation.

(3) To investigate the diversity and community composition of root-associated fungal communities and their interrelationship with soil and root nitrogen and carbon availability in temperate forests, the following hypotheses were tested: (i) the taxonomic community compositions differ among different biogeographical regions, but the trophic community compositions are similar; (ii) Root-associated fungal communities are driven more strongly by root than by soil chemistry because of the tight interaction of fungal communities with host plants; and (iii) different ecological groups of the root-associated fungi (symbiotroph, saprotroph and pathotroph) respond differently to environmental variation in soil and root N and carbon. To test these hypotheses, fine roots were sampled from 150 forest plots in the three Exploratory regions and related to root and soil chemistry. Root-associated fungi were identified by Illumina sequencing (MiSeq) and related to root and soil chemistry. Root-associated fungal community composition was explored using non-metric multidimensional scaling and analysis of similarities. Relationships between the abundance of root-associated fungi and abiotic variables were investigated using linear mixed effects models. Taxonomic community composition differed significantly among the three regions which was expected, but the difference in the taxonomic community composition was stronger than that in the trophic community composition which differed moderately between Schorfheide-Chorin and the two other regions but did not differ between Schwäbische-Alb and Hainich-Dün. Trophic fungal groups (symbiotroph, saprotroph and pathotroph) were driven by root NH_4^+ and NO_3^-, and N and carbon in roots were more important than those elements in soil for changes in the fungal groups. The abundance of fungal orders present in our analysis was driven by root NO_3^-. In agreement with my expectation, different fungal orders exhibited distinct patterns with the abiotic variables. Overall, these results support that functional resilience might be achieved by taxonomic divergence between Schwäbische Alb and Hainich-Dün. Biogeographical region and N in roots (NH_4^+ and NO_3^-) were drivers for changes in the abundance of root-associated fungal communities.

In conclusion, this study provides insights into the relationships between root-associated fungi and nitrogen in temperate forests. In young beech trees, ectomycorrhizal diversity controls N nutrition. How this works at the molecular levels remained enigmatic because NO_3^- was the preferred N source for beech underpinned by beech gene expression, while fungal genes were related to NH_4^+ uptake and

assimilation. Biogeographical regions and N-related variables (NO_3^-, NH_4^+) in roots are drivers for changes in root-associated fungal community composition across temperate forests. Future research should address taxon-specific fungal N uptake and the temporal dynamics of root-associated fungal communities. Developing a reference genome for *Fagus sylvatica* would be an important basis for better understanding the molecular mechanisms for beech-fungal interactions.

Zusammenfassung

Ektomykorrhizapilze bilden Symbiosen mit den Wurzeln vieler Pflanzenarten aus. Sie versorgen die Pflanzen mit Nährstoffen und werden im Gegenzug von den Pflanzen mit Kohlenhydraten aus der Photosynthese versorgt. In natürlichen Ökosystemen bilden Pflanzenwurzeln nicht nur mit Ektomykorrhizapilzen, sondern auch mit einer Vielzahl anderer Mikroben Interaktionen aus. Das Interesse an wurzelassoziierten Pilzgemeinschaften wächst, da diese eine wichtige Rolle bei der Nährstoffversorgung und Fitness der Pflanzen spielen könnten. Stickstoff (N) ist ein essentieller Nährstoff für Pflanzenwachstum und –entwicklung, aber häufig ein limitierender Faktor in Waldökosystemen. Bis heute ist das Wissen über die Beziehungen zwischen wurzelassoziierten Pilzen mit ihren Umweltbedingungen und ihrer potentiellen Funktion bei der N-Ernährung der Bäume gering. Ziel dieser Dissertation war es, die Umweltbedingungen und Funktionen von wurzelassoziierten Pilzgemeinschaften im Zusammenhang mit N zu untersuchen.

Buchen (*Fagus sylvatica*) wurden als Wirtspflanzen ausgewählt, um die Beteiligung von Ektomykorrhizapilzen bei der N-Versorgung der Pflanzen zu untersuchen. Es wurde die Infrastruktur der Biodiversitäts-Exploratorien genutzt, welche 150 Waldplots (je 100 x 100m) entlang drei biogeographischer Regionen mit einer Größe von 422 bis 1300 km^2 (Schwäbische Alb, Hainich-Dün und Schorfheide-Chorin) umfasst, entlang eines 700 km langen Transektes durch Deutschland. Die Biodiversitäts-Exploratorien stellen eine gut organisierte Plattform für großräumige und langfristige Forschung zu funktioneller Biodiversität und den Auswirkungen von Landnutzung und geographischer Region zur Verfügung. In den drei Versuchsregionen wurde ein Buchen-Transplantationsexperiment etabliert, bei dem junge Buchen, die aus verschiedenen Plots der drei Exploratorien stammten, in bestimmten Plots der Exploratorien nebeneinander angepflanzt wurden.

Die Ziele dieser Arbeit waren, (1) den Einfluß von ektomykorrhizaler Diversität und der Zusammensetzung ihrer Gemeinschaft auf die Ernährung mit anorganischem N und der Vitalität von Buchen verschiedener Herkunft zu beurteilen, (2) Erkenntnisse über die molekularen Mechanismen der Interaktionen zwischen Ektomykorrhizapilzen und Buchen zu bekommen, und (3) die wurzel-assoziierten Pilzgemeinschaften und ihre Beziehung zu Boden- und Wurzel-Stickstoff und -Kohlenstoff in temperaten Wäldern zu untersuchen.

(1) Um den Einfluß der ektomykorrhizalen Pilzgemeinschaft und der Bodenbeschaffenheit auf die Ernährung mit anorganischem N und der Vitalität von Buchen zu untersuchen, wurden die Hypothesen aufgestellt, daß (i) die Leistungsfähigkeit und N-Ernährung von Buchen verschiedener Herkunft sich in derselben Umwelt unterscheiden, (ii) die Leistungsfähigkeit und N-Ernährung von Buchenherkünften sich aufgrund einer unterschiedlichen Zusammensetzung der Ektomykorrhizagemeinschaft unterscheidet, und (iii) die Buchenherkünfte der drei verschiedenen biogeographischen Gebiete die N-Form bevorzugen, die in ihrer ursprünglichen Umwelt vorherrscht. Um diese Hypothesen zu untersuchen, wurde ein Gewächshausexperiment durchgeführt, bei dem Buchen von drei verschiedenen Herkünften aus Bucheckern angezogen wurden. Die Diversität der Ektomykorrhizapilze wurde beeinflußt, indem die Buchensetzlinge in zwei verschiedene Bodenarten gepflanzt wurden: Beide Bodenarten stammten aus dem Gebiet Hainich-Dün. Einmal wurde der Boden so belassen, wie er dem Wald entnommen wurde; für den zweiten Ansatz wurde er zunächst sterilisiert und dann mit Bakterien reinokuliert. Nach zwei Jahren der Exposition wurden Biomasseentwicklung und Photosynthese als Vitalitätsindikatoren der Buchen gemessen, die Gemeinschaften der Ektomykorrhizapilze identifiziert und die N-Ernährung der Buchen durch die Nachverfolgung von zugeführtem, mit ^{15}N markiertem NH_4^+ und NO_3^- untersucht. Die Effekte des Bodens und der Herkunft, sowie ihre Interaktion wurden mittels „allgemeiner linearer Modelle“ (general linear model) analysiert. Es wurden Ähnlichkeitsanalysen (analysis of similarity) der ektomykorrhizalen Gemeinschaften zwischen den verschiedenen Bodentypen und den unterschiedlichen Herkünften angewendet. Kanonische Korrespondenzanalysen (canonical correspondence analysis) wurden durchgeführt, um die Beziehungen zwischen pflanzlicher Biomasse, Stickstoff und ektomykorrhizalen Gemeinschaften zu erforschen. Entgegen der Erwartungen konnten keine genetisch bedingten Auswirkungen auf Wachstum, Stickstoffgehalt und ^{15}N-Aufnahme festgestellt werden. Eine Verbesserung der Leistungsfähigkeit und der Langzeit-N-Ernährung wurden an den Buchenherkünften festgestellt, die in unbehandelter Walderde kultiviert und von einer größeren ektomykorrhizalen Diversität kolonisiert waren, im Vergleich zu denen, die in sterilisierter Erde kultiviert wurden. Eine erhöhte Diversität von Ektomykorrhizapilzen hatte jedoch keinen Einfluß auf die ^{15}N-Aufnahme. Verschiedene Buchenherkünfte bevorzugten NO_3^-gegenüber NH_4^+.Insgesamt deuten die Ergebnisse darauf hin, daß die Leistungsfähigkeit von Buchen und deren N-Ernährung von Bodenmikroben

kontrolliert werden, insbesondere von der Zusammensetzung der Ektomykorrhizagemeinschaft.

(2) Um Einblicke in die molekularen Mechanismen der Ektomykorrhizapilz-Buchen-Interaktion zu erhalten, wurden die Transkriptome von Buchen und Pilzen charakterisiert und die Reaktionen von Pilz- und Buchen-Gentranskripten auf unterschiedliche Umweltbedingungen untersucht. Junge Buchen der Herkunft „Schorfheide-Chorin“ wurden in die drei biogeographisch unterschiedlichen Regionen der Biodiversitäts-Exploratorien gepflanzt. Nach zwei Jahren Wachstum unter Freilandbedingungen wurden die Buchenwurzeln geerntet und zur Untersuchung der Transkriptome verwendet. Nach einer Qualitätsfilterung wurden die bereinigten Sequenzen gegen Transkriptome von Buchen und Ektomykorrhizapilzen annotiert. Es wurden Transkripte mit signifikant verringerter oder erhöhter Häufigkeit in den drei Regionen nachgewiesen. Die Muster der Transkripthäufigkeit zwischen den drei Regionen wurden durch Hauptkomponentenanalyse (principal component analysis) sichtbar gemacht. Eine Gen-Ontologie-Analyse (gene ontology term analysis) wurde angewendet, um mögliche Funktionen von unterschiedlich exprimierten Genen zu erkennen. Das Transkriptom der Wurzeln aus Schorfheide-Chorin unterschied sich von denen in den anderen beiden Regionen. Die meisten unterschiedlich exprimierten und angereicherten Gen-Ontologie-Terme wurden zwischen Hainich-Dün und Schorfheide-Chorin nachgewiesen. Es wurden Buchengene (111) und Ektomykorrhizapilzgene (374) identifiziert, die mit der N-Aufnahme und –Assimilation in Verbindung gebracht wurden. Die nachgewiesenen Buchengene waren an der Nitrataufnahme und –assimilation beteiligt, während die Pilzgene bei der Aufnahme und Assimilation von Ammonium beteiligt waren.

(3) Um die Diversität und Gemeinschaftszusammensetzung von wurzelassoziierten Pilzgemeinschaften und ihre Wechselbeziehungen mit der Stickstoff- und Kohlenstoffverfügbarkeit von Böden und Wurzeln in temperaten Wäldern zu erforschen, wurden die folgenden Hypothesen getestet: (i) die taxonomischen Gemeinschaftszusammensetzungen der wurzelassoziierten Pilze unterscheiden sich in verschiedenen biogeographischen Regionen, aber die trophischen Gemeinschaftszusammensetzungen sind ähnlich; (ii) wurzelassoziierte Pilzgemeinschaften werden aufgrund der engen Interaktionen der Ektomykorrhiza mit ihren Wirtspflanzen stärker durch die Wurzel- als die Bodenchemie gesteuert; und (iii) verschiedene ökologische Gruppen der wurzelassoziierten Pilze (symbiotroph, saprotroph und pathotroph) reagieren unterschiedlich auf umweltbedingte

Veränderungen von Stickstoff und Kohlenstoff im Boden und in den Wurzeln. Um diese Hypothesen zu testen, wurden Feinwurzeln in 150 Waldplots der drei Exploratorien gesammelt und mit der Wurzel- und Bodenchemie korreliert. Dazu wurden die wurzelassoziierten Pilze durch Illumina-Sequenzierung (MiSeq) identifiziert. Die Zusammensetzung der wurzelassoziierten Pilzgemeinschaften wurde unter Verwendung von nichtmetrischer multidimensionaler Skalierung (non-metric multidimensional scaling) und Ähnlichkeitsanalysen (analysis of similarities) untersucht. Die Beziehungen zwischen der Häufigkeit von wurzelassoziierten Pilzen und abiotischen Variablen wurden mit linearen Mixed-Effekt-Modellen (linear mixed effects models) untersucht. Erwartungsgemäß unterschieden sich die Zusammensetzungen der taxonomischen Gemeinschaften in den drei Regionen signifikant, aber die Unterschiede der taxonomischen Gemeinschaftszusammensetzungen war größer, als die der trophischen, welche sich zwischen Schorfheide-Chorin und den anderen beiden Regionen nur geringfügig unterschied, zwischen der Schwäbischen Alb und Hainich-Dün unterschieden sie sich nicht. Es wurde eine Korrelation zwischen den trophischen Pilzgruppen (symbiotroph, saprotroph und pathotroph) und NH_4^+ und NO_3^- in den Wurzeln festgestellt, Stickstoff und Kohlenstoff in den Wurzeln hatten einen größeren Einfluß auf Veränderungen bei den Pilzgruppen, als diese Elemente im Boden. Die Abundanzen der in dieser Studie untersuchten Pilzbestände korrelierten mit dem NO_3^- in den Wurzeln. Gemäß den Erwartungen wiesen verschiedene Pilzordnungen unterschiedliche Muster mit den abiotischen Variablen auf. Insgesamt stützen diese Ergebnisse die Annahme, daß funktionale Resilienz durch taxonomische Divergenz zwischen Schwäbischer Alb und Hainich-Dün erreicht werden kann. Biogeographische Region und N in den Wurzeln (NH_4^+ und NO_3^-) waren vermutlich die treibende Kraft bei Veränderungen der Abundanz von wurzelassoziierten Pilzgemeinschaften in den Biodiversitäts-Exploratorien.

Zusammenfassend gibt diese Studie Einblicke in die Beziehungen zwischen wurzelassoziierten Pilzen und Stickstoff in temperaten Wäldern. Bei jungen Buchen wird die N-Ernährung durch die Ektomykorrhiza-Diversität kontrolliert. Wie das auf molekularer Ebene funktioniert, bleibt rätselhaft, da NO_3^-, gestützt durch die Buchen-Genexpression, die bevorzugte N-Quelle der Buchen war, während die Leistungsfähigkeit der Buchen auf molekularer Ebene durch die Mitwirkung von Pilzgenen erklärt werden könnte, die mit der Aufnahme und Assimilation von NH_4^+ in Zusammenhang stehen. Biogeographische Region und N-bezogene Variablen (NH_4^+ und NO_3^-) in Wurzeln waren die treibende Kraft für Veränderungen der

Zusammensetzung wurzelassoziierter Pilzgemeinschaften in gemäßigten Wäldern. Die künftige Forschung sollte sich mit Taxon-spezifischer pilzlicher N-Aufnahme und der zeitlichen Dynamik wurzelassoziierter Pilzgemeinschaften befassen. Die Entwicklung eines Referenzgenoms für *Fagus sylvatica* wäre eine wichtige Grundlage für ein besseres Verständnis der molekularen Mechanismen von Buche-Pilz-Interaktionen.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Temperate forest ecosystems

Forest ecosystems have always been playing an important role in human existence and spiritual life: providing food, shelter and materials, maintaining the atmospheric balance and being a source of artistic inspiration (Gilliam 2016). Temperate forests are situated in regions where there is a mild climate, mainly found around the middle latitudes between the tropics and the Arctic and Antarctic polar regions (Fig. 1). In the Northern Hemisphere, temperate forests occur in eastern North America, north-eastern Asia, and western and central Europe while in the Southern Hemisphere they are present in southern Chile, south-eastern Australia, Tasmania and parts of New Zealand (Gilliam 2016). Total area of temperate forests is about 10.4 million km^2, accounting for six percent of all the Earth's ecosystems (Frelich et al. 2015). Distinct cyclic seasonal changes are among the noticeable features of temperate forest climate; however, temporal patterns of seasonal changes vary substantially depending on the different forest regions and latitudes (Gilliam 2016). Temperate forests at low latitudes, for example, have longer growing period than those at high latitudes (Gilliam 2016). Due to historic extensive use and conversion by human, temperate forest biodiversity has been extensively changed compared to the past (Franklin 1998). A number of natural temperate forests and their associated organisms have been overexploited or completely destroyed, and the remaining natural forest ecosystems are fragmented and highly altered (Franklin 1998). Therefore, most of the temperate forests nowadays are managed or secondary forests.

Forest ecosystems in Germany belong to Central Europe's temperate forest region. Key climatic features of the region are that summers are relatively warm without frost while winters are quite cold, with temperatures seldom being under -20 ^{o}C in winter and over 30 ^{o}C in summer; the vegetation receives rainfall the year round (Leuschner and Ellenberg 2017). These climatic conditions are advantageous to the growth and development of deciduous broadleaved tree species which require relatively high precipitation of over 250 mm and mean temperatures in July over 15 ^{o}C (Frelich et al. 2015). The forested area of Germany is over 11.4 million hectares, accounting for around 32% of the total area of Germany. Despite an increasing demand on forest products, the 10-year period, from 2002 to 2012, witnessed an increase in the forested area, by 500,000 hectares (Friedrich et al. 2015). Over the past years, the federal governments in Germany have applied sustainable forest management practices (Häusler and Michael 2001). As a result, mixed forests have been established with a larger contribution of deciduous species such as beech and

oak, and forests have been becoming more diversely structured (Friedrich et al. 2015). In fact, forest structure is diverse with 76% of mixed forests, and forests with two or multiple canopy layers accounting for 68% of the total forest area (Friedrich et al. 2015).

Fig. 1.1 Global distribution of temperate forests. Green colour indicates the temperate forests. Source: http://www.ducksters.com

European beech (*Fagus sylvatica*) forest area was 1,680,072 hectares in 2012, accounting for 21% of total forested areas in Germany (Friedrich et al. 2015). Beech is an economically and ecologically important tree species; it is the most abundant deciduous tree species in German forests. Beech occur throughout Germany, but mostly in the highlands of southern and central parts such as the Schwäbische-Fränkische Alb, Plälzerwald, Eifel, Odenwald, Spessart and Solling (Friedrich et al. 2015). An increase of 12% or 151,000 hectares of the beech forest area was recorded between 1987 and 2002, followed by an increment of 6% or 102,000 hectares in 2012 (Friedrich et al. 2015). Beech is expected to be more popular in Germany in the coming years as a result of the sustainable forest management practices which aim to achieve increased mixed forest areas with deciduous species.

1.2 Climate change and its effects on temperate forests

An increase of up to around 1 ^{o}C in the Earth's surface temperature was recorded between 1850 and 2015; 2015 was the warmest year on record (EEA 2017). In the Northern Hemisphere, the 30-year period between 1983 and 2012 experienced the highest increase in surface temperature of the last 1400 years (IPCC 2014). By 2050, the global temperature is estimated to surpass 2 ^{o}C compared to the pre-industrial (between 1850 and 1900) level (EEA 2017). When the global surface temperature goes up, more frequent temperature extremes are to be expected in

most regions (IPCC 2014). The long-term projection suggests for Germany an annual average temperature increase by 1.6 to 3.8 ^{o}C until 2080 depending on the chosen climate scenarios (Schröter et al. 2005).

Increasing surface temperatures are also expected to result in changed precipitation patterns (Trenberth and Shea 2005). Globally, changes in precipitation will be not uniform among different parts of the Earth but depend on latitude and region (IPCC 2014). Change in average precipitation over land regions was low between 1901 and 1951, and medium afterwards (IPCC 2014). In the Northern Hemisphere, during the 20th century precipitation went up by 0.5 to 1% per decade in the middle and high latitudes while it decreased in the subtropical latitudes (Zebisch et al. 2005). In the north-eastern and north-western Europe, an annual increase of 70 mm per decade has been recorded since 1960, while there was a decrease of up to 90 mm per decade in some parts of southern Europe (EEA 2017). Similarly, the projected precipitation is expected to change considerably across regions and seasons and will increase in northern Europe and decrease in southern Europe (EEA 2017). The frequency and severity of droughts tend to have gone up in Europe, in particular in southern Europe (EEA 2017). In Germany, a small change of less than 10% in annual precipitation is projected from 1990 to 2080, but with an increase in winter precipitation and a decrease in summer precipitation (Schröter et al. 2005).

A large scale meta-analysis of over 1700 species ranging from plants, birds, insects to fishes showed that climate change caused widespread changes in phenology and species distribution (Parmesan and Yohe 2003). Growing seasons of a number of plant species in the temperate areas has been changed due to climate change (Gilliam 2016). Habitats (14%) and species (13%) in Europe have already been considered as being under pressure due to climate change; these figures are projected to increase double in the near future (EEA 2017). Depending on the magnitude of global average temperature changes by the end of the 21st century, forests in northern temperate regions are predicted to shift northward between 200 and 700 km (Frelich et al. 2015). Overall, it is clear that climate change will have profound effects on forests. However, many details especially with regard to the potential of temperate tree species to adapt to low precipitation are unclear.

1.3 Nitrogen in temperate forests

Many temperate forests are distributed on marginal soils with low N availability. N is essential for plant growth and development because it is an important component of genetic materials (DNA and RNA) and proteins (Ollivier et al. 2011). Under natural

conditions, there is high abundance of N as atmospheric N_2 as well as organic N compounds such as proteins or chitin in forests soils, but these N forms cannot be directly utilized by plants. To cope with low N nutrition, plants developed an association strategy with certain soil microorganisms such as N-fixing bacteria and mycorrhizal fungi to turn inaccessible forms of N into simple and readily available N (Ollivier et al. 2011). However, atmospheric N deposition has been increasing all over the world due to anthropogenic impacts (Fig. 2). Increased N deposition was reported to influence negatively the ectomycorrhizal and plant communities in temperate forest ecosystems (Suz et al. 2014, de Witte et al. 2017). NO_3^- and NH_4^+ are the main forms that are be taken up by forest tree species. A preference of some tree species for one inorganic form of nitrogen over the other has been reported (McFee and Stone 1968, Schulz et al. 2011, Jacob and Leuschner 2014, Dannenmann et al. 2016). Most coniferous species have a preference for NH_4^+ whereas the preference of broadleaved tree species for the two N forms is less clear (Zhang and Bai 2003).

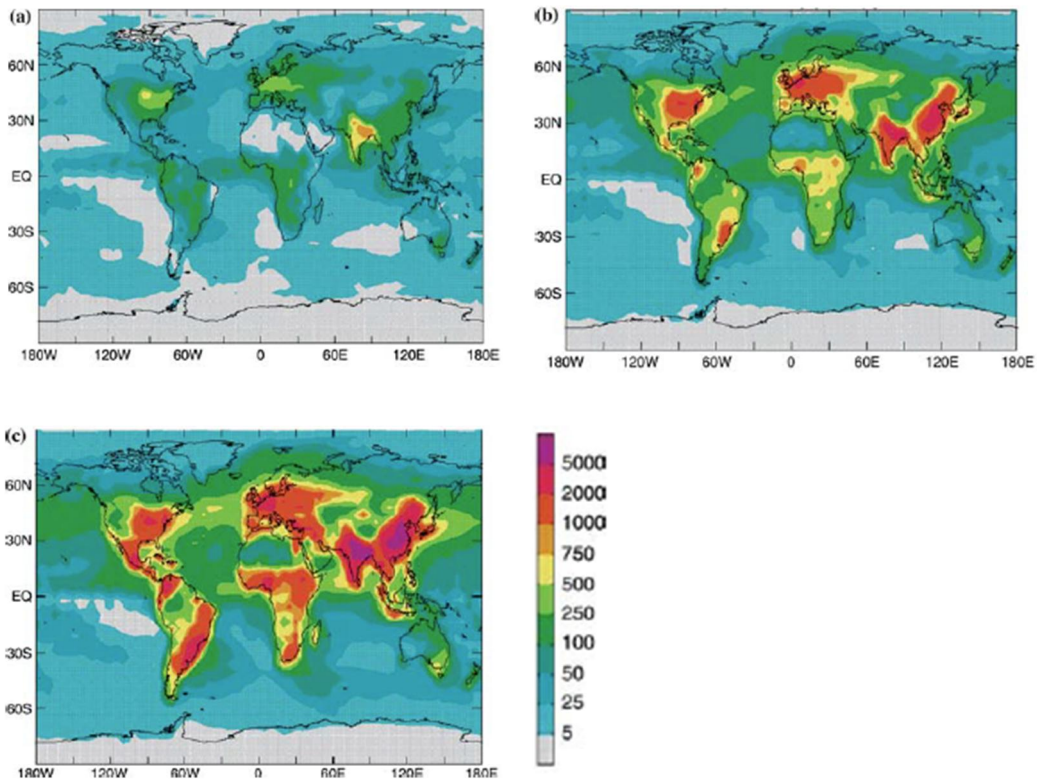

Fig. 1.2 Global spatial patterns of total inorganic nitrogen deposition in 1860 (a), 1990 (b) and 2050 (c). Unit scale is in mg N m^{-2} $year^{-1}$. Source: Galloway et al 2004

1.4 Root-associated fungi

Ectomycorrhizal fungi (EMF) are of vital importance for tree nutrition in temperate forests, especially in areas with low nutrient availability (Smith and Read 2008) . EMF form a symbiotic association with a large number of tree species in temperate forests, and they benefit host trees by enhanced nutrient supply in exchange of photosynthetic carbon (Smith and Read 2008, Courty et al. 2010).

However, in their natural habitats, plants form association with not only with EMF, but with many different microbes, resulting in multi-species entities. In other words, a plant and its associated microbiota form a holobiont (Lundberg et al. 2012, Vandenkoornhuyse et al. 2015). Root-associated fungi (RAF) are important components of the plant microbiome (Porras-Alfaro and Bayman 2011). RAF are defined as any fungus residing within or on plant roots (Dean et al. 2014). RAF are highly diverse in taxonomy and perform multiple ecological functions (Dean et al. 2014, Peršoh 2015). Key trophic groups of RAF include symbiotrophs, saprotrophs and pathotrophs, endophytes and mycoparasites (Kernaghan 2013). Because simple, readily usable sugars are scarce in soil (Nehls et al. 2007), root exudates may be critical for root-associated fungal communities. Root saprotrophs, which live on plant roots, may further obtain carbon from dead root cells or dead ectomycorrhizal hyphae while pathogenic fungi achieve carbon from living root cells (Kernaghan 2013).

To date, we know little about the environmental drivers of RAF and their potential role for tree N nutrition. To investigate how the RAF communities are composed, analyses in temperate forest ecosystems are required. I conducted greenhouse and field studies to investigate EMF and RAF on forest tree roots. Here, the Biodiveristy Exploratories were the perfect platform for this research purpose.

The Biodiversity Exploratories (DFG Priority Programme 1374) are a research infrastructure which is funded by the German Research Foundation (www.biodiversity-exploratories.de). The Biodiversity Exploratories consist of 150 forest plots (100m x 100m), which extend over various management types and land-used intensity, in three different regions across Germany: Schwäbische Alb, Hainich-Dün and Schoefheide-Chorin. The project covers various aspects of research regarding the relationships among biodiversity, land-use changes and ecosystem processes through the application of a large number of monitoring, observational and experimental approaches. (Fischer et al. 2010). It provides thereby, a well-organized platform to conduct large-scale and long-term research on

functional biodiversity under changing conditions of land use and geographical regions.

1.5 Goals of this thesis

The overarching aim of this study was to elucidate the diversity and function of root-associated fungal communities in relation to nitrogen in temperate forests. The following aims were addressed:

(1) to assess the impact of ectomycorrhizal community composition on inorganic nitrogen nutrition and performance of beech provenances (Chapter 2). I set up a greenhouse experiment, in which I manipulated the diversity and composition of the EMF community of the host plants and studied N uptake of beech by ^{15}N labeling;

(2) to obtain insights into molecular mechanisms of ectomycorrhizal fungi-beech interaction (Chapter 3). Young beech plants from the provenance "Schorfheide-Chorin" were planted into Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin and were used to study transcriptomes after two years of growth;

(3) to investigate the diversity and community composition of the root-associated fungi and their relationships with soil and root nitrogen and carbon in three biogeographical regions (Chapter 4). Roots were sampled in 150 forest plots in the three biogeographical regions across Germany and used to identify fungal communities by Illumina sequencing. Soil and root nitrogen and carbon were determined.

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CHAPTER 2

IMPACT OF ECTOMYCORRHIZAL COMMUNITY COMPOSITION AND SOIL TREATMENT ON INORGANIC NITROGEN NUTRITION AND PERFORMANCE OF BEECH (*FAGUS SYLVATICA* L.) PROVENANCES

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CHAPTER 3

BEECH AND FUNGAL TRANSCRIPTOMES IN RESPONSE TO DIFFERENT BIOGEOGRAPHICAL ENVIRONMENTS IN TEMPERATE FORESTS

3.1 Introduction

Plants are not anymore known as individuals but as entities of multi-species, whereby their associated microorganisms are of importance for the growth and development of holobiont (Bordenstein and Theis 2015, Mitter et al. 2016). As a result, the fitness of plants is shaped by the hologenome which includes the genome of the plant as well as those of associated microbes (Mitter et al. 2016). Indeed, research on plant microbiome has considered microbes as a reservoir of extra genes for host plants (Vandenkoornhuyse et al. 2015). Plant microbiome are recruited mostly from the surrounding soil, and they can also be transmitted by seeds and the air (Sánchez-Cañizares et al. 2017).

Although microbes can reside on or within different parts of plants, root-associated microbial communities have received most of the attention for their interaction with the plant (Lareen et al. 2016). High microbial diversity on roots is driven by root exudates in the root environment, and thus the root is a hot spot of the interaction between plants and microorganisms which is especially crucial for plant nutrition and health (Mitter et al. 2016, Pérez-Jaramillo et al. 2016). Soil fungal communities were determined more strongly by geographical distance of habitats than those of the roots, and the root-associated fungal communities were recruited from soil fungal communities (Danielsen et al. 2012, Goldmann et al. 2016). The dominant groups of root-associated fungi were ectomycorrhizal fungi (EMF) (Danielsen et al. 2012, Goldmann et al. 2016), which are important for tree nutrition and stress tolerance (Luo et al. 2009, Danielsen and Polle 2014, Kaling et al. 2018).

Beech is a dominant deciduous tree species in Central Europe of economic and ecological importance (Leuschner and Ellenberg 2017). Beech forms ectomycorrhizas with a variety of soil fungi. Roots of European beech trees are extensively colonized by ectomycorrhizal fungi, accounting for 85% to almost 100% of root tips. In mature beech forests, the roots are colonized by up to 85-90 different EMF species (Pena et al. 2010, Lang and Polle 2011). Sixty-one EMF were found and characterized during year-round monitoring of ectomycorrhizal diversity and community composition in a mature beech forest (Buée et al. 2005).

Nitrogen can be translocated by EMF to their host plants through extraradical mycelium (Melin and Nilsson 1953, Finlay et al. 1989). Since N is limited in many forest ecosystems, attention has been paid to the role of EMF in improving plant N supply (LeBauer and Treseder 2008). It was estimated that as much as 61-86% of N in arctic plants was supplied by mycorrhizal fungi (Hobbie and Hobbie 2006). NH_4^+

and NO_3^- are only forms of inorganic N which can be immediately accessed by plants, some plant species have a preference for one form over the other (Salsac et al. 1987, Forde and Clarkson 1999). Beech labeling study with $^{15}NH_4^+$ showed that $^{15}NH_4^+$ uptake by beech was correlated with $^{15}NH_4^+$ enrichment in ectomycorrhizal tips (Pena and Polle 2014). When exposed to both N forms, mycorrhizal beech plants showed a preference for NO_3^+ over NH_4^+ (Dannenmann et al. 2016, Leberecht et al. 2016, Chapter 2). Tracer study with both NH_4^+ and NO_3^- under field conditions showed that $^{15}NH_4^+$ uptake by EMF was higher than that of $^{15}NO_3^-$, but $^{15}NH_4^+$ uptake by beech was lower than that of $^{15}NO_3^-$ (Leberecht et al. 2016).

However, our understanding of the role of EMF to beech N supply at a molecular level remains scarce. Molecular approaches based on RNA sequencing can be used to broaden our understanding about the mechanism and function of beech-EMF interactions. The goals of the present study, therefore, were to characterize beech and fungal transcriptomes and to compare the functional contribution of fungal genes and those of beech genes to beech nitrogen nutrition. Young beech plants from the provenance “Schorfheide-Chorin” were planted in three biogeographical regions: Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin. After two years of growth under field conditions, fine roots of beech seedlings were harvested and used to study transcriptomes.

3.2 Materials and methods

3.2.1 Plant materials and experimental design

A beech transplantation experiment (BTE) was established in three regions: Hainich-Dün (H), Schorfheide-Chorin (S) and Schwäbische Alb (A) in the framework of the Biodiversity Exploratories by Kristina Schröter (Fig. 3.1, Appendix Table 3.1, Schröter 2015, Department of Forest Botany and Tree Physiology). Beech nuts of 12 provenances, four from each region, were collected in autumn 2011 (Appendix Table 3.1), treated and cultured under greenhouse conditions as characterized in the section of plant materials and growth conditions (Chapter 2). The only difference was that the substrate which was used for planting beech seedlings in this experiment was a sterilized mixture of soil and sand including four parts of soil (Fruhstorfer Erde Typ N, Hawite, Gruppe GmBH, Vechta, Germany), three parts of coarse sand (0.7-1.2 mm) and 1 part of fine sand (0.4-0.8 mm) (Melo Schwimmbadtechnik, Göttingen, Germany). In June 2012, the beech seedlings were acclimated to outdoor conditions under a shadowing net that removed 90% of incoming light. The seedlings were cultivated with Hakaphos Blau nutrient solution

(Compo GmbH, Münster, Germany) with only 40 % of the amount recommended by the manufacturer until outplanting in forest plots.

In autumn 2012, beech seedlings were outplanted in the three regions. Key characteristics of the regions were presented in Table 2.1 (Chapter 2). The experiment was conducted in nine forest plots in each region (Appendix Table 3.1). In each plot, 120 beech seedlings were planted in two blocks (Fig. 3.1b). Sixty beech seedlings, five seedlings from each beech provenance, were planted in one block in a randomized design (Fig. 3.1b). The size of one block was 2 m x 0.8 m with a distance of 20 cm between the beech seedlings (Fig. 3.1b). The distance between the two blocks was 60 cm. An example of plot S35 was presented (Fig. 3.1c).

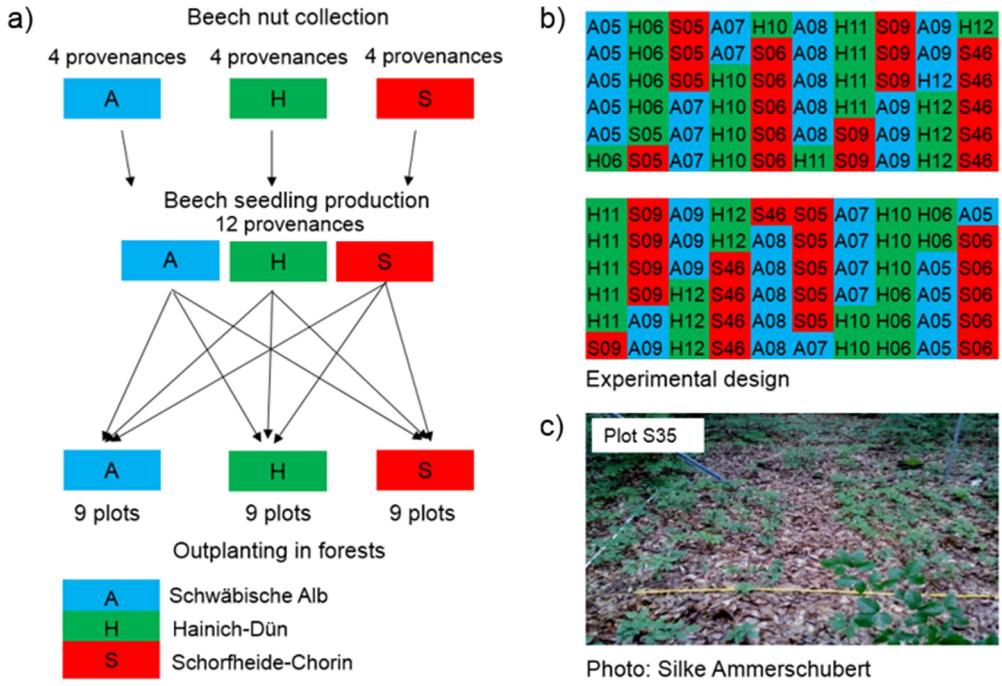

Fig. 3.1 Beech Transplantation Experiment**.** Steps of setting up the experiment from the beech nut collection to seedling outplanting in forests (a), experimental design of one plot (b) and picture of plot 35 (c)

3.2.2 Harvest

From 25 August to 12 September 2014, whole seedlings were harvested in 14 forest plots in three regions (Appendix Table 3.1). On the harvest day, in each plot two beech seedlings from the same provenance were harvested with a split tube (12.5 cm diameter). The fine roots were separated from coarse roots, gently washed with cold water and immediately shock frozen in liquid nitrogen in the field. To obtain

sufficient material for analyses, the root samples of two beech seedlings from the same provenance were pooled. Total collected root samples were 168 samples in three regions (48 samples from Hainich-Dün, 60 samples for each of the other two regions). The roots were transported in liquid nitrogen and stored at -80 °C.

3.2.3 RNA extraction

All 168 fine root samples were used for RNA extraction. Frozen fine roots were ground to a fine powder in liquid nitrogen with a ball mill (Type MM2, Retsch, Hann, Germany). Total RNA was extracted from the milled roots using a modified RNA extraction method (Chang et al. 2013). To an Eppendorf tube containing 150 mg of the roots, 800 µl pre-warmed CTAB buffer and 16 µl mercaptoethanol were added. One liter of the CTAB buffer contained: 2% hexadecyltrimethylammonium bromide (pH 8), 100mM trisaminomethane HCl, 25 mM ethylenediaminetetraacetic acid, 2M NaCl and 2% polyvinylpyrrolidone K30. Subsequently, the Eppendorf tube was well mixed and incubated at 20000 g for 15 min at 65 °C on a thermo-mixer (Thermo-mixer Comfort, Eppendorf, Hamburg, Germany). The Eppendorf tube was then cooled down to 22 °C for 15 min with repeated shaking at 100 rpm. To the Eppendorf tube, a solution of 800 µl of chloroform:isoamylalcohol (24:1) was added and incubated for 15 min at 22 °C for 1400 rpm on a thermo-mixer and then centrifuged for 5 min at 22 °C at 20000 g (5417R, Eppendorf, Hamburg, Germany). Then, the upper phase was transferred to a new 1.5 ml Eppendorf tube, and the washing step for the upper phase was repeated for a second time. 200 ml of 10M LiCl was added to the washed upper phase, mixed briefly and incubated for precipitation on ice at 4 °C overnight.

On the next day, the sample was centrifuged for 20 min at 4 °C at 20000 g, and the supernatant was carefully discarded. 400 µl SSTE buffer was added to the precipitate and incubated for 10 min at 42 °C on a shaker at 7400 g to dissolve the pellet completely. 100 ml of the SSTE buffer (pH 8) contained 0.5% sodium dodecyl sulfate, 10 mM trisaminomethane HCl, 1 mM ethylenediaminetetraacetic acid and 1 M NaCl. After 400 ml of chloroform:isoamyl alcohol (24:1) was added to the sample tube, it was mixed briefly and centrifuged for 5 min at 22 °C at 20000 g (5417R, Eppendorf, Hamburg, Germany). The upper phase was transferred into a 1.5 ml Eppendorf tube, and the RNA was precipitated by adding 800 µl of ethanol (96% (v:v), stored at -20 °C, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), incubating for 60 min at -80 °C and centrifuging 20 min at 4 °C at 20000 g. Five hundred µl of ethanol (70%, v:v) was added to the sample tube and centrifuged for 10 min at 22

°C at 14000 rpm to wash the pellet. The pellet was dried in a vacuum drier (Concentrator 5310, Eppendorf-Netheler-Hinz, Hamburg, Germany) at 45 °C for 3 min. The RNA was dissolved in 30 µl of RNase-free water (AppliChem GmbH, Darmstadt, Germany) and stored at -80 °C.

RNA concentration and purity were evaluated in a NanodropTM 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA). The RNA absorbance ratio at 260 nm and 280 nm for the samples ranged from 1.94 to 2.15. A value for the 260nm/280nm ratio of around 2 is usually considered as “pure” for RNA. RNA integrity was visualized by gel-electrophoresis. For this purpose, 0.6 g agarose was placed in a glass tube. Then, 35 ml distilled water and 5 ml 10x running buffer (1 liter of running buffer contained: 0.2M 3-(N-morpholino) propane sulfonic acid, 50 mM sodium acetate and 10 mM ethylenediaminetetraacetic acid (pH 8) were added to the tube and heated in 2 min in an 800-watt microwave oven to dissolve the mixture. In a fume hood, 10 ml formaldehyde (37%) was quickly added the tube. The mixture was used to make a gel using a plastic tray with a comb. After that the gel was placed on a gel running chamber containing 1x running buffer. The mixture of 1 µl RNA, 2 µl nuclease free water and 2 µl 2x loading buffer (for 1 ml: 660 µl formamid deionisiert, 140 µl of nuclease free water, 100 µl 10X running buffer, 80 µl formaldehyde (37%, v:v), 10 µl 10% bromophenol blue and 10 µl ethidium bromide) was loaded on the gel. The electrophoresis was run for 25 min at 100V, taken out and visualized at 300 nm excitation (Fluorescence-Multiimager, Bio-Rad, Munich, Germany). If an RNA sample was degraded (Sample ID 188, Fig. 3.2), the RNA extraction for that sample was repeated.

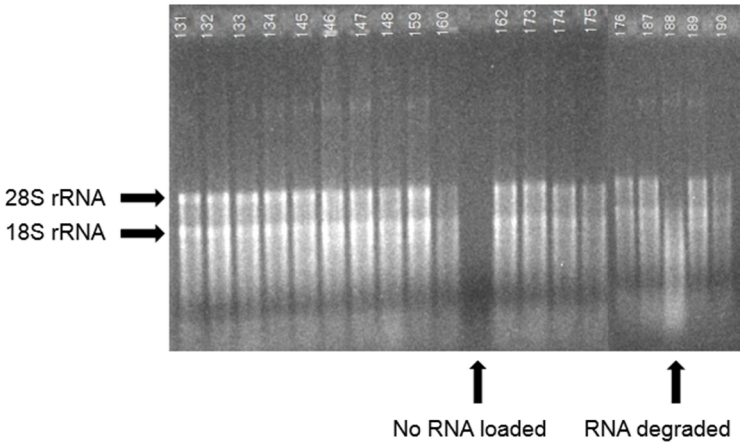

Fig. 3.2 Assessment of RNA intensity isolated from roots of beech seedlings by electrophoretic analysis. Numbers indicate the sample IDs. RNA bands were visualized using 1.2% agarose gel stained with ethidium bromide at the excitation wavelength of 300 nm.

3.2.4 Library preparation and RNA sequencing

In this study, seedlings from beech nuts of four Schorfheide-Chorin forest plots were selected for RNA sequencing (Appendix Table 3.1). Forty-eight RNA samples of the origin Schorfheide-Chorin, which came from four forest plots in each region, were used (Appendix Table 3.1). Four RNA samples of Schorfheide-Chorin origins from seedlings from the same plot were pooled with an equal concentration of 3 µg/ml, thereby 12 RNA samples (one per plot) were obtained and used for RNA sequencing. The RNA samples were sent to Chronix Biomedical GmbH (Chronix Biomedical, Inc., Göttingen, Germany) for library preparation and sequencing. The RNA quality was once again determined using Agilent 2100 Bioanalyzer System (Agilent Technologies, Santa Clara, CA, USA. RNA). The RNA integrity numbers (RIN) of these samples ranged from 4.0 to 6.6 (Table 3.1). The cDNA libraries were prepared using NEBNext Ultra RNA Library Prep Kit for Illumina (New England BioLabs, Frankfurt am Main, Germany), and single-end reads were sequenced with a length of 150 bp in an Illumina HiSeq 2000 (Illumina, San Diego, CA, USA).

3.2.5 Data analysis

Raw sequence data were preprocessed with the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Sequences, which were smaller than 75 bp or with a Phred score below 20, were discarded by using FASTQ Trimmer, and adapter sequences and primers, which were from NEBNext Ultra RNA Library Prep Kit for Illumina and used for RNA library preparation, were removed with FASTQ Clipper (http://hannonlab.cshl.edu/fastx_toolkit/) to obtain cleaned reads. These sequences were mapped against transcriptomes of *Fagus sylvatica* deposited in a transcriptome shotgun assembly sequence database in GenBank (ID number HADB00000000.1) and against the transcriptomes of 27 fungal species (Appendix Table 3.2) downloaded from MycoCosm (Grigoriev et al. 2014) using Bowtie 2 (Langmead and Salzberg 2012). Bowtie mapping files were summarized to transcript count tables in R (R Core Team 2017).

To detect transcripts with significantly increased or decreased abundance under different conditions, the DEseq package (Love et al. 2014) was applied in R. Genes with an adjusted p value < 0.05 were considered differentially expressed. The P values were adjusted to control the false discovery rate (Benjamini and Hochberg 1995). Principal component analysis (PCA) was used to visualize the pattern of transcript abundance among the three study regions. To obtain groups of genes according to functional role in the cell, Gene Ontology (GO) term enrichment was

analyzed with Ontologizer (Bauer et al. 2008) using gene annotations of beech (Polle et al, unpublished) and the lists of differentially expressed genes between study regions as input data. The P values were adjusted using the Bonferroni method to determine enriched GO terms (Miller 1966). The Ontologizer produces a list of GO annotations, and these enriched GO terms were subsequently summarized in a hierarchy using GOPathDrawer (http://coxpresdb.jp/top_draw.shtml).

To find out which fungal and beech genes were related to nitrogen, two approaches were used. The first was that all of the increased and decreased genes of beech and fungi among the three conditions were searched via nitrogen metabolism. In fact, the whole fungal transcriptome was sorted according to Enzyme commission (EC) number and mapped to KEGG pathway using KEGG mapper- Search & Color Pathway (Kanehisa et al. 2017). To investigate beech genes which were involved in nitrogen metabolism, Paintomics 3 was used with *Arabidopsis thaliana* selected and total beech transcriptome as input data file, (version 0.4.5, http://bioinfo.cipf.es/paintomics/). The second approach was to search genes of nitrate transporters and ammonium transporters in the fungal and beech transcriptomes. To confirm the the contribution of beech and fungal genes from the samples to beech N uptake and assimilation, transcriptome of beech gene pool and that of *Laccaria bicolor* were also used to map to N metabolism, and comparison of N-related genes between transcriptomes of the samples and gene pools were made.

3.3 Results

3.3.1 RNA-Seq output and read mapping

A total of 355.4 million reads from 12 samples with an average of about 29.6 million reads per sample were obtained by RNA sequencing (Table 3.1). The number of reads per sample ranged from 27.3 to 34.5 million reads (Table 3.1). After removing adapter and low-quality sequences, we obtained a total of above 211.8 million reads, with an average number of 17.7 ± 0.35 million reads per sample (Table 3.1). As a result, after quality filtering 60 percent of the reads remained for further analyses (Table 3.1).

Table 3.1 Summary of numbers of reads after RNA sequencing of twelve samples from three biogeographical regions

Sample	Region	Plot	RIN[#]	Numbers of reads/sample	Read numbers after quality filtering	Reads after quality filtering (%)
1	H	H5	6.2	31043735	18919446	61
2	H	H12	5.3	30228997	18225988	60
3	H	H16	5.0	28030977	16316726	58
4	H	H21	5.4	29343454	16945089	58
5	S	S34	4.2	34521582	20816106	60
6	S	S35	6.3	29614473	16971806	57
7	S	S37	5.1	28391141	16773063	59
8	S	S38	4.3	28546089	17165667	60
9	A	A5	4.0	27313438	16281344	60
10	A	A6	5.3	28970637	17598466	61
11	A	A39	6.6	28563535	17220387	60
12	A	A42	5.1	30827671	18604504	60
Mean			5.2±0.2	29616311	17653216	60
Sum				355395729	211838592	60

[#] RNA integrity number

When reads from the 12 samples were mapped against transcriptome of *Fagus sylvatica* (131000 “genes”, Table 3.2), 26.2 million hits were detected with an average of 2.2 million hits per sample (Table 3.2). Here, the beech “genes” were defined as follows: the assembled sequences obtained by de novo transcriptome assembly from a transcriptome shotgun assembly sequence database available in GenBank (ID number HADB00000000.1). The hits could subsequently be assigned to 51.4 thousand *Fagus sylvatica* gene IDs. On average, 43 hits per beech “gene” were found. For fungal data, 10.7 million hits were detected when the sequence reads of the 12 RNAseq samples were mapped against those of 27 EMF species, resulting in 895.2 thousand hits per sample (Table 3.2). The hits were annotated to fungal EC (enzyme commission) numbers, resulting in the assignment of a total of 169.7 thousand reads (Table 3.2). This corresponded to only four hits per fungal gene (Table 3.2). Because of the low coverage of hits per gene, the significance of a bulk differential expression analysis was questionable and therefore not attempted here.

Table 3.2 Summary of results of mapping transcriptomic data of 12 samples against transcriptomes of *Fagus sylvatica* and 27 ectomycorrhizal fungi

Kingdom	Gene pool	Numbers of hits	Mean number of hits per sample			Mean numbers of hits per gene
			mean	min	max	
Plant (Fagus)	131000	26168355	2180696	1890472	2649971	43
Fungi	190000	10742602	895217	541702	1325763	4

3.3.2 Analysis of transcript abundance

An overall analysis of the whole transcriptomes by principal component analysis (PCA) ordination plots showed that the root samples from the S regions were separated along PC1 from those of the H and A regions (Fig. 3.3). This pattern was consistent when the data of the transcript abundance of both beech and fungi (Fig. 3.3a) or beech transcript abundance data (Fig. 3.3b) or fungal transcript abundance data (Fig. 3.3c) were plotted. However, the differences appeared to be stronger between H and S than between A and S.

When comparing the expression of beech root-related genes among the three biogeographical regions, 536 differentially expressed genes (DEGs) were identified between the H and S regions, 7 DEGs between the H and A regions, and 42 DEGs between the S and A regions (Fig. 3.4a). Three out of 7 genes, which were expressed differentially between A and H region, were also expressed differentially between H and S region (Fig. 3.4b). Twenty-three out of 42 of differentially expressed genes between S and A region, showed differential expression between H and S region (Fig. 3.4b). As a result, the difference in transcript abundances between H and S was higher than in the other combinations of the three regions. Twenty-three transcripts were common to H and A among the DEGs (Appendix Table 3.6). Among the common genes were transporters for iron, sulfate, phosphate and nitrate suggesting a difference in beech nutrient uptake between S and the other two regions.

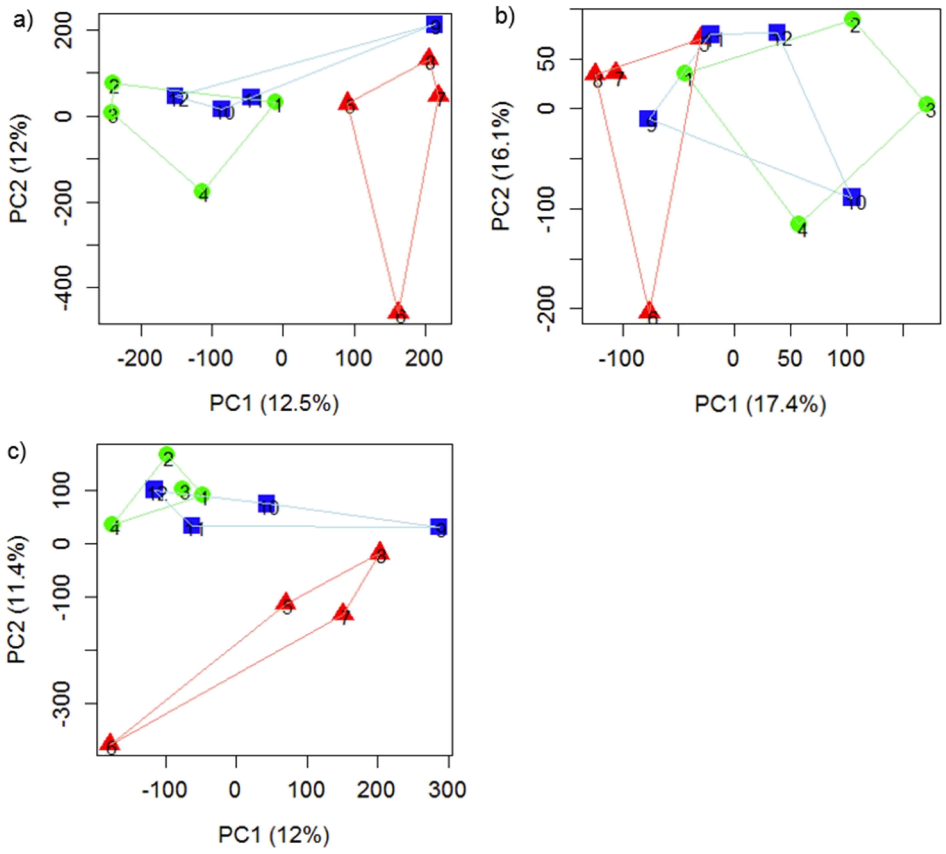

Fig. 3.3 Principal component analysis (PCA) for transcript abundance from three different biogeographical regions. Transcript abundance of both beech and fungi (a), beech transcript abundance (b) and fungal transcript abundance (c). Hainich-Dün (green circle), Schorfheide-Chorin (red triangle) and Schwäbische Alb (blue square). Sample numbers are shown according to Table 3.1.

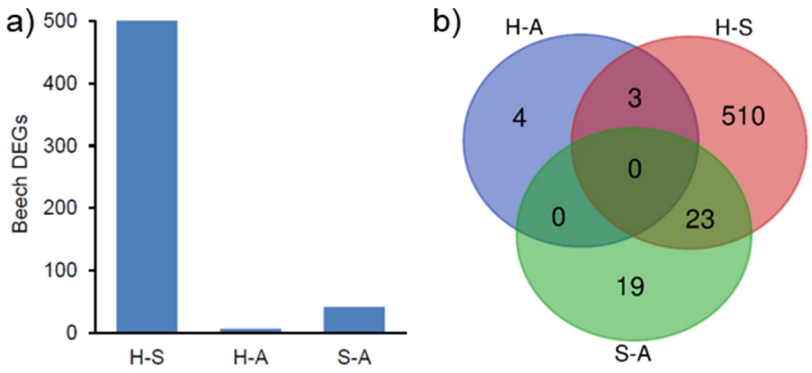

Fig. 3.4 Numbers of differently expressed genes (DEGs) of beech (a) and Venn diagrams for beech DEGs in pairwise comparison (b) among the three biogeographic regions

3.3.3 Analysis of Gene Ontology (GO) terms

Beech DEGs were grouped according to different functional categories by GO term analysis. Based on the DEGs between H and S, ninety-two GO terms were found to be enriched (Fig. 3.5). In this set, 87 GO terms belonged to the category of “biological processes”, and five GO terms were in the “molecular function” group.

In the category “biological processes”, GO terms for biosynthesis and metabolism of olefin, alkene, ceramide, salicylic acid, phenol-and benzene-containing compounds, phosphorus, reactive oxygen species, toxin, camalexin, indole phytoalexin and monocarboxylic acid were detected (Fig. 3.5a). Furthermore, GO terms for the category “stimuli” were found: immune, acid, light intensity, chemicals, chitin, organic nitrogen, other organisms, boron deprivation (Fig. 3.5b). Other groups of GO terms with functions in the regulation of “stimuli” were present such as regulation of stress response, cell death, multi-organism process, immune system process, autophagy, cellular macromolecule biosynthesis, gene expression, RNA metabolic process, nitrogen and nucleobase-containing compound metabolic process, hydrogen peroxide, reactive oxygen species metabolic process (Fig. 3.5c). Among 87 GO terms in the category of “biological process”, there were two N-related GO terms, GO:0010243 (response to organic nitrogen), GO: 0051171 (regulation of nitrogen compound metabolic process). There were 64 beech genes in GO:0010243 and 65 beech genes in GO:0051171 (Appendix Table 3.3).

The five GO terms in the category “molecular functions”, were mainly related to nucleic acid binding. These GO terms were GO:0001067 (regulatory region nucleic acid binding), GO:0003677 (DNA binding), GO:0001071 (nucleic acid binding transcription factor activity), GO:0000975 (regulatory region DNA binding), and GO:0003700 (transcription factor activity, sequence-specific DNA binding).

There were only two enriched GO terms for beech DEGs between the S and A regions. These were GO:0019745 (pentacyclic triterpenoid biosynthesis process) and GO:0019742 (pentacyclic triterpenoid metabolic process). No enriched GO terms in the differential expression data between H and A plots was found.

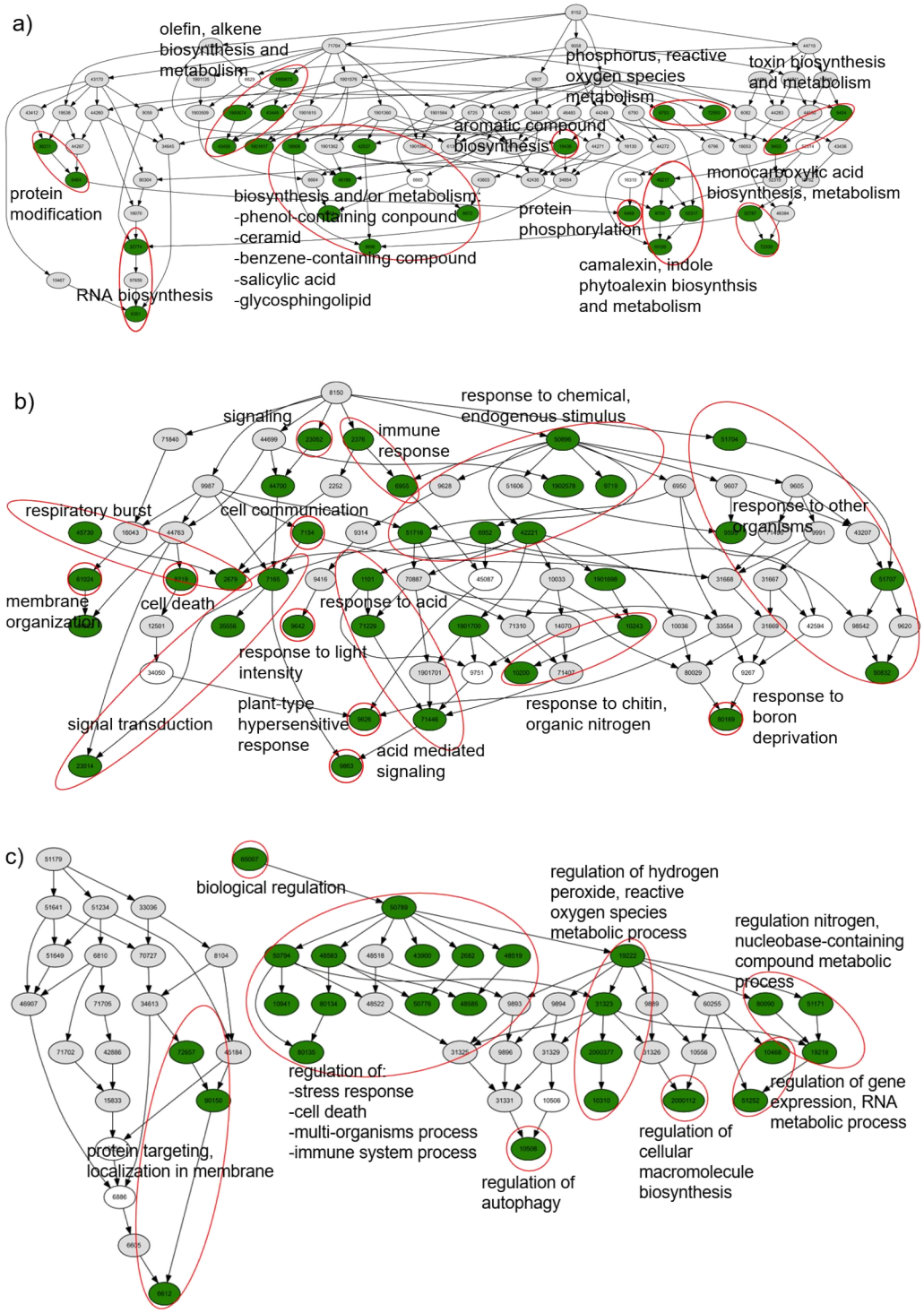

Fig. 3.5 Hierarchy of enriched GO terms of DEGs in relation to biological processes between H and S regions. Enriched GO terms are shown in green, others in white. The original figure (Appendix Fig. 3.1) was cut into three parts (a, b, c). Some of the connections between the three parts were removed and can be found on the original figure (Appendix Figure 3.1).

3.3.4 Beech and fungal genes related to nitrogen uptake and assimilation

Beech genes, which were related to N uptake and assimilation, were detected by mapping the beech transcriptome to N metabolism and keyword searching for nitrate and ammonium transporters in the beech transcriptome, resulting 111 hits (Table 3.3, Appendix Table 3.4). Among them, 80 beech "genes" were putatively involved in N metabolism (Fig. 3.6) and further in N transport such as nitrate transporters (5 NRT2.5 and 4 NRT2.7) (Table 3.3, Fig. 3.6). Three beech genes (nitrate reductase, 1 NIA1 and 2 NIA2) were likely involved in nitrate reduction, from nitrate (NO_3^-) to nitrite (NO_2^-). Subsequently, NO_2^- reduction to NH_4^+ could be catalyzed by beech nitrite reductase, of which 4 NIR1 were detected (Table 3.3, Fig. 3.6). Beech genes of carbonic anhydrase (5 BCA5, 1 ACA1, 1 ACA4, 3 ACA7, 1 CA1 and 5 CA2) and acetamidase/formamidase family protein (3 AT4G37560) might further contribute to NH_4^+ production (Table 3.3, Fig. 3.6). The rest of the beech genes were involved in L-glutamate biosynthesis in glutamate metabolism (Fig. 3.6). These genes included glutamine synthetase (2 GS2, 1 GLN1.3 and 7 GSR_1), glutamate synthase (9 GLT1, 6 GLU1, and 1 GLU2) (Fig. 3.6, Table 3.3).

In regard to N-related fungal genes, 374 fungal genes were detected in the fungal transcriptome of the 27 EMF species, all of which were putatively involved in ammonium uptake and assimilation (Table 3.4). We found 102 fungal genes of ammonia permease in the fungal transcriptome (Table 3.4, Appendix Table 3.5) but no nitrate transporters. Among the detected N-related fungal genes 272 genes were found to be involved in NH_4^+ production and glutamate metabolism (Fig. 3.7, Table 3.4, Appendix Table 3.5). For instance, 25 genes (Nitrilase) from 14 EMF species were detected that may take part in the nitrite reduction to ammonia (Table 3.4, Fig. 3.7). Three fungal genes (carbamoyl-phosphate synthase (ammonia)) from three fungal species may take part in carbamoyl phosphate biosynthesis (Table 3.4, Fig. 3.7), and 91 fungal genes (carbonate dehydratase) from 27 EMF species were able to catalyze a reaction which produces carbonic acid from CO_2 (Table 3.4, Fig. 3.7). The rest of the fungal genes (153 genes) can potentially participate in L-glutamate biosynthesis in glutamate metabolism from ammonia and L-glutamine (Table 3.4, Fig. 3.7).

When transcriptomes of the beech gene pool were used to map to N metabolism, the same result as mapped with the samples was obtained, indicating that due to the constraints of the data base further N-related genes may not have been found (Appendix Fig. 3.2). To test the data base coverage, the transcriptome of *Laccaria*

bicolor (Martin et al. 2008) was mapped against the N metabolism, revealing the absence of genes related to nitrite reduction to ammonia and carbamoyl phosphate biosynthesis. Most of the genes related to glutamate metabolism were present in N metabolism (Appendix Fig. 3.3).

Overall, while beech genes were involved in all the steps of nitrate uptake and assimilation (Fig. 3.6), fungal genes were related to ammonium uptake and assimilation in N metabolism (Fig. 3.7).

Table 3.3 N-related genes in the beech transcriptome. These genes obtained from mapping the beech transcriptome against N metabolism and from doing keyword search for nitratre and ammonium transporters using the beech transcriptome

Gene name	AGI ID	Putative function	Fasyl IDs
ACA1	AT3G52720	alpha carbonic anhydrase 1	1621113
ACA4	AT4G20990	alpha carbonic anhydrase 4	2250854
ACA7	AT1G08080	alpha carbonic anhydrase 7	1776839, 0593659, 0569751
AT1G51720	AT1G51720	Amino acid dehydrogenase family protein	2569622, 2566762, 1774376, 1271906, 0129825,1015882
AT4G37560	AT4G37560	Acetamidase/Formamidase family protein	2224379, 1685580, 0614143
BCA5	AT4G33580	beta carbonic anhydrase 5	1320821, 0239657, 2155958, 2558280, 0028039
CA1	AT3G01500	carbonic anhydrase 1	1394408
CA2	AT5G14740	carbonic anhydrase 2	1886503, 2568912, 0144715, 0138595, 0119002
GDH1	AT5G18170	glutamate dehydrogenase 1	2430631, 2279097 1182924, 2559332 2028990
GDH2	AT5G07440	glutamate dehydrogenase 2	0450109, 1384250, 2567469, 2308625, 0284218, 2115554 1488290, 0976755
GLN1.3	AT3G17820	glutamine synthetase 1.3	0391853
GLT1	AT5G53460	NADH-dependent glutamate synthase 1	1319886, 2291512, 0792729, 1680520, 1169052, 0160678 1058148, 0076001, 0076000
GLU1	AT5G04140	glutamate synthase 1	1897902, 2567974, 2567363, 1169208, 1622159, 1460472
GLU2	AT2G41220	glutamate synthase 2	0603322
GS2	AT5G35630	glutamine synthetase 2	0700968, 0170119
GSR_1	AT5G37600	glutamine synthase clone R1	0952505, 2340284, 1832704, 1163541, 1114376, 0614595 2546236
NIA1	AT1G77760	nitrate reductase 1	0609862
NIA2	AT1G37130	nitrate reductase 2	2169193, 1594841
NIR1	AT2G15620	nitrite reductase 1	0948436, 0829064, 0819600, 0075946
NRT2.5	AT1G12940	nitrate transporter 2.5	0845329, 0321329, 0688632, 0554271, 2449251
NRT2.7	AT5G14570	high affinity nitrate transporter 2.7	1283739, 2528693, 0036908, 0027662
NRT1.1	AT1G12110	nitrate transporter 1.1	0092364, 2558839
NRT1.5	AT1G32450	nitrate transporter 1.5	0078523, 0117415, 0760882, 1458839, 1463770, 2418771 2564957
NRT1.7	AT1G69870	nitrate transporter 1.7	0224199, 2042055, 2273703, 2568183
NRT1:2	AT1G69850	nitrate transporter 1:2	0281961, 0662237, 1230789, 1235428, 1316850, 1342999 2125755, 2558101
AMT1;1	AT4G13510	ammonium transporter 1;1	0129718, 0375136, 1065322, 1834562, 2569115
AMT2	AT2G38290	ammonium transporter 2	0341208, 1489616, 1594973, 1678058, 2239635

AGI: Arabidopsis Genome Initiative

Fasyl: *Fagus sylvatica*

Table 3.4 N-related genes in the fungal transcriptome. These genes obtained from mapping the fungal transcriptome against N metabolism and from doing keyword search for nitratre and ammonium transporters using the fungal transcriptome

KOG ID	EC number	Function	Number of fungal genes*	Number of fungal species*
KOG1587, KOG0382	4.2.1.1	Carbonate dehydratase	91	27
KOG0370	6.3.4.16	Carbamoyl-phosphate synthase (ammonia)	3	3
KOG2250	1.4.1.2	Glutamate dehydrogenase	33	26
KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)	15	14
KOG0399	1.4.1.13	Glutamate synthase (NADPH)	20	20
KO0683	6.3.1.2	Glutamate-ammonia ligase	85	25
KOG0805, KOG1231	3.5.5.1	Nitrilase	25	14
KOG0682		Ammonia permease	102	27
Sum			**374**	

EC: Enzyme Commission number

KOG: Eucaryotic Orthologous Groups of protein

* details of number of fungal genes and species in Appendix Table 3.2

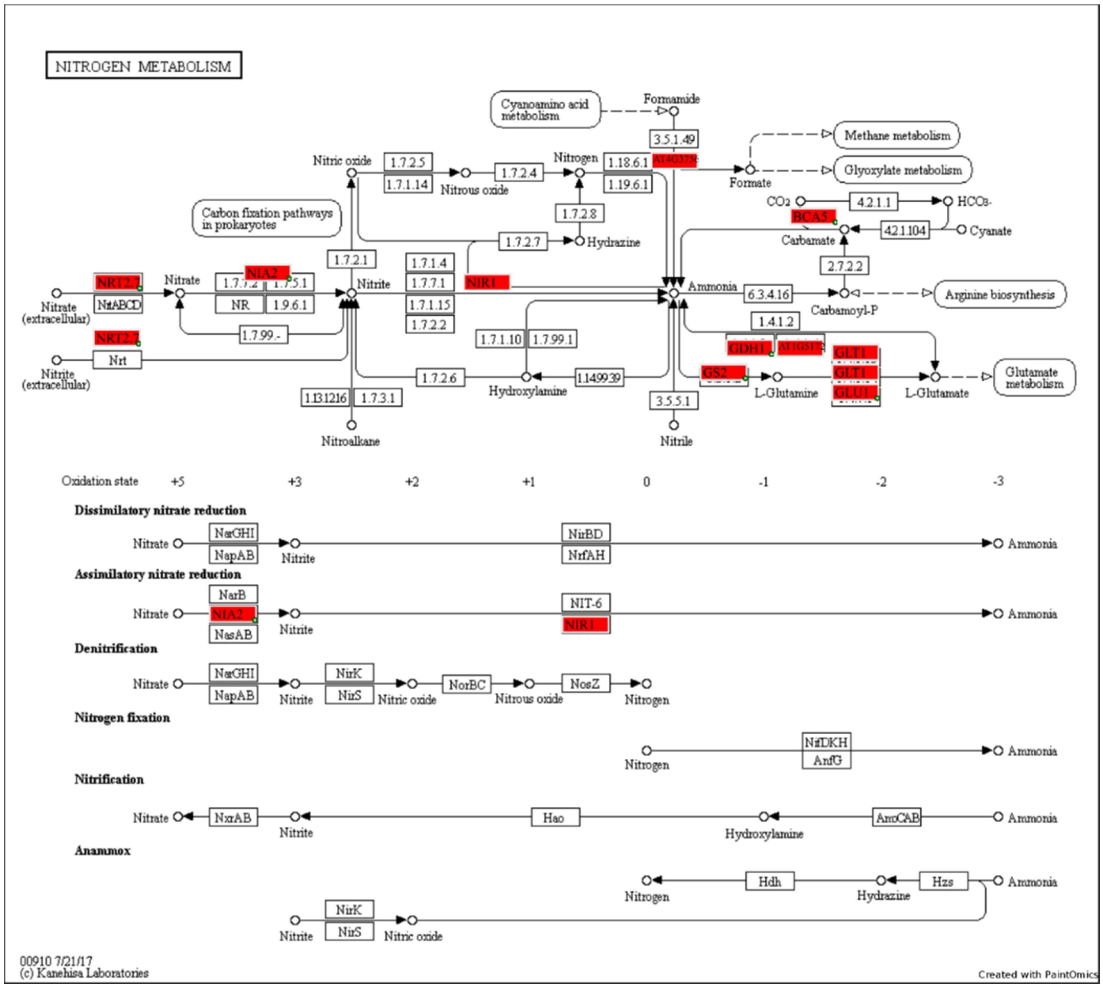

Fig. 3.6 Nitrogen metabolism of beech. Red colours indicate beech genes involved in nitrogen metabolism. The result was obtained from Paintomics 3 using list of genes from the beech transcriptome of the sample in this study according to AGI ID as input data.

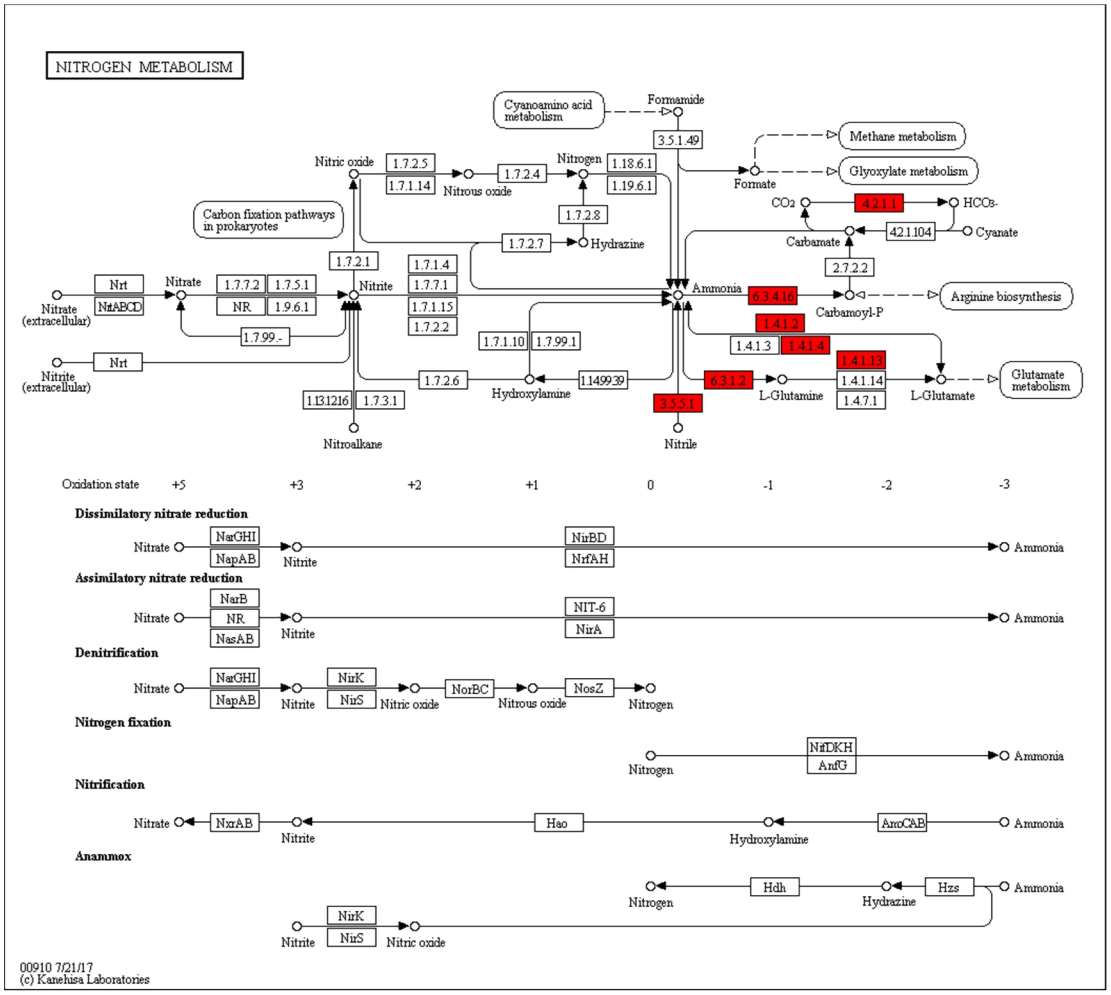

Fig. 3.7 Nitrogen metabolism of fungi. Red colour indicates fungal genes involved in nitrogen metabolism. The result was obtained from KEGG mapper-Search&Color Pathway using EC number from the fungal transcriptome as input data

3.4 Discussion

3.4.1 Limitations of RNA-Seq of beech roots

The objectives of the present study were to characterize beech and fungal transcriptomes in three biogeographical environments and investigate the role of EMF in beech N supply at a molecular level. Since the beech genome has not been sequenced, mapping with the whole genome of beech is not possible (Müller et al. 2017). Therefore, the transcriptomes from the samples in this study were mapped against a beech transcriptome generated in a previous project on the basis of different tissues (leaves, wood and roots). Annotations of beech genes can be based on those of tree species which are genetically close to beech such as oak (Fagaceae). However, although the oak genome has been sequenced (Plomion et al. 2016) most genes of the oak genome have been not annotated yet. The best

annotated tree species is poplar (Tuskan et al. 2004) whose gene annotations have, however, been mainly based on those of *Arabidopsis thaliana*. Therefore, in this study Fasyl IDs were assigned by their sequence similarities to genes of the model plant *Arabidopsis thaliana*.

Transcriptomes of twenty-seven EMF species downloaded from MycoCosm were used in this study (Grigoriev et al. 2014, Appendix Table 3.2). These species were selected because they are associated with beech and commonly found in the temperate forests in the three study regions (Schröter 2015). One limitation in this study is that because many EMF species were used for mapping the transcriptomes, on average only four hits per fungal genes were discovered (Table 3.2). This made further analysis such as differential expression analysis and GO term analysis of differentially expressed fungal transcriptomes unreliable, therefore, these analyses were not conducted for fungal transcriptomes in this study. To overcome those drawbacks, I suggest to use root tips, instead of fine roots, for RNA extraction and sequencing to achieve a higher abundance of fungal transcripts than in the present study. Secondly, information about abundance of EMF species on the root tips used for RNA-Seq should be obtained, and only the genomes of the most abundant EMF could then be used for mapping. Thirdly, the depth of RNA sequencing should be enhanced, then more fungal transcripts would be detected. Thereby the coverage of hits per gene for fungi can be enhanced.

3.4.2 Differentially expressed genes of beech in three biogeographical regions

Principal component analysis (PCA) showed that patterns of transcript abundances were different between S and the other two regions when beech and fungal transcriptomes, beech transcriptomes or fungal transcriptomes were plotted (Fig 3.3a,b,c), and higher numbers of DEGs were observed between S and the two other regions than between A and H region (Fig. 3.4a). The difference may be related to different environmental conditions in the S region compared with the A and H regions (Table 2.1, Chapter 2). Indeed, in S region has higher temperature, lower precipitation and more sandy soil than the other two regions (Table 2.1, Chapter 2).

Transcriptome studies of beech roots have not been conducted before. Recent studies on drought stress-related transcriptomes and individual genes from beech leaf samples showed different gene expression between drought stress and control treatments and between regions with different levels of water availability (Carsjens et al. 2014, Müller et al. 2017). Different expression levels of beech genes were also detected between two different phenological stages and between different time

points of ozone exposure, but elevated ozone showed small effects on the gene expression level of beech plants (Olbrich et al. 2009, 2010, Lesur et al. 2015). Here, I speculated that a clearer pattern of PCA plots with S region more separated from A and H regions would be observed and higher number of DEGs between S and A regions will be detected when the number of biological samples used for sequencing was enhanced. Future research should address the question to what extent different EMF colonizing the roots are contributing to the observed differences.

Here, I found that 23 beech DEGs were in common between A and H (Fig 3.4b). These genes may be related to higher environmental stress in the S region compared to the other regions (Fig. 3.4b). Therefore, functions of these genes were checked (Appendix Table 3.6). It was found that 4 beech genes were related to plant defense response to pathogens. These genes ID included 1232754 and 1905152 (pathogenesis-related family protein), 2556709 (disease resistance protein (CC-NBS-LRR class) family and 0654506 (Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family) (Appendix Table 3.6). Moreover, among the 23 genes, five beech genes associated with nutrition transport were detected 2568183 (nitrate transporter 1.7), 1051267 (phosphate transporter 1;4), 2568106 (phosphate transporter 1.7), 1656484 (vacuolar iron transporter 1) and 0020415 (sulfate transporter 91). A lower concentration of NO_3^- in the soil S than in the other two regions might have caused the enhanced expression of nitrate transporter (Table 2.1, Chapter 2). As a result, the DEGs might be related to the difference in the root pathogen and nutrition status between S and the other two regions which need to be further investigated because it was found that dead root tips in S region were significantly more frequent than in the H and A regions (Chapter 4).

3.4.3 N-related genes of beech and fungi

The analysis of functions of beech DEGs revealed 64 genes belonging to GO:0010243 (response to organic nitrogen) and 65 genes belonging to GO:0051171 (regulation of nitrogen compound metabolic process) (Appendix Table 3.3). In a recent transcriptome study of young beeches in response to drought stress, two enriched GO terms related to N including GO:0050463 (nitrate reductase [NAD(P)H] activity) and GO:0050464 (nitrate reductase (NADPH) activity) were also detected (Müller et al. 2017). Based on the results from GO term analysis, it is of interest to know whether the N-related genes were involved in N metabolism.

Here, I uncovered that beech genes involved in nitrate uptake and fungal genes involved in ammonium uptake and turn over were enhanced in H compared to S.

These results suggesting differences between fungal and plant genes for N usage are in agreement with a number of studies. In a field labeling experiment with both NH_4^+ and NO_3^-, EMF took up more $^{15}NH_4^+$ than $^{15}NO_3^-$ regardless of water supply, season, and site exposure, while young beech trees preferred $^{15}NO_3^-$ over $^{15}NH_4^+$ (Leberecht et al. 2016). In another study in which metatranscriptomic analysis were compared between ectomycorrhizal roots of *Piloderma*-Pinus symbiosis and soil, it was also found that a gene encoding NH_4^+ transporter (PiAMT) was highly expressed (Liao et al. 2014); furthermore, a high level of expression of genes encoding glutamine synthase and glutamate-related transporters were detected in fungal hyphae and ectomycorrhizal roots pointing to glutamate metabolism. It was concluded that the glutamine pathway is the key for NH_4^+ assimilation and N use in *Piloderma* (Liao et al. 2014). Also, our results were in line with a study on oak root in response to ectomycorrhizal symbiosis (Sebastiana et al. 2014). When comparing ectomycorrhizal roots and non-mycorrhizal roots, 2238 differentially expressed transcripts were detected. Among them, a high level of expression for a transcript encoding a putative carbonic anhydrase (CA, EC 4.2.1.1) was detected. This enzyme is capable of converting CO_2 to bicarbonate HCO_3^-. For N transport, NH_4^+ and amino acid transporters were detected to be differently expressed (Sebastiana et al. 2014). Nitrogen assimilation-related genes such as glutamine synthetase, nitrite reductase, nitrite transporter were also differentially expressed when forming the symbiosis (Sebastiana et al. 2014). Furthermore, research on diversity of expressed genes in spruce and beech forest soil by metatranscriptomics showed that 12-13% of the transcripts, which encode proteins with enzymatic activities, could be assigned to an EC (Enzymatic Commission) number (Damon et al. 2012). Key enzymes in pathways related to soil nutrient assimilation were glutamine synthase (EC 6.3.1.2), NADPH-glutamate dehydrogenase (EC 1.4.1.4), glutamate synthase (EC 1.4.1.13) for ammonium, adenylyl transferase (EC 2.7.7.4), aldehyde reductase (EC 1.1.1.21) (Damon et al. 2012). Regarding inorganic nutrient transport, ammonium transporter (T.C.1.A.11.), phosphate transporters, but no nitrate (T.C.2.A.1.8) and sulfate (T.C.2.A.53.1) transporters were detected (Damon et al. 2012). These results are in agreement with those of this study. Overall, the results suggest the possible contribution by EMF to beech NH_4^+ supply and assimilation.

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CHAPTER 4

ROOT-ASSOCIATED FUNGAL COMMUNITIES AND THEIR RELATIONSHIPS WITH SOIL AND ROOT NITROGEN AND CARBON AVAILABILITY IN TEMPERATE FORESTS

4.1 Introduction

Root-associated fungal (RAF) communities, important components of plant microbiomes, are defined as any fungi residing in and on plant roots (Porras-Alfaro and Bayman 2011, Dean et al. 2014). To date, fungi from most phyla (Basidiomycota, Ascomycota, Glomeromycota, Chytridiomycota and Zygomycota) have been detected in RAF communities (Zhang et al. 2016, Kolaříková et al. 2017). These fungi are from different trophic groups: symbiotic fungi, root pathogens, saprotrophs, root endophytes and mycoparasites (Kernaghan 2013, Peršoh 2015). In forest ecosystems, RAF community composition of distinct host species changed significantly under human disturbance (Zhang et al. 2016), in different habitats (Goldmann et al. 2015, Vannette et al. 2016, Maghnia et al. 2017) and along a successional chronosequence (Kolaříková et al. 2017). Previous studies focused on RAF community composition of specific tree species at small scales, but little is known about changes in RAF community composition of mixed root samples at a large geographical scale. Therefore, it is of great interest to evaluate if differences in the taxonomic composition across biogeographic regions lead to changes in the functional composition of RAF communities.

Environmental factors such as soil properties (Maghnia et al. 2017), elevation (Gorzelak et al. 2012) and vegetation type (Bougoure et al. 2007) have been reported to affect RAF community structure. In soil, most of the variation in the fungal microbiome was explained by soil properties, whereas other factors (geographic location, climate, vegetation) were less important (Goldmann et al. 2016). Root-associated fungi are recruited from the surrounding soil but are less affected by the abiotic environment compared to soil fungi (Danielsen et al. 2012, Goldmann et al. 2016). RAF communities may be strongly influenced by the surrounding biotic environment, but this possibility has not yet received much attention.

It has been suggested that the coexistence of different trophic groups in plant root systems is primarily due to host plant carbon (C) partitioning (Kernaghan 2013). Therefore, carbon is expected to be a driver of RAF community structure. Symbiotic fungi generally obtain carbon from host trees in exchange for mineral nutrients while root saprotrophic fungi are supposed to live mainly on root surfaces and utilize carbon from dead root cells (Kernaghan 2013). Pathogenic fungi often obtain carbon from living root cells (Kernaghan 2013). Ectomycorrhizal abundance and diversity is driven by the carbon production of beech (Druebert et al. 2009, Pena et al. 2010). In

boreal forests, root and RAF play an important role in the long-term carbon sequestration (Clemmensen et al. 2013). As much as 50 to 70% of stored carbon in boreal forested islands stemmed from root and root-associated microorganisms (Clemmensen et al. 2013), and the significance of RAF was stressed to contribute significantly to the regulation of C dynamics in boreal forests. It is also known that up to 30% of total carbon produced by forest trees through photosynthesis is supposed to be converted into RAF biomass (Kernaghan 2013). Despite the tight links of root carbon sources and associated fungal life styles, it is unknown whether differences in root pools may drive changes in RAF community structures.

Nitrogen (N) is one of the most essential elements for tree growth and development (Lupi et al. 2013), but it is not available in sufficient quantity in a number of forest ecosystems (LeBauer and Treseder 2008). In some forest ecosystems increased nitrogen deposition leads to decreased diversity of mycorrhizal fungal communities (Lilleskov et al. 2002, 2008, Cox et al. 2010, Suz et al. 2014, de Witte et al. 2017). However, the relationships between soil and root nitrogen and RAF communities have not been addressed.

The goal of this study was to investigate the taxonomic and functional composition of RAF communities in temperate forests in relation to soil and root carbon and nitrogen pools. We hypothesized that (i) the taxonomic community compositions of RAF differ among different biogeographical regions, while the trophic community composition remains similar because of functional stability of fungal communities across three study regions; (ii) RAF communities are strongly driven by root than by soil chemistry because of host plant species effects, and (iii) different ecological groups of the RAF (symbiotroph, saprotroph, and pathotroph) respond differently to soil and root C and N. To test the hypotheses, we collected soil and fine root samples from 150 forest plots in three large-scale regions of 422 to 1300 km² (Schwäbische Alb (A), Hanich-Dün (H) and Schorfheide-Chorin (S)) located along a transect of about 700 km across Germany. We measured carbon-related parameters (soil and root carbon, root carbohydrates), nitrogen-related parameters (soil and root N, NH_4^+ and NO_3^-) and determined RAF communities using deep sequencing.

4.2 Materials and methods

4.2.1 Study site description

This study was conducted in the Biodiversity Exploratories (http://www.biodiversity-exploratories.de/startseite/) encompassing three biogeographic regions located in the south-west (Schwäbische Alb), centre (Hainich-Dün) and north-east (Schorfheide-Chorin) of Germany (Fischer et al. 2010). Key bioclimatic and geographic data have been summarized in Table 4.1. Briefly, Schwäbische Alb (A) has a cooler and moister climate than the two other regions. The soils in A are silty and have higher pH values than those in Hainich-Dün (H) and Schorfheide-Chorin (S) (Table 4.1). Intermediate climate is characteristic of H, while warm and dry conditions with sandy acidic soils characterizes the S region. In each region, 50 forest plots (100x100 m) were established. The locations of the plots can be found under http://www.biodiversity-exploratories.de/exploratories/.

Table 4.1 Key biogeographic characteristics of three study regions. Source: (Fischer et al. 2010) with additional information about atmospheric N input (Schwarz et al. 2014, personal communication)

	Schorfheide-Chorin (S)	Hainich-Dün (H)	Schwäbische Alb (A)
Location	North-east Germany	Central Germany	South-west Germany
Size (km^2)	1300	1300	422
Geology	Young glacial landscape	Calcareous bedrock	Calcareous bedrock with karst phenomena
Altitude a.s.l. (m)	3-140	285-550	460-860
Longtitude east-to-west (decimal degree)	14.14796-13.39094	10.77917-10.17332	9.58024-9.02362
Latitude north-to-south (decimal degree)	53.22390-52.79023	51.37872-50.93735	48.53435-48.34996
Annual mean temperature (^oC)	8-8.5	6.5-8	6-7
Annual mean precipitation (mm)	500-600	500-800	700-1000
Total N atmospheric input (kg ha^{-1})*	16.65 ± 1.14	13.84 ± 0.84	17.09 ± 1.44
NO_3^- input (kg ha^{-1})*	7.48 ± 0.51	7.85 ± 0.48	10.52 ± 0.89
NH_4^+ input (kg ha^{-1})*	6.36 ± 0.44	4.10 ± 0.25	4.69 ± 0.40
Dissolved organic N input (kg ha^{-1})*	3.10 ± 0.21	1.89 ± 0.12	1.95 ± 0.16

*Data for the vegetation period 2010 (end of March to beginning of November)

4.2.2 Root and soil sampling

In early May 2014, 150 mineral topsoil samples (0-10 cm) and 150 root samples were collected in 150 forest plots. In each forest plot, two transects of 40 m length were established. Fourteen soil cores were taken with a split tube (56 mm in diameter) along the transects, and these were taken from north to south and from west to east at the sampling points 2.5, 8.5, 14.5, 20.5, 26.5, 32.5 and 38.5 m from the starting points. The collected soil cores were mixed into one composite sample per plot. An aliquot of soil from the composite sample was used for element analysis. Fine roots from each composite were separated from soil, gently washed with cool tap water, and aliquots were immediately frozen in liquid nitrogen in the field and stored at -80 ^{o}C.

4.2.3 Analysis of root and soil carbon and nitrogen

Fine root samples were dried at 60 ^{o}C for one week and ground to a fine powder with a ball mill (Type MM400, Retsch GmbH, Hann, Germany). Aliquots of 0.7-1 mg were weighed into 4mm x 6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) using a microbalance (Model: Cubis MSA 2.7S-000-DM, Sartorious, Goettingen, Germany). Samples were analyzed in an Elemental Analyzer (Model SHNC-O EA1108, Carlo Erba Instruments, Milan, Italy). Acetanilide (71.09% C, 10.36% N) was used as the standard for quantification of N and C. Total carbon and nitrogen contents in soils were analyzed on ground subsamples by dry combustion in a CN analyzer “Vario Max” (Elementar Analysensysteme GmbH, Hanau, Germany).

4.2.4 Analysis of non-structural carbohydrates

Non-structural carbohydrates in fine roots were determined spectrophotometrically using an enzymatic method (Schopfer 1989). Frozen root samples were milled and extracted in dimethyl sulfoxide and 25% HCl (80:20, v:v). A solution of glucose, fructose and sucrose (100 mg l^{-1} per compound) was used as the reference. For the assay, the following enzymes were used: 10 µl hexokinase/glucose-6-phosphate dehydrogenase (3 mg ml^{-1}, 340 U hexokinase ml^{-1} and 170 U glucose-6-phosphate dehydrogenase ml^{-1} at 25 ^{o}C, Roche Diagnostics GmbH, Mannheim, Germany) for glucose determination, 5 µl phosphoglucose isomerase (10 mg ml^{-1}, 350 U mg^{-1} at 25 ^{o}C, Roche Diagnostics GmbH, Mannheim, Germany) for fructose and 10 µl of invertase (30 mg ml^{-1}, 200-300 U mg^{-1}, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) for sucrose. For starch analysis, amyloglucosidase (10 mg ml^{-1}, 14 U mg^{-1}

at 25 °C, Roche Diagnostics GmbH, Mannheim, Germany) in 50 µM citrate buffer pH 4.6 (1:5, v:v)) was used to convert starch to glucose and then the glucose assay was used. The enzymes were added to a 12.5 x 12.2 x 4.5 disposable UV cuvette (Brand, Wertheim, Germany) containing 250 µl mixture (4 mM NaDP, 10 mM ATP, 9 mM MgSO4 and 0.75 M triethanolamin, pH 7.6) and 400 µl of double-distilled water and 100 µl of root extract. After mixing and incubation for 5 min in dark, the absorbance for glucose was measured at 340 nm using a spectrophotometer (Type UV-DU640, Beckmann, California, USA). The obtained absorbances were used to calculate the concentrations of the carbohydrates using the following formula.

$$C\ (mg\ ml^{-1}) = \frac{\text{test volume (ml)} * \text{molecular weight of carbohydrate (g mol}^{-1})}{\varepsilon\ NADPH * \text{cuvette thickness (cm)} * \text{sample volume (ml)}} * \Delta E$$

In which, C (mg ml^{-1}) = concentration of the carbohydrate, Molecular weight of the carbohydrate = 180.16 (g mol^{-1}), ε NADPH = extinction coefficient of NADPH = 6.3 (l * $mmol^{-1}$ * cm^{-1}), ΔE= the difference of the absorbances between the blank and the sample measurements, and cuvette thickness = 1 (cm).

4.2.5 Analysis of soil and root ammonium and nitrate

Ammonium (NH_4^+) and nitrate (NO_3^-) of soil samples were extracted from 20 g of fresh soil with 40 ml of 1 mM $CaCl_2$ solution. The samples were thoroughly shaken for 10 min and filtered for one hour using folded filter papers MN 280 (Macherey-Nagel, Düren, Germany). To remove all organisms, the filtrates were further filtered through a 0.2 µm sterile Corning® syringe filters (CLS431218 Sigma, Sarstedt, Nümbrecht, Germany), and then kept at -80°C until further analysis. The ammonium and nitrate concentrations in the filtrate were spectrophotometrically analyzed using ammonium (100683) and nitrate (109713) test kits (Merck, Darmstadt, Germany) following the manufacturer's instructions. The extinction of the assays was measured using UV-Vis spectrophotometer (Shimadzu 1601, Hannover, Germany) at 690 nm for NH_4^+, and 340 nm for NO_3^-.

Root samples of 50 mg of frozen fine roots were extracted in 0.4 ml of a mixture of methanol (VVA, Cologne, Germany) and chloroform (Th.Geyer GmbH, Renningen, Germany) (3.5:1.5, v:v, Winter et al. 1992). After incubation at 40 °C for 60 min, 0.2 ml demineralized water was added to the sample solution (3 ml g^{-1}), shaken and centrifuged for 5 min at 5000 rpm (Model 5417R, Eppendorf AG, Hamburg, Germany). The above procedure of water shaking and centrifuging of the sample solution were carried out twice. The lower hydrophilic phase was collected and dried

in a SpeedVac at 35 °C. The residue was resuspended in 400 µl demineralized water and centrifuged shortly at 5000 rpm (Model 5417R, Eppendorf AG). Subsequently, ammonium and nitrate were determined spectrophotometrically using commercially available kits (Nitrate Test 109713 and Ammonium Test 100683, Merck Chemicals GmbH, Darmstadt, Germany) according to the manufacturer's instructions. Standard curves were generated in the range from 0 to 100 µg for NH_4^+ using standard solution (Test Kit 119812, Merck Chemicals GmbH, Darmstadt, Germany) and from 0 to 1000 µg for NO_3^- (Test Kit 119811). The extinctions of sample and standard solutions were measured at 690 nm for NH_4^+ and 340 nm for NO_3^- (Type UV-DU640, Beckmann, California, USA).

4.2.6 Measuring soil pH and soil moisture

Twenty-five milliliters of 0.01 M $CaCl_2$ was added to 10 g soil. The soil pH was measured with a WTW pH meter 538 (Wissenschaftlich-Technische-Werkstätten GmbH, Germany). Two measurements per sample were taken, and final value was calculated as the average value of the two measurements. To determine soil moisture, soil samples were dried at 105 °C to a constant weight. Weights were taken before and after soil drying. The percentage of water in soil was calculated as follows:

$$\text{Soil water content (\%)} = \frac{(\text{moist soil weight-dry soil weight})}{\text{dry soil weight}} * 100$$

4.2.7 DNA extraction and amplification of ITS2

Frozen samples of fine roots were ground in a ball mill (Type MM400, Retsch GmbH, Haan, Germany). Total DNA from roots was extracted using the innuPREP Plant DNA Kit (Analytik Jena AG, Jena, Germany) according to the manufacturer's instructions. Fungal ribosomal internal transcribed spacer (ITS) region 2 was amplified with a Mastercycler Gradient (Eppendorf AG, Hamburg, Germany) using the forward primer and adapter ITS3KYO2 (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGATGAAGAACGYAGYRAA-3') (Toju et al. 2012) and the reverse primer and adapter ITS4 (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATG C-3') (White et al. 1990). Primers were purchased from Microsynth AG (Balgach, Switzerland). The PCR mixture (50 µl) contained 30.5 µl sterile nuclease-free water, 10 µl 5X Phusion High-Fidelity buffer with $MgCl_2$, 1 µl of 10 mM dNTPs Mix, 0.5 µl of Phusion High-Fidelity DNA polymerase (2U/µl) (all chemicals from Thermo Fisher Scientific), 2.5 µl of each primer (10 µM) and 3 µl of DNA template (diluted 1:20).

PCR reactions were performed in triplicate under the following conditions: initial denaturation for 2 min at 98 °C; 25 cycles of 98 °C for 10 sec, 48 °C for 20 sec and 72 °C for 20 sec; final extension step of 5 min at 72 °C. PCR products were checked by agarose gel electrophoresis (1.2 % agarose, Biozym Scientific GmbH, Hessisch Oldendorf, Germany) for appropriate size (Thermo Scientific™ GeneRuler™ 1kb DNA Ladder, Life Techlonogies GMbH, Darmstadt, Germany) and purified using a magnetic bead-based Magsi-NGS*PREP* kit (Steinbrenner Laborsysteme GmbH, Wiesenbach, Germany) according to the manufacturer's instructions. PCR pro ducts were stained using GelRed (0.01 µl mL^{-1}, GelRed™ Nucleic Acid, Biotium Inc., VWR International GmbH, Darmstadt, Germany) and visualized under ultraviolet light (Intas Science Imaging Instruments GmbH, Göttingen, Germany). Gel running conditions were 90V for 25 min in an electrophoresis system (Power Pac 200, Biorad Laboratories Ltd., München, Germany). Purified PCR products were quantified using a Qubit dsDNA HS assay Kit in a Qubit 3.0 Fluorometer (Thermo Fischer Scientific, Dreieich, Germany) and pooled at equimolar concentrations for sequencing. Amplicons were sent to Göttingen Genomics Laboratory (G2L) at the Department of Microbiology and Applied Genetics in Göttingen University, Germany for sequencing using the dual index paired-end approach (v3, 2 x 300 bp) for the Illumina MiSeq platform.

4.2.8 Sequence processing and analyses

Demultiplexing of raw sequences was performed by CASAVA data analysis software (Illumina). Paired-end sequences were merged using PEAR v0.9.10 (Zhang et al. 2014) with default parameters. Subsequently, we used Quantitative Insights into Microbial Ecology (QIIME) v1.9.1 (Caporaso et al. 2010) with the *split_library_fastq.py* script to remove the sequences with an average quality (Phred) score lower than 20 or containing unresolved nucleotides. For the removal of reverse and forward primer sequences, cutadapt v1.12 (Martin 2011) with default settings was applied. Before operational taxonomic unit (OTU) clustering, we employed USEARCH v9.2.64 (Edgar 2010) with the UPARSE (Edgar 2013) algorithm to remove sequences to shorter 140 bp, discard singleton reads, merge identical sequences (dereplication) and sort sequences by cluster size. Operational taxonomic units were clustered at 97% sequence identity using USEARCH. Chimeric DNA sequences were detected and removed using UCHIME2 algorithm (Edgar et al. 2011) with a reference dataset from the UNITE database Version 7.1 (Abarenkov et al. 2010) available at https://unite.ut.ee/repository.php. The merged paired-end sequences were mapped to chimera-free OTUs. The OTU table was

generated using USEARCH. Taxonomic assignment of OTUs was performed with *parallel_assign_taxonomy_blast.py* against the UNITE database Version 7.2. Extrinsic domain OTUs and unclassified OTUs were removed from the data set by employing *filter_otu_table_py*. Finally, all unidentified fungal OTUs were BLASTed against the nt database (May 2017) to remove non-fungal OTUs, and only fungal classified reads were kept. For comparisons all samples were rarefied by random selection to the same number of reads utilizing the lowest number of sequences present in one of the samples (total 8400 reads). The OTUs were deposited under a sequence read archive (SRA) accession number SRP140604. The OTUs were assigned to functional guilds using FunGuild (Nguyen et al. 2016)

4.2.9 Statistical analysis and calculations

Statistical analyses were conducted using R statistical software version 3.4.1 (R Core Team 2017). Data distribution and homogeneity of the variance were checked by visual inspection using histograms and residual plots. When the data did not show a normal distribution, the data were log-transformed for statistical analyses. We used ANOVA analyses to compare the means of soil and root variables among the three biogeographical regions. Generalized linear models (Poisson regression, chi-square test) were used to compare the means of count data-related variables such as fungal OTU richness and read abundance among the study regions. Pairwise differences between two study regions were compared using Tukey's Honestly Significant Difference (HSD) test. Linear mixed effects models were applied to explore the relationships between soil-and root-related variables and root fungal read abundance using function lmer() in "lme4" package after checking assumptions and performing the selection of the best variables. Study region were included in the models as random effects. The best-fit models by Akaike's information criterion (AIC) in a stepwise algorithm, which are based on the best predictor variables, were constructed using step() function in "stats" package in R. The variance inflation factor (VIF) was used to detect the existence and severity of multicollinearity. A predictor with VIF > 10 is considered as severe multicollinearity and was removed from models. We assessed the relative importance of predictor variables using the method developed by Lindeman, Merenda and Gold (lmg) with calc.relimp() function in "relaimpo" package (Grömping 2006). Non-metric multidimensional scaling (NMDS) ordination of RAF fungal communities was conducted using function metaMDS() in "vegan" package (Oksanen et al. 2018). Data in tables and figures are shown as mean ± standard error (SE). P value ≤ 0.05 was used to indicate a significant difference in statistical analyses.

4.3 Results

4.3.1 Soil and root chemistry varies among biogeographic regions

Soil chemistry, soil pH and soil moisture differed among the three biogeographical regions (Table 4.2). Soil carbon and nitrogen were lowest in S and highest in the A region (Table 4.2). The soil was more acid in S than in A plots. Soil moisture, which was correlated with annual mean temperature ($r=-0.53$, $p<0.001$, Appendix Fig. 4.1), was lower in S than in the A region (Table 4.2). In contrast, the concentration of soil NH_4^+ was highest in S and lowest in A region while the concentration of soil NO_3^- exhibited the opposite order (Table 4.2).

The concentration of carbon, glucose and fructose in roots were higher in S than the other two regions (Table 4.2). Root starch concentrations were higher in A than in H and S (Table 4.2). Root N concentrations were highest in A region and lowest in S region. The concentrations of root NO_3^- were significantly higher in forest plots in the S region than the other two regions while there was no difference in root NH_4^+ among the three regions ($p=0.173$, Table 4.2).

Table 4.2 Characterization of soil and root carbon and nitrogen, soil pH and soil moisture in three biogeographical regions. A = Schwäbische Alb, H = Hainich-Dün, S = Schorfheide-Chorin. Significant differences at $p \leq 0.05$ are indicated with bold letters. Data are means of n = 50 plots per region ± SE

Variables	Unit	Study region			P value			
		A	H	S	A-H-S	A-H	A-S	H-S
Soil variables								
Soil C	mg kg^{-1}	62520 ± 1920	36850 ± 1550	19790 ± 650	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Soil N*	mg kg^{-1}	4680 ± 140	2760 ± 110	1110 ± 40	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Soil NH_4^+*	mg kg^{-1}	0.32 ± 0.04	0.44 ± 0.07	0.67 ± 0.06	**<0.001**	0.385	**<0.001**	**<0.002**
Soil NO_3^-	mg kg^{-1}	2.35 ± 0.17	1.31 ± 0.10	0.71 ± 0.08	**<0.001**	**<0.001**	**<0.001**	**<0.002**
Soil pH*		5.23 ± 0.10	4.80 ± 0.12	3.55 ± 0.02	**<0.001**	**0.002**	**<0.001**	**<0.001**
Soil moisture	%	60.19 ± 1.92	43.57 ± 0.99	14.58 ± 0.61	**<0.001**	**0.001**	**<0.001**	**<0.001**
Root variables								
Root C	mg kg^{-1}	436550 ± 5440	421310 ± 5570	488360 ± 2930	**<0.001**	0.068	**<0.001**	**<0.001**
Root glucose*	mg kg^{-1}	2530 ± 130	2080 ± 90	4360 ± 260	**<0.001**	0.058	**<0.001**	**<0.001**
Root fructose	mg kg^{-1}	1250 ± 90	1060 ± 60	1950 ± 170	**<0.001**	0.471	**0.004**	**<0.001**
Root starch*	mg kg^{-1}	4680 ± 850	1830 ± 280	3640 ± 870	**0.022**	**0.019**	0.154	0.651
Root N*	mg kg^{-1}	13870 ± 450	12150 ± 260	11510 ± 340	**<0.001**	**0.006**	**<0.001**	0.236
Root NH_4^+*	mg kg^{-1}	29.81 ± 3.21	22.43 ± 1.05	29.57 ± 1.98	0.173	0.443	0.797	0.156
Root NO_3^-	mg kg^{-1}	1716.2 ± 123.8	1735.1 ± 89.0	2207.5 ± 136.7	**0.006**	0.993	**0.012**	**0.017**

* Data were subjected to log10 transformation before ANOVA analysis

4.3.2 Characterization of the abundance and richness of root-associated fungi

A total of 5030679 quality-filtered reads, which were clustered into 4765 OTUs, were present in 150 root samples from the three biogeographical regions. After removal of plant sequences, we acquired 4758344 fungal reads that were grouped into 3815 OTUs. The highest number of reads in a sample was 101929 while the lowest number was 8471 reads. On average, there were 31722 fungal reads per sample. After rarefaction to 8400 reads per sample, the data set for the comparison of root-associated fungi consisted of 126000 reads accounting for 3366 OTUs.

Mean fungal OTU richness per plot was highest in H and lowest in the S region (Table 4.3). The total OTU richness encompassing 50 plots per region were 1913, 2230 and 1215 for A, H and S regions, respectively (Appendix Fig. 4.2). Michaelis Menten Fit, which was additionally used to assess OTU richness per plot, also showed lowest fungal richness in S and highest in H (Table 4.3). Mean Shannon diversity (*H'*) index per plot was marginally lower in the S than in the H region ($p=0.058$, Table 4.3). Evenness (E_H) did not differ among the three study regions (Table 4.3).

Table 4.3 Operational taxonomic unit (OTU) richness and diversity estimates of root-associated fungi in three biogeographical regions. A = Schwäbische Alb, H = Hainich-Dün, and S = Schorheide-Chorin. Differences at $p < 0.05$ are indicated with bold letters. Data are means of n = 50 plots per region ± SE. Generalized linear model (Poisson) were used for analysis of the count data (OTU richness). ANOVA was used to analyze Michaelis Menten Fit, Shannon and Evenness.

Parameter	Mean ± standard error			p value			
	A	H	S	A-H-S	A-H	A-S	H-S
OTU richness	157 ± 7	201 ± 8	119 ± 3	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Michaelis Menten Fit*	182 ± 9	240 ± 9	135 ± 4	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Shannon (*H'*)	4.23 ± 0.1	4.37 ± 0.1	4.04 ± 0.1	0.072	0.591	0.382	0.058
Evenness (E_H)	0.85 ± 0.0	0.83 ± 0.0	0.85 ± 0.0	0.624	0.760	0.973	0.622

* An additional method to estimate OTU richness in the three regions

4.3.3 Taxonomic and trophic community structures of root-associated fungi

To explore community structure of root associated fungi, we conducted nonmetric multidimentional scaling (NMDS) analyses (Fig. 4.1). Based on the abundance of fungal taxa, the three regions were clearly separated (Fig. 4.1a). This finding was supported by ANOSIM analysis showing significant differentiation of the fungal taxa ($p<0.001$, $R=0.46$, Table 4.4, Fig. 4.1a). We further classified the fungal taxa according to trophic guilds. The differentiation of symbiotroph (SYM), saprotroph (SAP) and pathotroph (PAT) among different regions was less obvious than that based on taxonomy (Fig. 4.1b). Only S was separated from A and H (Fig. 4.1b, Table 4.4). When we tested the community structures of each trophic group separately, we found that SYM and PAT showed similar separation among the three regions as the whole RAF community. SAP differed only between H and S (Table 4.4).

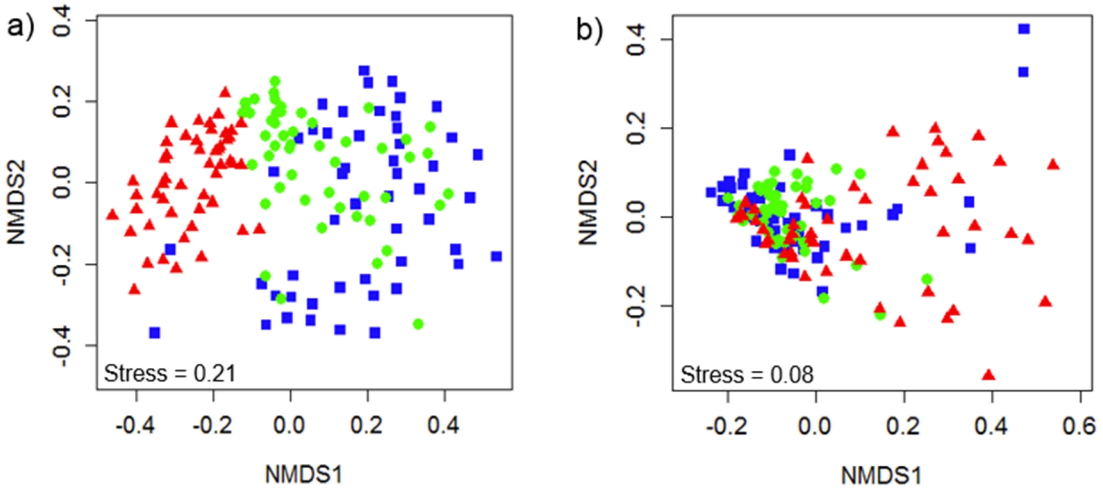

Fig. 4.1 NMDS plots by OTU taxonomic (a) and trophic (b) composition of root associated fungal communities of 150 forest plots in three regions based on Bray-Curtis dissimilarity matrix. A= Schwäbische Alb (blue square), H=Hainich-Dün (green circle), and S=Schorfheide-Chorin (red triangle).

SYM were the largest group among the fungi which could be assigned to a functional guild. The abundance of SYM was lower in S (42% of total reads) than in A (57%) and H 60%) (Fig. 4.2a). About 30% of total reads could not be assigned to any trophic groups (Fig. 4.2a). Saprotrophic (SAP) and pathogenic (PAT) fungi formed the smaller groups (Fig. 4.2a). The mean abundance of trophic groups differed significantly among the three study regions (Appendix Table 4.1). The only exception were pathogens where pairwise comparison of fungal OTU richness

between A and S regions did not show significant differences (p=0.251, Appendix Table 4.1).

Table 4.4 Similarity of root-associated fungal communities according to OTU taxonomic and trophic composition, symbiotroph (SYM), saprotroph (SAP) and pathotroph (PAT) among the three biogeographical regions. A = Schwäbische Alb, H = Hainich-Dun and S = Schorfheide-Chorin.

		OTU taxonomic composition	Trophic composition	SYM	SAP	PAT
p value	All regions	**<0.001**	**<0.001**	**<0.001**	**<0.05**	**<0.001**
	A-H	**<0.001**	0.114	0.131	0.845	0.132
	A-S	**<0.001**	**<0.001**	**<0.01**	0.060	**<0.001**
	H-S	**<0.001**	**<0.001**	**<0.001**	**0.035**	**<0.001**
R value	All regions	0.460	0.111	0.095	0.031	0.211
	A-H	0.166	0.023	0.023	0.003	0.030
	A-S	0.687	0.135	0.093	0.040	0.354
	H-S	0.468	0.187	0.177	0.047	0.246

Data show *p* and R values after ANOSIM based on Bray-Curtis measure. *p* values ≤ 0.05 are shown in bold

Taxonomic composition according to phyla and orders varied among the three study regions (Fig. 4.2b, Appendix Table 4.2). The most dominant phylum in all three regions was that of Basidiomycota, accounting for 77%, 79% and 64% of total reads for A, H and S, respectively (Fig. 4.2b, Appendix Table 4.2). The second common phylum was that of Ascomycota, with 14% of total reads in A, 14% in H and 29% in S (Fig. 4.2b, Appendix Table 4.2). Approximately 5% of the sequences could not be assigned to any fungal order. The remaining fungal reads stemmed from two small phyla, Zygomycota and Chytridiomycota, with less than 0.5% of total reads in each region (Fig. 4.2b, Appendix Table 4.2). Two most abundant orders within Basidiomycota were Agaricales and Russulales, accounting for between 50% and 56% of total fungal reads (Fig. 4.2b). The most abundant fungal order in the phylum of the Ascomycota were Helotiales which made up to 20% of total reads in S, compared to 7% in A and 5% in S region (Fig. 4.2b, Appendix Table 4.2). We found that Agaricales and Russulales forming mycorrhizas were dominant in A, H and in S (Fig. 4.2b, Appendix Table 4.2).

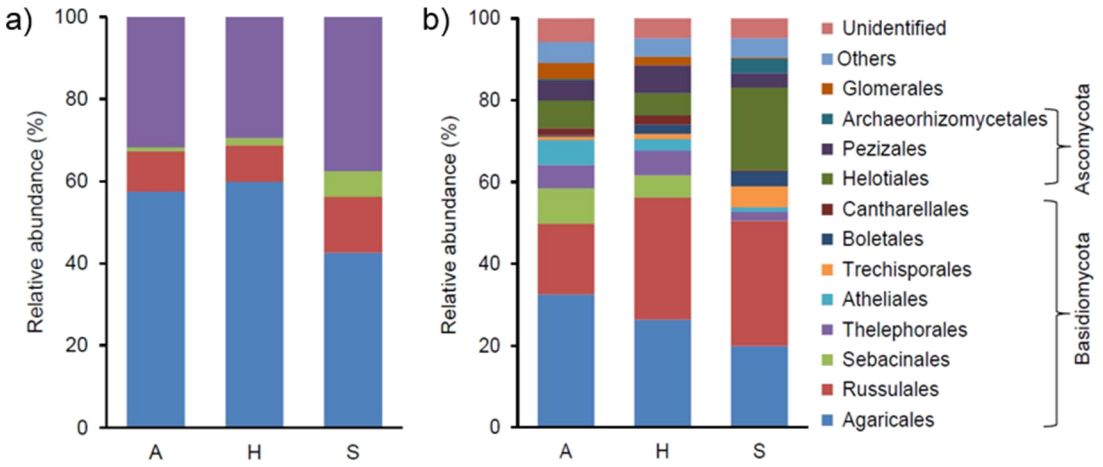

Fig. 4.2 Relative abundance of root-associated fungal communities in three study regions. The abundance of trophic groups (a), blue=symbiotroph, red=saprotroph, green=pathotroph, purple=unknown. The OTU taxonomic orders (b), fungal orders with a relative abundance < 1% were grouped in Others. A=Schwäbische Alb, H = Hainich-Dün, and S = Schorfheide-Chorin.

4.3.4 Drivers for the changes in root-associated fungal communities in three biogeographic regions

We tested whether the abundance of SYM, SAP or PAT were driven by soil- or root-related properties. We found that root NO_3^- was positively correlated with SYM and negatively correlated to SAP and PAT (Fig. 4.3a). In contrast to root NO_3^-, root NH_4^+ was negatively correlated to SYM, but positively correlated with SAP or PAT abundances (Fig. 4.3a). As a result, all of the trophic groups were driven by root NH_4^+ and NO_3^- but in a contrasting manner (Fig. 4.3a). Root glucose was positively correlated to SAP and negatively correlated to PAT (Fig. 4.3a). Soil water content was positively correlated to SYM, but negatively to SAP and not correlated to PAT (Table 4.3a). SYM responded positively to soil NH_4^+ but negatively to soil pH while SAP and PAT showed no responses to the factors (Table 4.3a). Regarding the relative importance of soil and root variables, soil water content and study site were important, explaining over 20% and 30% variances for SYM and PAT respectively (Fig. 4.3b). For SYM and SAP, the proportion of variance explained by root-related variables was higher than by soil-related variables (Fig 4.3b). Therefore, across the whole biogeographic range studied here, carbon and nitrogen in roots were the most important variables in explaining changes in the fungal groups than those elements in soil (Fig. 4.3b). However, within a distinct region these clear relationships were not observed anymore (Appendix Fig. 4.3).

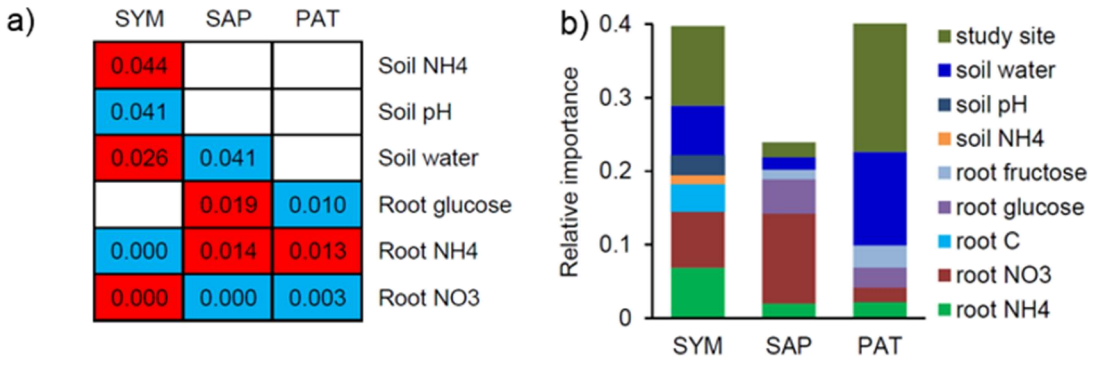

Fig. 4.3 Significant relationships between the abundance of trophic groups and soil or root chemistry (a) and relative importance of abiotic variables to trophic fungal groups (b) in the three biogeographical regions. SYM=symbiotroph, SAP=saprotroph, PAT=pathotroph. Blue colors in (a) indicate negative correlations while red colors stand for positive correlations. P values were shown numerically where significant effects were found.

Since the fungal composition changed among the regions, we wondered whether the divergent behavior within and among the regions might have been caused by different effects of environmental drivers on phylogenetically different fungi. To address this question, we explored the relationships of fungal orders with soil- and root-related factors. Different fungal orders showed specific response patterns to soil and root chemistry, soil pH and soil moisture (Fig. 4.4a). Root NO_3^- was related to seven out of ten fungal orders which contain 76% of the total fungal sequences (Fig. 4.4a). Other important drivers were soil pH and soil water (Fig. 4.4a). Soil pH was significantly related to five fungal orders accounting for 41% of the total sequences while soil water was correlated to four fungal orders with 42% of the total sequences. Study site contributed to the explained variances of all fungal orders, with more than 20% of the variance for each of the Helotiales and Sebacinales (Fig. 4.4b). Soil pH and water content explained a majority of variances of several fungal orders such as Sebacinales (45%), Trechisporales (38%) and Helotiales (30%) (Fig. 4.4b). Root NO_3^- is important for Glomerales, explaining 30.3% of variance for changes of the fungal order (Fig. 4.4b). Other root and soil variables contributed to less than 10% of variance for changes in the fungal orders (Fig. 4.4b).

Furthermore, we compared whether closely related orders are more similarly influenced by soil and root factors (Fig. 4.4a,b). Helotiales and Pezizales, which are from subphylum Pezizomycotina (Hibbett et al. 2007), showed different responses to the environmental factors. Helotiales was much stronger related to soil factors and study site than Pezizales (Fig. 4.4a,b). Glomerales, which is from Glomeromycota,

showed a distinct behavior compared to other orders (Fig 4.4a,b). We did not find the similar response for the rest of 7 fungal orders (Fig. 4.4a,b) which belongs to the same taxa of Agaricomycotina (Hibbett et al. 2007). At a deeper classification level, Agaricales, Atheliales and Boletales, belonging to Agaricomycetidae, showed no similar responses (Fig. 4.4a,b). As a result, we found no phylogenetic relationships in relation to similar responses of root-associated fungi to abiotic factors.

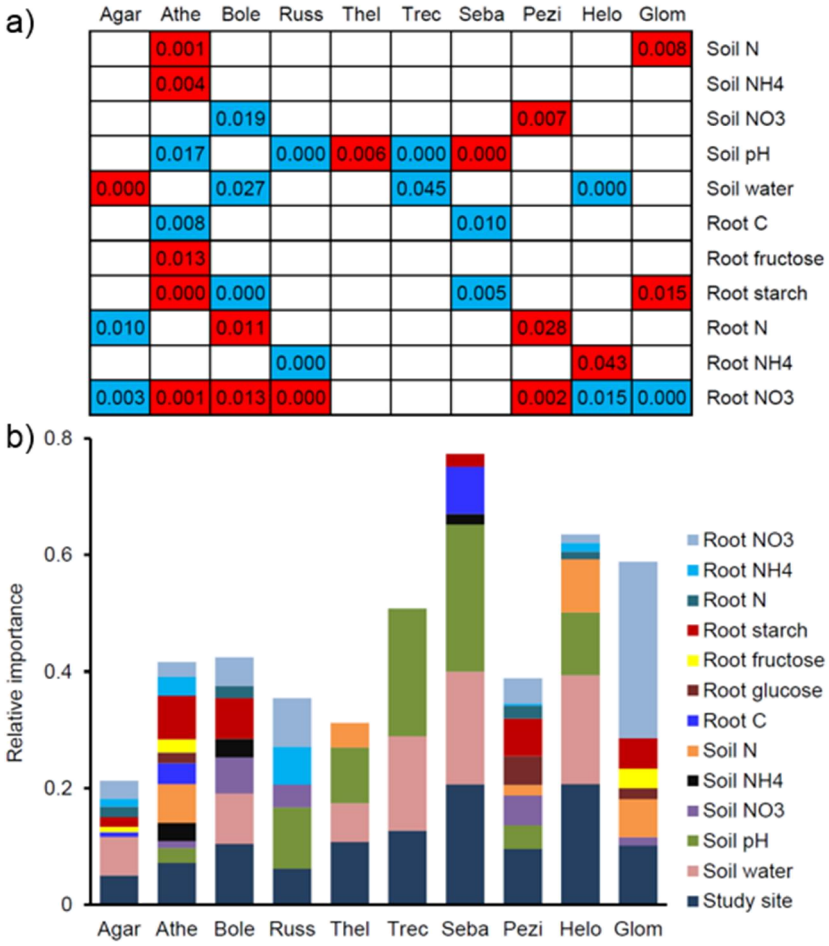

Fig. 4.4 Significant relationships between the abundance of root-associated fungal orders and soil and root chemistry (a) and relative importance of abiotic variables to taxonomic fungal orders (b) in the three study regions. Orders with a relative abundance > 2% were included in the analysis. Blue colors in (a) indicate negative correlations while red colors stand for positive correlations. P values were represented numerically where significant effects were found. Agar=Agaricales, Athe= Atheliales, Bole=Boletales, Russ=Russulales, Thel=Thelephorales, Trec=Trechisporales, Seba=Sebacinales, Pezi= Pezizales, Helo=Helotiales, Glom=Glomerales.

4.4 Discussion

4.4.1 Differentiation in taxonomic and trophic communities of root-associated fungi across the three biogeographical regions

A strong differentiation of RAF communities among the three biogeographical regions (Fig. 4.1, Table 4.4) is in agreement with our hypothesis that differences in soil and root chemistry at each studied region lead to different RAF community assemblages. The taxonomic dissimilarity of RAF communities could be explained by differences in composition of soil fungal communities in the three study regions. This result is supported by previous studies on the same sampling system. It was reported that RAF communities are inherited from soil fungal communities, and that fungal community structures in soil differ significantly across temperate forest ecosystems (Wubet et al. 2012, Goldmann et al. 2015, 2016). Furthermore, the difference in tree species composition among the three study regions. In our sampling plot systems, there are pine plots in S but not in A and H regions whereas spruce plots occur in A and H but not in S region. In our study, difference in composition of trophic groups are less strong than the taxonomic composition, and no difference in the composition of tropic groups between A and H was observed (Fig. 4.1b, Table 4.4). This result suggests that functional resilience of RAF communities can still be achieved by taxonomic divergence between A and H, however, it cannot be obtained between S and the other two biogeographic regions.

We found Basidiomycota being the most abundant fungal phyla in RAF communities following by Ascomycota (Fig. 4.2b, Appendix Table 4.2), although Ascomycota is the largest phylum in the fungal kingdom (James et al. 2006, Beimforde et al. 2014). This result is in agreement with previous studies which investigated root and soil fungal communities in forest ecosystems (Mathiesen and Ohlson 2008, Wubet et al. 2012, McGuire et al. 2013, Goldmann et al. 2015, 2016, de Witte et al. 2017, Philpott et al. 2018). The reason for this is possibly explained by the contribution of ectomycorrhizal communities, which are more dominant members of the Basidiomycota than Ascomycota (Smith and Read 2008), in temperate forests (Goldmann et al. 2015).

Our results showed that there was a significant difference in mean read abundance of trophic groups per plot among three study regions (Appendix Table 4.1), and SYM is the most abundant group compared to SAP and PAT (Fig. 4.2a, Appendix Table 4.1). This is consistent with earlier finding suggesting that the abundance of

ecological guilds differed a cross an elevational gradient in temperate forests (Veach et al. 2017) and the abundance of ectomycorrhizal fungi is dominant compared to pathogenic and endophytic fungi in the roots of two tree species *Salix caprea* and *Betula pendula* (Kolaříková et al. 2017). Similarly, ectomycorrhizal fungi were found to be dominant in our results. This is in agreement with previous observations on mycorrhizas in top soil in the same sampling system, where richness and diversity of ectomycorhizal communities, investigated by DNA sequencing from ectomycorrhizal root tips, is lowest in S compared to the other two study regions (Pena et al. 2017). However, a more recent study in oak, beech and spruce temperate forests reported that saprotrophic fungi were dominant, accounting for 67% of fungal sequences in litter and 53% in soil whereas percentages of ectomycorrhizal fungi were 3% of fungal sequences in litter and 36% in soil (Bahnmann et al. 2018). We suggest that the composition of guilds is determined by habitat as the compositions of trophic groups are different from those of soil and litter. High abundance of SYM in this study may be because of that unlike litter and soil, there are more symbiotic fungi, especially ectomycorrhizal fungi associated with forest tree root systems (Kolaříková et al. 2017).

Furthermore, we found the most abundance of PAT and SAP in S compared to the other two regions (Fig. 4.2a). This is possibly due to the fact that the abundance of dead root tips is significantly different among the three regions, with the highest amount being in S and the lowest in the A plots (Appendix Fig. 4.4). Higher abundance of dead root tips in S are possibly related to environmental factors because soils are more acid and less moist in S than the other regions (Table 4.2). However, we found no relations between soil pH and soil water content and the abundance of death root tips. We suggested that fungal pathogens in roots could play a role in this situation.

4.4.2 Root and soil factors correlated with trophic and taxonomic groups of root-associated fungi

Previous studies reported that C/N ratio is of importance for RAF communities (Maghnia et al. 2017) and soil fungal communities (Wubet et al. 2012, Goldmann et al. 2015). Because of the importance of carbon and nitrogen exchange in RAF-plant interactions, and to further understand the interaction at a biogeographical scale we investigated the relationships of RAF communities with a number of C- and N related variables in both roots and soils.

We found that trophic groups responded differently to root and soil carbon and nitrogen variables (Fig. 4.3a), and this is in line with our expectation. In our study, root NH_4^+ and root NO_3^- were significantly related to SYM, SAP and PAT and contributed to large explained variance for changes in SYM and SAP (Fig. 4.3a, b). Previous studies focused on the relationships of abiotic factors with the whole fungal communities and mycorrhizal fungi. For example, soil pH and C/N ratio were reported to be drivers for both soil and root fungal communities as the whole (Wubet et al. 2012, Maghnia et al. 2017). Other studies in forest ecosystems found negative relationships of nitrogen input and ectomycorrhizal communities (De Witte et al. 2017, Suz et al. 2014, Lilleskov et al. 2002). We showed that carbon and nitrogen in roots were more important than those in soil for the trophic groups (Fig. 4.3a, b). This finding can be explained by control of RAF communities by host trees than by soil chemistry. For example, it is suggested that host plants in temperate forests make RAF communities more stable than soil fungal communities (Goldmann et al. 2016).

Generally, different fungal orders responded distinct patterns to the abiotic variables (Fig. 4.4a) which is consistent with our hypothesis. In our study, root NO_3^-, soil pH and water were important drivers for root-associated fungal orders. (Fig. 4.4a,b). Among those, soil pH has been commonly shown to be a driver for changes in soil and root fungal communities (Suz et al. 2014, Goldmann et al. 2015, Bahnmann et al. 2018). In another study, however, soil pH was significantly related to the whole fungal community, especially in the Pezizomycota and Agaricomycota, and was also correlated to ectomycorrhizal genera such as Inocybe, Phialophora and Sebacina (Wubet et al. 2012). In the present study, in general, the different relationship patterns of fungal order can be explained by the preference of different orders to different environmental conditions. The order Agaciales, which was positively correlated to soil water and negatively correlated to root NO_3^- (Fig. 4.4a), tends to reside under the conditions of higher moisture and lower concentration of root NO_3^- in A than in H and S plots (Appendix Table 4.2). Similarly, Russulales, negatively correlated with soil pH and positively correlated to root NO_3^- (Fig. 4.4a), prefers to reside under lower pH and higher root NO_3^- in S than the other two regions (Appendix Table 4.2). Helotiales, the most abundant order of Ascomycota in our study and negatively correlated to soil water, has more sequence abundance in drier S region than H and A (Fig. 4.4a). This order had more abundance in S region although it was negatively related to root NO_3^-. Root NO_3^- could be of minor importance to Helotiales because root NO_3^- only explained 1.4% variance for

changes in Helotiales communities (Fig. 4.4b). The different proportion of trophic groups in the same order cannot explain the difference in response patterns of fungal orders to abiotic variables. In our study, Russulales (99.9% SYM sequences) and Boletales (88% SYM, 0.4% SAP and 0% PAT, 11.8 UNK), had more abundance in S than in A region. In contrast, Glomerales (100% SYM) and Sebacinales (82.8% SYM, 17.2 UNK sequences) occurred more in A than in S and H, this trend is also true for other orders of Thelephorales and Atheliales. For orders containing both SYM and SAP, Helotiales (16.9% SYM, 17.1% SAP, 2.2% PAT, 63.7% UNK) had more sequence abundance in S than in A and H region; however, Pezizales (54.7% SYM, 36.2% SAP, 0% PAT and 9.1% UNK) was more abundant in A and H than in S regions. Overall, the results indicated that soil pH, soil moisture and root NO_3^- were the most important drivers for the changes in root fungal orders, and that phylogenetically different fungi responded differently to abiotic factors in roots and soils.

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CHAPTER 5

CONCLUSION AND OUTLOOK

5.1 Conclusion

This study was carried out to explore environmental drivers and functions of root-associated fungal communities in relation to N in temperate forests. Greenhouse and field research approaches were used. A greenhouse experiment was conducted in order to know whether the diversity of EMF and beech provenance affect beech nitrogen and performance. To further understand the role of EMF in beech N supply at molecular level, root samples from a field beech transplantation experiment were harvested and used to study beech and fungal transcriptomes. To investigate the environmental drivers of RAF, another field approach was undertaken to understand whether the RAF community composition in temperate forests changes among biogeographical regions, and to explore the relationships between the abundance of taxonomic and trophic groups of RAF and nitrogen-related and other important abiotic variables across temperate forests.

Comparing the performance of beech provenances between two soil treatments indicated that the provenances from three biogeographical regions showed no difference in long-term N nutrition and short-term ^{15}N uptake. Hainich-Dün provenance performed better in original Hainich-Dün forest soil than Schorfheide-Chorin and Schwäbische Alb provenances. The result indicates that beech intra-specific diversity has no effects on beech N nutrition and that soil fungal communities, which were adapted locally to Hanich-Dün soil, may play a role in improved performance of Hainich-Dün provenance. Beech plants in original forest soil, which were colonized with higher ectomycorrhizal diversity, showed better performance and improved nitrogen nutrition than those in γ-irradiated soil regardless of tested beech provenance. This finding suggests that EMF may control beech growth and nitrogen supply over two years of experiment (Chapter 2), although the effects of increased EMF diversity on short-term ^{15}N uptake were not detected. The tested beech provenances exhibited a preference for NO_3^- over NH_4^+.

Root transcriptome analysis indicates that pattern of transcript abundance of the beech roots samples from Schorfheide-Chorin was different from those from Hainich-Dün and Schwäbische Alb. The majority of differentially expressed genes and enriched gene ontology terms were found between Hainich-Dün and Schorfheide-Chorin. Beech genes (111) and ectomycorrhizal genes (134) were detected to be related to N uptake and assimilation. Nitrogen-related beech genes were detected to be involved in NO_3^- transport and assimilation, whereas the fungal genes were found to be related to NH_4^+ assimilation. These results are in line with

those of chapter 2, suggesting that differences in the molecular regulation of N-uptake lead to segregation of N utilization und support complementary resource use.

The analysis of RAF and their relationships with N-related variables and other environmental variables indicated that biogeographical regions affected the RAF community composition. Stronger effects were observed for the taxonomic fungal community compositions than for the trophic fungal community compositions. Root NO_3^- and NH_4^+ were drivers for changes in the abundance of trophic fungal community composition, and root N and C were more important for the changes in the abundance of the trophic communities than those elements in soil. The results suggest the stronger control of trophic fungal communities by the host trees than soil chemistry. Furthermore, the differences in response pattern of root-associated fungal orders to environmental variables indicate no genetically determined relationships of RAF communities to root and soil N.

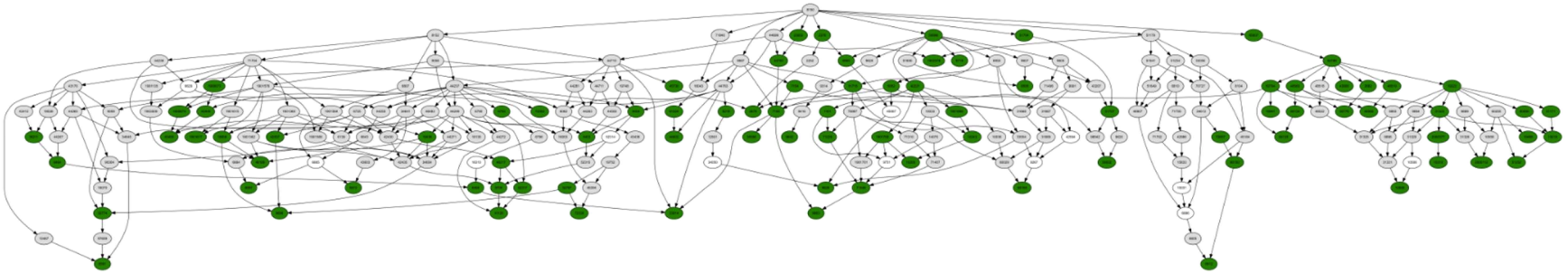
Taken key results from the three main chapters into consideration, comparing the N uptake by beech of the two N forms indicates that more NO_3- was taken up by beech than NH_4^+ (Chapter 2). A question was whether the higher uptake of NO_3^- than NH_4^+ by beech is contributed by beech or EMF or by both beech and EMF. Transcriptome analysis showed that beech genes were involved in NO_3^- uptake and assimilation whereas fungal genes were related to NH_4^+ assimilation (Chapter 3). There were no nitrate transporters were detected in fungal transcriptome (Chapter 3). The results suggest that beech might be mainly responsible for NO_3^- uptake itself, and EMF assist beech in additional NH_4^+ uptake and assimilation. A majority of root-associated fungal orders in our field data analysis were correlated with root NO_3^-. Among them, root NO_3^- was negatively related to Agaricales and positively related to Pezizales (Chapter 4). These results support the findings regarding improved beech performance in O soil compared to T soil (Chapter 2). In fact, *Tuber rufum*, which belongs to Pezizales and was present in O soil but not in T soil, might play a role in improved beech performance. The presence of *Tuber rufum* in O soil may indirectly improve beech $^{15}NO_3^-$ uptake by accelerating glutamate metabolism. High abundance of *Hebeloma incarnatulum* which belongs to Agaricales in T soil may constrain the beech performance because Agaricales was negatively related to root NO_3^- (Chapter 4). Furthermore, because there was no correlation between root NO_3^- and Thelephorales, *Tomentella sublilacina* (belonging to Thelephorales), which occurred in both soil types, may have little contribution to beech performance (Chapter 2). Due to low abundance of Mytilinidiales (8 reads) to which *Cenococum geophilum* belongs, correlation between Mytilinidiales and abiotic variables were not

made. Root NH_4^+ was related with fewer root-associated fungal orders and less important than root NO_3^- (Chapter 4). Overall, NO_3^- was important for changes in RAF communities, beech nutrition and performance. The results also suggest the important role of specific EMF species in beech N supply and performance.

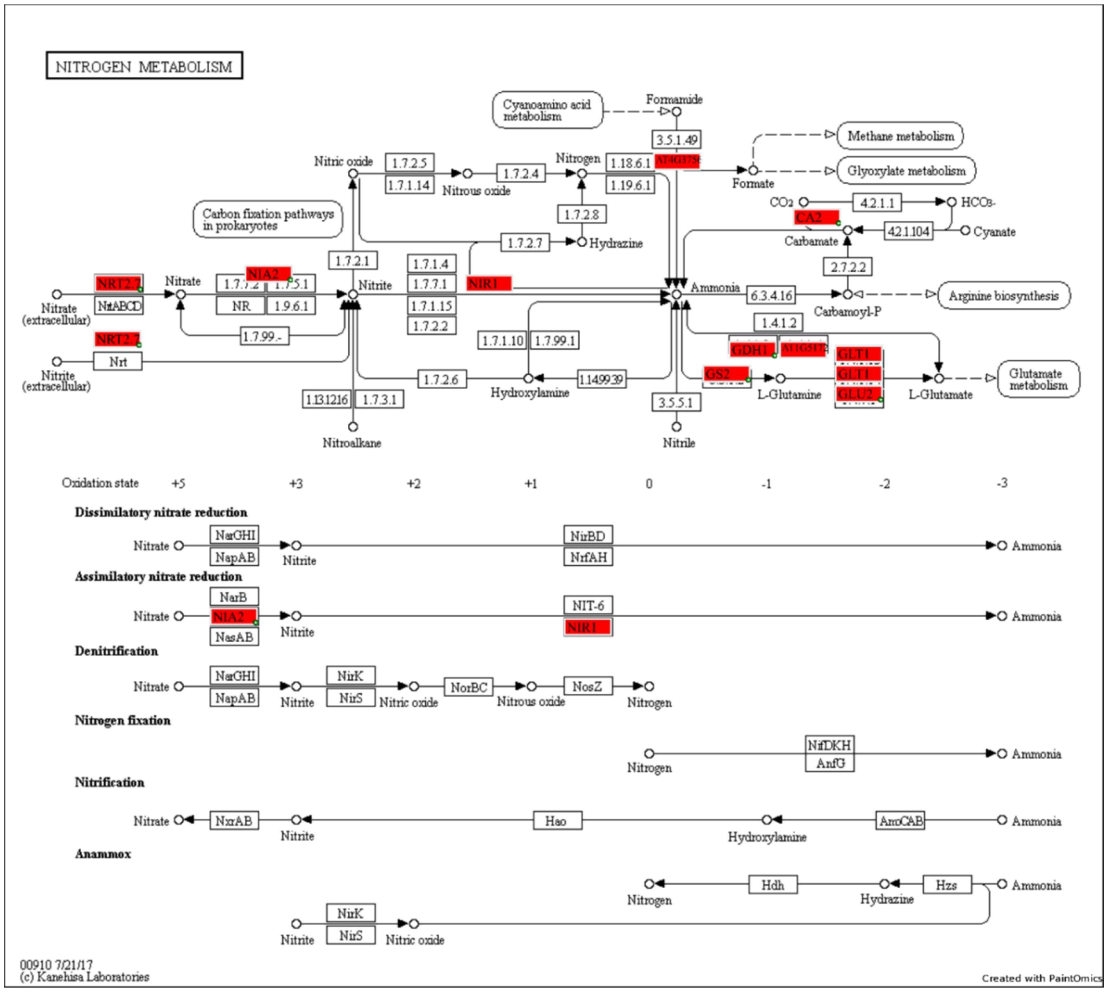
5.2 Outlook

The improved biomass production was observed for Hanich-Dün beech provenance in O soil supporting the role of adapted soil microbial communities. However, conclusion regarding effects of local microbial communities on beech growth need to be confirmed by further testing provenances in different soil origins in future studies. Moreover, specific EMF species were suggested to play an important role in beech N supply; therefore, future research should address taxon-specific N uptake to understand better beech nitrogen. Because metatranscriptomic approach is a snapshot of the gene expression at a specific time point, temporal root sampling should be required to obtain further understanding about the molecular mechanism of beech-EMF interaction over time. In this study, the present approach was taken based on mixed root samples from multiple plant species in temperate forests. Separation of roots according to plant species should be conducted and used for sequencing to obtain insights into relationships of environmental variables and RAF communities of individual tree species. Furthermore, root sampling for research on RAF community composition and relationships between RAF and abiotic variables was collected at one time point. Seasonal sampling should, therefore, be conducted to obtain knowledge about year-round dynamics of RAF communities. It is also of great interest to explore RAF communities at different soil depths.

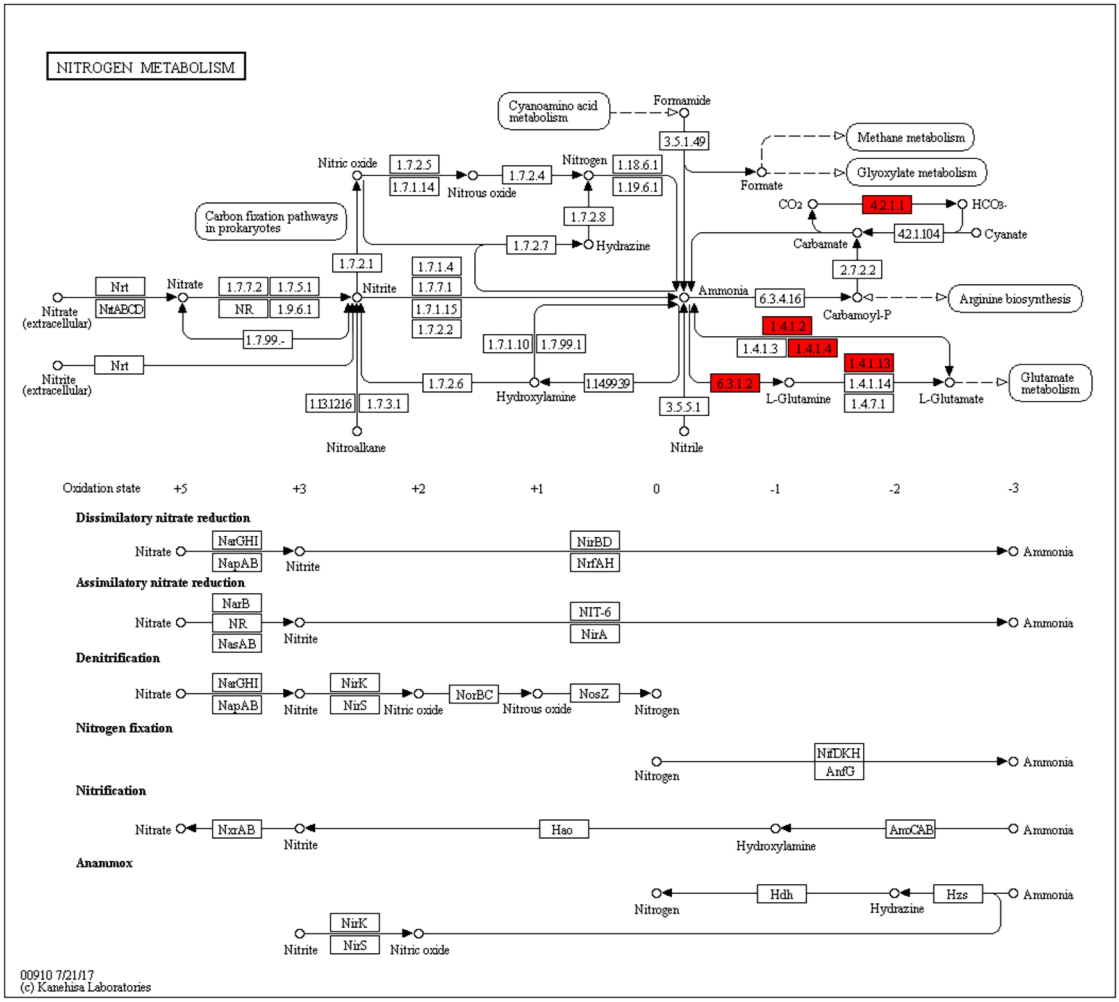
APPENDICES



Appendix Fig. 3.1 Hierarchy of enriched GO terms of DEGs between H and S regions. Enriched GO terms were coded green, while white color indicate GO terms which were not enriched.



Appendix Fig. 3.2 Nitrogen metabolism of beech. Red colours indicate beech genes involved in nitrogen metabolism. The result was obtained from Paintomics 3 using list of genes from the beech transcriptome database in GenBank (ID number HADB00000000.1) according to AGI ID as input data.



Appendix Fig. 3.3 Nitrogen metabolism of *Laccaria bicolor*. Red colour indicates fungal genes involved in nitrogen metabolism. The result was obtained from KEGG mapper-Search&Color Pathway using EC number from the fungal transcriptome as input data

Appendix Table 3.1 Field experimental plots where beech nuts were collected, the seedlings were planted out, harvested and used for RNA Seq

Study region	Plot	Collection plot	Planting plot	Harvest plot	Plot for RNA Seq
Schwäbische Alb	5	x	x	x	x
	6		x	x	x
	7	x	x		
	8	x			
	9	x	x		
	29		x	x	
	39		x	x	x
	41		x		
	42		x	x	x
	50		x		
Hainich-Dün	5		x	x	x
	6	x			
	8		x		
	10	x			
	11	x	x		
	12	x	x	x	x
	16		x	x	x
	21		x	x	x
	26		x		
	27		x		
	29				
	35		x		
Schorfheide-Chorin	5	x	x		
	6	x			
	9	x	x		
	31		x		
	34		x	x	x
	35		x	x	x
	37		x	x	x
	38		x	x	x
	46	x	x		
	49		x	x	

Appendix Table 3.2 A list of ectomycorrjizal fungi which is associated with beech and commonly observed in temperate forests for mapping fungal transcriptomic data

	Ectomycorrhiza	Abbreviation	Information contributor*
1	Boletus edulis	Boled	Andrea Polle
2	Paxillus involutus	Paxin	Andrea Polle
3	Rhizopogon vinicolor	Rhivi	Andrea Polle
4	Scleroderma citrinum	Sclci	Andrea Polle
5	Suillus brevipes	Suibr	Andrea Polle
6	Suillus luteus	Suilu	Andrea Polle
7	Amanita muscaria	Amamu	Kristina Schröter
8	Clavulina sp	ClaPMI390	Kristina Schröter
9	Laccaria amethystina	Lacam	Kristina Schröter
10	Laccaria bicolor	Lacbi	Kristina Schröter
11	Lactifluus volemus	Lacvol	Kristina Schröter
12	Russula brevipes	Rusbre	Kristina Schröter
13	Russula dissimulans	Rusdis	Kristina Schröter
14	Russula rugulosa	Rusrug	Kristina Schröter
15	Sebacina vermifera	Sebve	Kristina Schröter
16	Acephala macrosclerotiorum	Acema	Silke Ammerschubert
17	Cenococcum geophilum	Cenge	Silke Ammerschubert
18	Hebeloma cylindrosporum	Hebcy	Silke Ammerschubert
19	Hymenoscyphus varicosporoides	Hymva	Silke Ammerschubert
20	Lactarius quietus	Lacqui	Silke Ammerschubert
21	Mortierella elongata	Morel	Silke Ammerschubert
22	Phialocephala scopiformis	Phisc	Silke Ammerschubert
23	Piloderma croceum	Pilcr	Silke Ammerschubert
24	Tricholoma matsutake	Trima	Silke Ammerschubert
25	Tuber borchii	Tubbor	Silke Ammerschubert
26	Wilcoxina mikolae	Wilmi	Silke Ammerschubert
27	Xerocomus badius	Xerba	Silke Ammerschubert

* The person who contributed information about fungal species which are associated with beech in 150 forest plots in the Biodiversity Exploraories

Appendix Table 3.3 N-related beech "genes" which belong to GO:0010243 and GO:0051171

GO:0010243 (64 genes)
fasyl_0039072, fasyl_0209646, fasyl_0315645, fasyl_0335115, fasyl_0405009, fasyl_0506550, fasyl_0654202, fasyl_0656465, fasyl_0696282, fasyl_0750520, fasyl_0905213, fasyl_0952951, fasyl_0972042, fasyl_0975780, fasyl_0981716, fasyl_1191726, fasyl_1227766, fasyl_1261849, fasyl_1387574, fasyl_1529761, fasyl_1566326, fasyl_1602866, fasyl_1604473, fasyl_1740886, fasyl_1795448, fasyl_1812836, fasyl_1814668, fasyl_1818317, fasyl_1847386, fasyl_1883823, fasyl_1886503, fasyl_1950264, fasyl_1965879, fasyl_1968305, fasyl_2025207, fasyl_2084470, fasyl_2167877, fasyl_2192216, fasyl_2294623, fasyl_2400233, fasyl_2440069, fasyl_2458112, fasyl_2482184, fasyl_2494511, fasyl_2508042, fasyl_2521052, fasyl_2530495, fasyl_2558898, fasyl_2561234, fasyl_2561287, fasyl_2561852, fasyl_2562295, fasyl_2564152, fasyl_2564585, fasyl_2567425, fasyl_2568542, fasyl_2568910, fasyl_2569308, fasyl_2569882, fasyl_2569888, fasyl_2570184, fasyl_2571491, fasyl_2572571, fasyl_2572860
GO: 0051171 (65 genes)
fasyl_0000917, fasyl_0039072, fasyl_0056283, fasyl_0133768, fasyl_0209646, fasyl_0269187, fasyl_0315645, fasyl_0387823, fasyl_0471418, fasyl_0531870, fasyl_0546407, fasyl_0551049, fasyl_0592673, fasyl_0632798, fasyl_0654202, fasyl_0656465, fasyl_0696282, fasyl_0750520, fasyl_0881101, fasyl_0973606, fasyl_0979330, fasyl_1218425, fasyl_1227766, fasyl_1397641, fasyl_1470509, fasyl_1522087, fasyl_1529761, fasyl_1559642, fasyl_1686239, fasyl_1693434, fasyl_1731194, fasyl_1780685, fasyl_1795448, fasyl_1812198, fasyl_1812836, fasyl_1816289, fasyl_1818317, fasyl_1856028, fasyl_1867532, fasyl_1895060, fasyl_2004906, fasyl_2117386, fasyl_2137253, fasyl_2192216, fasyl_2294623, fasyl_2375059, fasyl_2428951, fasyl_2440069, fasyl_2458112, fasyl_2482184, fasyl_2500953, fasyl_2508042, fasyl_2558274, fasyl_2558898, fasyl_2561852, fasyl_2562745, fasyl_2564152, fasyl_2564836, fasyl_2567425, fasyl_2567951, fasyl_2569308, fasyl_2569888, fasyl_2569927, fasyl_2570184, fasyl_2572571

Appendix Table 3.4 N-related genes in the beech transcriptome. These genes obtained from mapping the transcriptome against N metabolism and from doing keyword search for nitrate and ammonium transporters using the beech transcriptome. Fasyl: Fagus sylvatica, H: Hainich Dün; S: Schorfheide-Chorin, A: Schwäbische Alb, Yellow colour: genes involved in N metabolism, Log2fc: log2 fold change

	Fasyl ID	Gene name	AGI ID	H-S		H-A		S-A		Function
				Log2fc	p value	Log2fc	p value	Log2fc	p value	
1	fasyl_1621113	ACA1	AT3G52720	-0.817	1.000	-0.745	1.000	0.073	1.000	alpha carbonic anhydrase 1
2	fasyl_2250854	ACA4	AT4G20990	-0.237	1.000	-0.183	1.000	0.054	1.000	alpha carbonic anhydrase 4
3	fasyl_1776839	ACA7	AT1G08080	-0.284	1.000	-0.446	1.000	-0.161	1.000	alpha carbonic anhydrase 7
4	fasyl_0593659	ACA7	AT1G08080	0.032	0.983	0.519	1.000	0.487	0.678	alpha carbonic anhydrase 7
5	fasyl_0569751	ACA7	AT1G08080	-0.351	0.789	0.096	1.000	0.448	0.781	alpha carbonic anhydrase 7
6	fasyl_2569622	AT1G51720	AT1G51720	-0.003	0.998	-0.117	1.000	-0.115	0.912	Amino acid dehydrogenase family protein
7	fasyl_2566762	AT1G51720	AT1G51720	0.261	0.771	0.134	1.000	-0.127	0.935	Amino acid dehydrogenase family protein
8	fasyl_1774376	AT1G51720	AT1G51720	0.025	0.981	0.339	1.000	0.314	0.698	Amino acid dehydrogenase family protein
9	fasyl_1271906	AT1G51720	AT1G51720	0.156	1.000	-0.376	1.000	-0.532	1.000	Amino acid dehydrogenase family protein
10	fasyl_0129825	AT1G51720	AT1G51720	-0.590	1.000	0.181	1.000	0.771	1.000	Amino acid dehydrogenase family protein
11	fasyl_1015882	AT1G51720	AT1G51720	0.021	1.000	0.245	1.000	0.223	1.000	Amino acid dehydrogenase family protein
12	fasyl_2224379	AT4G37560	AT4G37560	0.171	0.897	-0.222	1.000	-0.393	0.769	Acetamidase/Formamidase family protein
13	fasyl_1685580	AT4G37560	AT4G37560	0.131	0.937	-0.027	1.000	-0.158	0.940	Acetamidase/Formamidase family protein
14	fasyl_0614143	AT4G37560	AT4G37560	0.060	0.968	-0.149	1.000	-0.210	0.889	Acetamidase/Formamidase family protein
15	fasyl_1320821	BCA5	AT4G33580	0.058	1.000	-0.139	1.000	-0.196	1.000	beta carbonic anhydrase 5
16	fasyl_0239657	BCA5	AT4G33580	-0.495	0.612	-0.509	1.000	-0.015	0.994	beta carbonic anhydrase 5
17	fasyl_2155958	BCA5	AT4G33580	-0.638	0.410	-0.409	1.000	0.229	0.890	beta carbonic anhydrase 5
18	fasyl_2558280	BCA5	AT4G33580	0.540	0.480	0.368	1.000	-0.172	0.917	beta carbonic anhydrase 5
19	fasyl_0028039	BCA5	AT4G33580	0.738	0.345	0.606	1.000	-0.132	1.000	beta carbonic anhydrase 5
20	fasyl_1394408	CA1	AT3G01500	-0.812	1.000	-0.558	1.000	0.254	1.000	carbonic anhydrase 1
21	fasyl_1886503	CA2	AT5G14740	-0.804	0.037	-0.364	1.000	0.440	0.579	carbonic anhydrase 2
22	fasyl_2568912	CA2	AT5G14740	-0.128	0.933	0.405	1.000	0.533	0.656	carbonic anhydrase 2
23	fasyl_0144715	CA2	AT5G14740	-0.083	0.961	0.476	1.000	0.559	0.648	carbonic anhydrase 2
24	fasyl_0138595	CA2	AT5G14740	-0.720	0.164	-0.332	1.000	0.387	0.709	carbonic anhydrase 2
25	fasyl_0119002	CA2	AT5G14740	0.272	0.811	0.599	1.000	0.327	0.821	carbonic anhydrase 2
26	fasyl_2430631	GDH1	AT5G18170	-0.064	0.961	-0.134	1.000	-0.071	0.968	glutamate dehydrogenase 1
27	fasyl_2279097	GDH1	AT5G18170	0.030	1.000	0.191	1.000	0.161	1.000	glutamate dehydrogenase 1
28	fasyl_1182924	GDH1	AT5G18170	0.189	1.000	0.696	1.000	0.506	1.000	glutamate dehydrogenase 1
29	fasyl_2559332	GDH1	AT5G18170	-0.322	0.735	-0.223	1.000	0.099	0.956	glutamate dehydrogenase 1

30	fasyl_2028990	GDH1	AT5G18170	-0.456	0.528	-0.045	1.000	0.410	0.691	glutamate dehydrogenase 1
31	fasyl_0450109	GDH2	AT5G07440	0.391	1.000	0.099	1.000	-0.292	1.000	glutamate dehydrogenase 2
32	fasyl_1384250	GDH2	AT5G07440	1.181	0.032	0.516	1.000	-0.665	0.562	glutamate dehydrogenase 2
33	fasyl_2567469	GDH2	AT5G07440	0.309	0.778	0.425	1.000	0.117	0.952	glutamate dehydrogenase 2
34	fasyl_2308625	GDH2	AT5G07440	0.879	0.204	0.258	1.000	-0.622	0.610	glutamate dehydrogenase 2
35	fasyl_0284218	GDH2	AT5G07440	0.334	0.755	0.554	1.000	0.220	0.894	glutamate dehydrogenase 2
36	fasyl_2115554	GDH2	AT5G07440	1.277	0.011	0.569	1.000	-0.708	0.493	glutamate dehydrogenase 2
37	fasyl_1488290	GDH2	AT5G07440	0.832	0.234	0.437	1.000	-0.394	0.793	glutamate dehydrogenase 2
38	fasyl_0976755	GDH2	AT5G07440	-0.029	0.988	0.236	1.000	0.265	1.000	glutamate dehydrogenase 2
39	fasyl_0391853	GLN1.3	AT3G17820	0.143	1.000	0.295	1.000	0.152	1.000	glutamine synthetase 1.3
40	fasyl_1319886	GLT1	AT5G53460	-0.323	1.000	-0.173	1.000	0.151	1.000	NADH-dependent glutamate synthase 1
41	fasyl_2291512	GLT1	AT5G53460	-0.453	1.000	-0.221	1.000	0.232	1.000	NADH-dependent glutamate synthase 1
42	fasyl_0792729	GLT1	AT5G53460	-0.489	0.429	-0.174	1.000	0.314	0.774	NADH-dependent glutamate synthase 1
43	fasyl_1680520	GLT1	AT5G53460	-0.406	0.621	-0.502	1.000	-0.096	0.956	NADH-dependent glutamate synthase 1
44	fasyl_1169052	GLT1	AT5G53460	0.211	1.000	0.012	1.000	-0.200	1.000	NADH-dependent glutamate synthase 1
45	fasyl_0160678	GLT1	AT5G53460	-0.539	0.384	-0.204	1.000	0.335	0.763	NADH-dependent glutamate synthase 1
46	fasyl_1058148	GLT1	AT5G53460	-0.278	1.000	-0.358	1.000	-0.080	1.000	NADH-dependent glutamate synthase 1
47	fasyl_0076001	GLT1	AT5G53460	-0.688	0.150	-0.215	1.000	0.473	0.585	NADH-dependent glutamate synthase 1
48	fasyl_0076000	GLT1	AT5G53460	-0.488	1.000	-0.159	1.000	0.328	1.000	NADH-dependent glutamate synthase 1
49	fasyl_1897902	GLU1	AT5G04140	-0.082	0.963	0.091	1.000	0.173	1.000	glutamate synthase 1
50	fasyl_2567974	GLU1	AT5G04140	0.078	0.953	-0.096	1.000	-0.174	0.901	glutamate synthase 1
51	fasyl_2567363	GLU1	AT5G04140	0.188	0.868	-0.050	1.000	-0.239	0.863	glutamate synthase 1
52	fasyl_1169208	GLU1	AT5G04140	-0.060	0.958	-0.166	1.000	-0.106	0.936	glutamate synthase 1
53	fasyl_1622159	GLU1	AT5G04140	-0.004	0.998	-0.256	1.000	-0.251	0.826	glutamate synthase 1
54	fasyl_1460472	GLU1	AT5G04140	0.018	0.987	-0.119	1.000	-0.137	0.905	glutamate synthase 1
55	fasyl_0603322	GLU2	AT2G41220	0.041	0.979	-0.049	1.000	-0.089	0.962	glutamate synthase 2
56	fasyl_0700968	GS2	AT5G35630	-0.486	1.000	0.166	1.000	0.652	1.000	glutamine synthetase 2
57	fasyl_0170119	GS2	AT5G35630	-0.206	1.000	0.035	1.000	0.241	1.000	glutamine synthetase 2
58	fasyl_0952505	GSR_1	AT5G37600	0.207	0.851	-0.081	1.000	-0.287	0.825	glutamine synthase clone R1
59	fasyl_2340284	GSR_1	AT5G37600	-0.194	1.000	0.076	1.000	0.270	1.000	glutamine synthase clone R1
60	fasyl_1832704	GSR_1	AT5G37600	-0.461	0.639	0.100	1.000	0.561	0.645	glutamine synthase clone R1
61	fasyl_1163541	GSR_1	AT5G37600	0.538	1.000	0.170	1.000	-0.369	1.000	glutamine synthase clone R1
62	fasyl_1114376	GSR_1	AT5G37600	0.298	1.000	0.303	1.000	0.000	1.000	glutamine synthase clone R1
63	fasyl_0614595	GSR_1	AT5G37600	0.109	1.000	-0.239	1.000	-0.348	1.000	glutamine synthase clone R1
64	fasyl_2546236	GSR_1	AT5G37600	-0.925	1.000	-0.119	1.000	0.807	1.000	glutamine synthase clone R1
65	fasyl_0609862	NIA1	AT1G77760	-0.229	0.885	0.093	1.000	0.321	0.864	nitrate reductase 1
66	fasyl_2169193	NIA2	AT1G37130	-0.480	0.680	0.219	1.000	0.699	0.589	nitrate reductase 2
67	fasyl_1594841	NIA2	AT1G37130	-0.358	0.793	0.258	1.000	0.616	0.664	nitrate reductase 2
68	fasyl_0948436	NIR1	AT2G15620	0.335	0.790	0.459	1.000	0.124	0.955	nitrite reductase 1

69	fasyl_0829064	NIR1	AT2G15620	-0.100	1.000	-0.005	1.000	0.095	1.000	nitrite reductase 1
70	fasyl_0819600	NIR1	AT2G15620	0.275	0.856	0.276	1.000	0.001	1.000	nitrite reductase 1
71	fasyl_0075946	NIR1	AT2G15620	0.560	0.640	0.385	1.000	-0.175	1.000	nitrite reductase 1
72	fasyl_0845329	NRT2.5	AT1G12940	0.467	1.000	0.354	1.000	-0.113	1.000	nitrate transporter2.5
73	fasyl_0321329	NRT2.5	AT1G12940	0.260	1.000	0.133	1.000	-0.127	1.000	nitrate transporter2.5
74	fasyl_0688632	NRT2.5	AT1G12940	0.273	0.829	0.402	1.000	0.129	0.951	nitrate transporter2.5
75	fasyl_0554271	NRT2.5	AT1G12940	0.330	1.000	-0.253	1.000	-0.583	1.000	nitrate transporter2.5
76	fasyl_2449251	NRT2.5	AT1G12940	0.413	0.699	0.365	1.000	-0.048	0.984	nitrate transporter2.5
77	fasyl_1283739	NRT2.7	AT5G14570	1.175	1.000	0.447	1.000	-0.728	1.000	high affinity nitrate transporter 2.7
78	fasyl_2528693	NRT2.7	AT5G14570	-0.831	1.000	0.215	1.000	1.046	1.000	high affinity nitrate transporter 2.7
79	fasyl_0036908	NRT2.7	AT5G14570	0.104	1.000	0.379	1.000	0.275	1.000	high affinity nitrate transporter 2.7
80	fasyl_0027662	NRT2.7	AT5G14570	0.263	1.000	0.607	1.000	0.344	1.000	high affinity nitrate transporter 2.7
81	fasyl_0092364	NRT1.1	AT1G12110	-0.104	0.952	-0.200	1.000	-0.096	0.968	nitrate transporter 1.1
82	fasyl_2558839	NRT1.1	AT1G12110	-0.235	0.864	-0.131	1.000	0.104	0.963	nitrate transporter 1.1
83	fasyl_0078523	NRT1.5	AT1G32450	0.997	1.000	0.800	1.000	-0.197	1.000	nitrate transporter 1.5
84	fasyl_0117415	NRT1.5	AT1G32450	-0.291	0.852	-0.627	1.000	-0.335	0.864	nitrate transporter 1.5
85	fasyl_0760882	NRT1.5	AT1G32450	-0.351	0.807	-0.517	1.000	-0.166	0.943	nitrate transporter 1.5
86	fasyl_1458839	NRT1.5	AT1G32450	-0.345	0.812	-0.476	1.000	-0.131	0.957	nitrate transporter 1.5
87	fasyl_1463770	NRT1.5	AT1G32450	0.353	1.000	0.688	1.000	0.335	1.000	nitrate transporter 1.5
88	fasyl_2418771	NRT1.5	AT1G32450	-0.884	1.000	0.005	1.000	0.889	1.000	nitrate transporter 1.5
89	fasyl_2564957	NRT1.5	AT1G32450	1.423	0.029	1.096	1.000	-0.327	0.867	nitrate transporter 1.5
90	fasyl_0224199	NRT1.7	AT1G69870	-0.861	1.000	-0.300	1.000	0.562	1.000	nitrate transporter 1.7
91	fasyl_2042055	NRT1.7	AT1G69870	-1.051	1.000	0.208	1.000	1.259	1.000	nitrate transporter 1.7
92	fasyl_2273703	NRT1.7	AT1G69870	-0.842	1.000	-0.061	1.000	0.780	1.000	nitrate transporter 1.7
93	fasyl_2568183	NRT1.7	AT1G69870	-1.449	0.003	0.376	1.000	1.826	0.000	nitrate transporter 1.7
94	fasyl_0281961	NRT1:2	AT1G69850	0.570	1.000	0.383	1.000	-0.186	1.000	nitrate transporter 1:2
95	fasyl_0662237	NRT1:2	AT1G69850	-0.264	0.844	0.129	1.000	0.393	1.000	nitrate transporter 1:2
96	fasyl_1230789	NRT1:2	AT1G69850	-0.021	0.991	-0.483	1.000	-0.462	1.000	nitrate transporter 1:2
97	fasyl_1235428	NRT1:2	AT1G69850	-0.117	1.000	0.050	1.000	0.167	1.000	nitrate transporter 1:2
98	fasyl_1316850	NRT1:2	AT1G69850	0.104	0.945	0.118	1.000	0.014	0.993	nitrate transporter 1:2
99	fasyl_1342999	NRT1:2	AT1G69850	-0.153	0.896	-0.456	1.000	-0.303	0.803	nitrate transporter 1:2
100	fasyl_2125755	NRT1:2	AT1G69850	-0.797	1.000	-0.027	1.000	0.770	1.000	nitrate transporter 1:2
101	fasyl_2558101	NRT1:2	AT1G69850	-0.367	1.000	-0.145	1.000	0.222	1.000	nitrate transporter 1:2
102	fasyl_0129718	AMT1;1	AT4G13510	0.263	0.856	0.290	1.000	0.027	1.000	ammonium transporter 1;1
103	fasyl_0375136	AMT1;1	AT4G13510	-0.249	1.000	-0.185	1.000	0.064	1.000	ammonium transporter 1;1
104	fasyl_1065322	AMT1;1	AT4G13510	-0.132	1.000	-0.744	1.000	-0.612	1.000	ammonium transporter 1;1
105	fasyl_1834562	AMT1;1	AT4G13510	0.036	1.000	-0.250	1.000	-0.286	1.000	ammonium transporter 1;1
106	fasyl_2569115	AMT1;1	AT4G13510	-0.263	0.698	-0.228	1.000	0.035	0.982	ammonium transporter 1;1
107	fasyl_0341208	AMT2	AT2G38290	-0.338	0.704	-0.481	1.000	-0.143	0.928	ammonium transporter 2

108	fasyl_1489616	AMT2	AT2G38290	0.333	1.000	0.102	1.000	-0.231	1.000	ammonium transporter 2
109	fasyl_1594973	AMT2	AT2G38290	0.413	0.723	0.304	1.000	-0.110	0.962	ammonium transporter 2
110	fasyl_1678058	AMT2	AT2G38290	0.046	1.000	0.002	1.000	-0.044	1.000	ammonium transporter 2
111	fasyl_2239635	AMT2	AT2G38290	0.036	0.978	-0.099	1.000	-0.135	0.918	ammonium transporter 2

Appendix Table 3.5 N-related genes in the fungal transcriptome. These genes obtained from mapping the fungal transcriptome against N metabolism and from doing keyword search for nitrate and ammonium transporters using the fungal transcriptome. Yellow colour: genes involved in N metabolism, KOG: Eucaryotic Orthologous Groups, EC number: Enzyme Commission number.

	Fungal ID	Fungal species*	KOG ID	EC number	Function
1	Acema1.173726	Acema	KOG1578	4.2.1.1	Carbonate dehydratase
2	Acema1.252825	Acema	KOG0382	4.2.1.1	Carbonate dehydratase
3	Acema1.42563	Acema	KOG0382	4.2.1.1	Carbonate dehydratase
4	Acema1.563058	Acema	KOG1578	4.2.1.1	Carbonate dehydratase
5	Acema1.602334	Acema	KOG1578	4.2.1.1	Carbonate dehydratase
6	Amamu1.184360	Amamu	KOG1578	4.2.1.1	Carbonate dehydratase
7	Amamu1.187012	Amamu	KOG1578	4.2.1.1	Carbonate dehydratase
8	Boled1.945473	Boled	KOG1578	4.2.1.1	Carbonate dehydratase
9	Boled1.959507	Boled	KOG1578	4.2.1.1	Carbonate dehydratase
10	Cenge3.532183	Cenge	KOG0382	4.2.1.1	Carbonate dehydratase
11	Cenge3.658527	Cenge	KOG1578	4.2.1.1	Carbonate dehydratase
12	Cenge3.673061	Cenge	KOG1578	4.2.1.1	Carbonate dehydratase
13	ClaPMI390.2054122	ClaPMI390	KOG1578	4.2.1.1	Carbonate dehydratase
14	Hebcy2.31455	Hebcy	KOG1578	4.2.1.1	Carbonate dehydratase
15	Hebcy2.422688	Hebcy	KOG1578	4.2.1.1	Carbonate dehydratase
16	Hebcy2.440671	Hebcy	KOG1578	4.2.1.1	Carbonate dehydratase
17	Hebcy2.441841	Hebcy	KOG1578	4.2.1.1	Carbonate dehydratase
18	Hebcy2.60663	Hebcy	KOG1578	4.2.1.1	Carbonate dehydratase
19	Hebcy2.7784	Hebcy	KOG1578	4.2.1.1	Carbonate dehydratase
20	Hymvar1.365650	Hymvar	KOG1578	4.2.1.1	Carbonate dehydratase
21	Hymvar1.418299	Hymvar	KOG1578	4.2.1.1	Carbonate dehydratase
22	Hymvar1.429608	Hymvar	KOG0382	4.2.1.1	Carbonate dehydratase
23	Hymvar1.46314	Hymvar	KOG1578	4.2.1.1	Carbonate dehydratase
24	Hymvar1.473328	Hymvar	KOG0382	4.2.1.1	Carbonate dehydratase
25	Hymvar1.528692	Hymvar	KOG0382	4.2.1.1	Carbonate dehydratase
26	Lacam2.106447	Lacam	KOG0382	4.2.1.1	Carbonate dehydratase
27	Lacam2.245258	Lacam	KOG1578	4.2.1.1	Carbonate dehydratase
28	Lacam2.672024	Lacam	KOG1578	4.2.1.1	Carbonate dehydratase
29	Lacam2.677295	Lacam	KOG1578	4.2.1.1	Carbonate dehydratase
30	Lacam2.86035	Lacam	KOG1578	4.2.1.1	Carbonate dehydratase
31	Lacam2.96908	Lacam	KOG0382	4.2.1.1	Carbonate dehydratase
32	Lacbi2.237838	Lacbi	KOG0382	4.2.1.1	Carbonate dehydratase
33	Lacbi2.237860	Lacbi	KOG0382	4.2.1.1	Carbonate dehydratase
34	Lacbi2.237880	Lacbi	KOG0382	4.2.1.1	Carbonate dehydratase
35	Lacbi2.292325	Lacbi	KOG1578	4.2.1.1	Carbonate dehydratase
36	Lacbi2.296408	Lacbi	KOG1578	4.2.1.1	Carbonate dehydratase
37	Lacbi2.299838	Lacbi	KOG1578	4.2.1.1	Carbonate dehydratase
38	Lacbi2.310771	Lacbi	KOG1578	4.2.1.1	Carbonate dehydratase
39	Lacbi2.437952	Lacbi	KOG0382	4.2.1.1	Carbonate dehydratase
40	Lacbi2.438072	Lacbi	KOG0382	4.2.1.1	Carbonate dehydratase
41	Lacbi2.455107	Lacbi	KOG0382	4.2.1.1	Carbonate dehydratase
42	Lacbi2.577322	Lacbi	KOG0382	4.2.1.1	Carbonate dehydratase
43	Lacbi2.674253	Lacbi	KOG1578	4.2.1.1	Carbonate dehydratase
44	Lacbi2.674272	Lacbi	KOG1578	4.2.1.1	Carbonate dehydratase
45	Lacbi2.684751	Lacbi	KOG1578	4.2.1.1	Carbonate dehydratase
46	Lacqui1.1597150	Lacqui	KOG1578	4.2.1.1	Carbonate dehydratase
47	Lacvol1.1227871	Lacvol	KOG1578	4.2.1.1	Carbonate dehydratase
48	Lacvol1.1446031	Lacvol	KOG1578	4.2.1.1	Carbonate dehydratase
49	Lacvol1.1455320	Lacvol	KOG1578	4.2.1.1	Carbonate dehydratase
50	Lacvol1.383293	Lacvol	KOG1578	4.2.1.1	Carbonate dehydratase
51	Morel2.124836	Morel	KOG1578	4.2.1.1	Carbonate dehydratase
52	Morel2.83280	Morel	KOG1578	4.2.1.1	Carbonate dehydratase
53	Morel2.892886	Morel	KOG0382	4.2.1.1	Carbonate dehydratase
54	Paxin1.106375	Paxin	KOG1578	4.2.1.1	Carbonate dehydratase

55	Paxin1.165648	Paxin	KOG1578	4.2.1.1	Carbonate dehydratase
56	Paxin1.72176	Paxin	KOG1578	4.2.1.1	Carbonate dehydratase
57	Phisc1.603197	Phisc	KOG1578	4.2.1.1	Carbonate dehydratase
58	Phisc1.603554	Phisc	KOG0382	4.2.1.1	Carbonate dehydratase
59	Phisc1.631256	Phisc	KOG1578	4.2.1.1	Carbonate dehydratase
60	Phisc1.654722	Phisc	KOG0382	4.2.1.1	Carbonate dehydratase
61	Phisc1.681262	Phisc	KOG1578	4.2.1.1	Carbonate dehydratase
62	Pilcr1.14524	Pilcr	KOG1578	4.2.1.1	Carbonate dehydratase
63	Pilcr1.1895	Pilcr	KOG0382	4.2.1.1	Carbonate dehydratase
64	Rhivi1.850253	Rhivi	KOG1578	4.2.1.1	Carbonate dehydratase
65	Rusbre1.1090347	Rusbre	KOG1578	4.2.1.1	Carbonate dehydratase
66	Rusdis1.1246726	Rusdis	KOG1578	4.2.1.1	Carbonate dehydratase
67	Rusdis1.1246728	Rusdis	KOG1578	4.2.1.1	Carbonate dehydratase
68	Rusrug1.983034	Rusrug	KOG1578	4.2.1.1	Carbonate dehydratase
69	Sclci1.119680	Sclci	KOG1578	4.2.1.1	Carbonate dehydratase
70	Sclci1.121623	Sclci	KOG1578	4.2.1.1	Carbonate dehydratase
71	Sclci1.124779	Sclci	KOG1578	4.2.1.1	Carbonate dehydratase
72	Sclci1.31017	Sclci	KOG1578	4.2.1.1	Carbonate dehydratase
73	Sebve1.72644	Sebve	KOG1578	4.2.1.1	Carbonate dehydratase
74	Suibr2.298092	Suibr	KOG1578	4.2.1.1	Carbonate dehydratase
75	Suibr2.746699	Suibr	KOG1578	4.2.1.1	Carbonate dehydratase
76	Suibr2.834634	Suibr	KOG1578	4.2.1.1	Carbonate dehydratase
77	Suilu3.536259	Suilu	KOG1578	4.2.1.1	Carbonate dehydratase
78	Suilu3.807555	Suilu	KOG1578	4.2.1.1	Carbonate dehydratase
79	Suilu3.808474	Suilu	KOG1578	4.2.1.1	Carbonate dehydratase
80	Trima3.1291571	Trima	KOG1578	4.2.1.1	Carbonate dehydratase
81	Tubbor1.1050178	Tubbor	KOG1578	4.2.1.1	Carbonate dehydratase
82	Tubbor1.1052338	Tubbor	KOG0382	4.2.1.1	Carbonate dehydratase
83	Tubbor1.1124951	Tubbor	KOG0382	4.2.1.1	Carbonate dehydratase
84	Tubbor1.172039	Tubbor	KOG0382	4.2.1.1	Carbonate dehydratase
85	Tubbor1.891513	Tubbor	KOG0382	4.2.1.1	Carbonate dehydratase
86	Wilmi1.585265	Wilmi	KOG0382	4.2.1.1	Carbonate dehydratase
87	Wilmi1.589141	Wilmi	KOG1578	4.2.1.1	Carbonate dehydratase
88	Wilmi1.640319	Wilmi	KOG0382	4.2.1.1	Carbonate dehydratase
89	Wilmi1.646552	Wilmi	KOG0382	4.2.1.1	Carbonate dehydratase
90	Xerba1.1424789	Xerba	KOG1578	4.2.1.1	Carbonate dehydratase
91	Xerba1.1511264	Xerba	KOG1578	4.2.1.1	Carbonate dehydratase
92	Lacbi2.445655	Lacbi	KOG0370	6.3.4.16	Carbamoyl-phosphate synthase (ammonia)
93	Morel2.37499	Morel	KOG0370	6.3.4.16	Carbamoyl-phosphate synthase (ammonia)
94	Suilu3.11962	Suilu	KOG0370	6.3.4.16	Carbamoyl-phosphate synthase (ammonia)
95	Acema1.701138	Acema	KOG2250	1.4.1.2	Glutamate dehydrogenase
96	Amamu1.159038	Amamu	KOG2250	1.4.1.2	Glutamate dehydrogenase
97	Boled1.984825	Boled	KOG2250	1.4.1.2	Glutamate dehydrogenase
98	Cenge3.657361	Cenge	KOG2250	1.4.1.2	Glutamate dehydrogenase
99	ClaPMI390.2057137	ClaPMI390	KOG2250	1.4.1.2	Glutamate dehydrogenase
100	Hebcy2.443951	Hebcy	KOG2250	1.4.1.2	Glutamate dehydrogenase
101	Hymvar1.427869	Hymvar	KOG2250	1.4.1.2	Glutamate dehydrogenase
102	Lacam2.135068	Lacam	KOG2250	1.4.1.2	Glutamate dehydrogenase
103	Lacam2.674757	Lacam	KOG2250	1.4.1.2	Glutamate dehydrogenase
104	Lacbi2.182694	Lacbi	KOG2250	1.4.1.2	Glutamate dehydrogenase
105	Lacbi2.309733	Lacbi	KOG2250	1.4.1.2	Glutamate dehydrogenase
106	Lacqui1.1743849	Lacqui	KOG2250	1.4.1.2	Glutamate dehydrogenase
107	Morel2.136790	Morel	KOG2250	1.4.1.2	Glutamate dehydrogenase
108	Morel2.20596	Morel	KOG2250	1.4.1.2	Glutamate dehydrogenase
109	Paxin1.9921	Paxin	KOG2250	1.4.1.2	Glutamate dehydrogenase
110	Phisc1.666944	Phisc	KOG2250	1.4.1.2	Glutamate dehydrogenase
111	Pilcr1.816852	Pilcr	KOG2250	1.4.1.2	Glutamate dehydrogenase
112	Pilcr1.98451	Pilcr	KOG2250	1.4.1.2	Glutamate dehydrogenase
113	Rhivi1.844494	Rhivi	KOG2250	1.4.1.2	Glutamate dehydrogenase
114	Rhivi1.864780	Rhivi	KOG2250	1.4.1.2	Glutamate dehydrogenase
115	Rusbre1.995021	Rusbre	KOG2250	1.4.1.2	Glutamate dehydrogenase
116	Rusdis1.1147059	Rusdis	KOG2250	1.4.1.2	Glutamate dehydrogenase
117	Rusrug1.1089297	Rusrug	KOG2250	1.4.1.2	Glutamate dehydrogenase
118	Sclci1.32964	Sclci	KOG2250	1.4.1.2	Glutamate dehydrogenase

119	Sebve1.15711	Sebve	KOG2250	1.4.1.2	Glutamate dehydrogenase
120	Suibr2.824256	Suibr	KOG2250	1.4.1.2	Glutamate dehydrogenase
121	Suibr2.842796	Suibr	KOG2250	1.4.1.2	Glutamate dehydrogenase
122	Suilu3.72908	Suilu	KOG2250	1.4.1.2	Glutamate dehydrogenase
123	Suilu3.798838	Suilu	KOG2250	1.4.1.2	Glutamate dehydrogenase
124	Trima3.1425595	Trima	KOG2250	1.4.1.2	Glutamate dehydrogenase
125	Tubbor1.1076602	Tubbor	KOG2250	1.4.1.2	Glutamate dehydrogenase
126	Wilmi1.584754	Wilmi	KOG2250	1.4.1.2	Glutamate dehydrogenase
127	Xerba1.1225020	Xerba	KOG2250	1.4.1.2	Glutamate dehydrogenase
128	Acema1.690110	Acema	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
129	Cenge3.637358	Cenge	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
130	ClaPMI390.2049040	ClaPMI390	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
131	Hebcy2.78090	Hebcy	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
132	Hymvar1.235082	Hymvar	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
133	Lacam2.675815	Lacam	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
134	Lacbi2.292653	Lacbi	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
135	Morel2.131839	Morel	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
136	Morel2.132437	Morel	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
137	Phisc1.457315	Phisc	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
138	Pilcr1.11511	Pilcr	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
139	Sebve1.17555	Sebve	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
140	Trima3.1378607	Trima3	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
141	Tubbor1.1068674	Tubbor	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
142	Wilmi1.405230	Wilmi	KOG2250	1.4.1.4	Glutamate dehydrogenase (NADP+)
143	Acema1.737509	Acema	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
144	Amamu1.97466	Amamu	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
145	Boled1.899184	Boled	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
146	Cenge3.575254	Cenge	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
147	ClaPMI390.2077267	ClaPMI390	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
148	Hebcy2.438587	Hebcy	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
149	Lacam2.674478	Lacam	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
150	Lacbi2.183838	Lacbi	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
151	Morel2.132072	Morel	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
152	Paxin1.73378	Paxin	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
153	Phisc1.691103	Phisc	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
154	Pilcr1.63545	Pilcr	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
155	Rhivi1.770144	Rhivi	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
156	Sclci1.1218577	Sclci	KOG0399	1.4.1.13	Glutamate synthase (NADPH)

157	Sebve1.327024	Sebve	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
158	Suibr2.725303	Suibr	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
159	Suilu3.799154	Suilu	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
160	Trima3.1440003	Trima	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
161	Wilmi1.585891	Wilmi	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
162	Xerba1.1581850	Xerba	KOG0399	1.4.1.13	Glutamate synthase (NADPH)
163	Acema1.107182	Acema	KOG0683	6.3.1.2	Glutamate--ammonia ligase
164	Acema1.361082	Acema	KOG0683	6.3.1.2	Glutamate--ammonia ligase
165	Amamu1.166750	Amamu	KOG0683	6.3.1.2	Glutamate--ammonia ligase
166	Amamu1.185211	Amamu	KOG0683	6.3.1.2	Glutamate--ammonia ligase
167	Boled1.850637	Boled	KOG0683	6.3.1.2	Glutamate--ammonia ligase
168	Boled1.912339	Boled	KOG0683	6.3.1.2	Glutamate--ammonia ligase
169	Boled1.937395	Boled	KOG0683	6.3.1.2	Glutamate--ammonia ligase
170	Cenge3.673039	Cenge	KOG0683	6.3.1.2	Glutamate--ammonia ligase
171	Cenge3.680736	Cenge	KOG0683	6.3.1.2	Glutamate--ammonia ligase
172	ClaPMI390.2053778	ClaPMI390	KOG0683	6.3.1.2	Glutamate--ammonia ligase
173	ClaPMI390.2059115	ClaPMI390	KOG0683	6.3.1.2	Glutamate--ammonia ligase
174	ClaPMI390.2076846	ClaPMI390	KOG0683	6.3.1.2	Glutamate--ammonia ligase
175	Hebcy2.438051	Hebcy	KOG0683	6.3.1.2	Glutamate--ammonia ligase
176	Hebcy2.440776	Hebcy	KOG0683	6.3.1.2	Glutamate--ammonia ligase
177	Hebcy2.64545	Hebcy	KOG0683	6.3.1.2	Glutamate--ammonia ligase
178	Hymvar1.168440	Hymvar	KOG0683	6.3.1.2	Glutamate--ammonia ligase
179	Hymvar1.394337	Hymvar	KOG0683	6.3.1.2	Glutamate--ammonia ligase
180	Lacam2.102647	Lacam	KOG0683	6.3.1.2	Glutamate--ammonia ligase
181	Lacam2.672562	Lacam	KOG0683	6.3.1.2	Glutamate--ammonia ligase
182	Lacam2.680307	Lacam	KOG0683	6.3.1.2	Glutamate--ammonia ligase
183	Lacbi2.183088	Lacbi	KOG0683	6.3.1.2	Glutamate--ammonia ligase
184	Lacbi2.191578	Lacbi	KOG0683	6.3.1.2	Glutamate--ammonia ligase
185	Lacbi2.299654	Lacbi	KOG0683	6.3.1.2	Glutamate--ammonia ligase
186	Lacbi2.582908	Lacbi	KOG0683	6.3.1.2	Glutamate--ammonia ligase
187	Lacbi2.684579	Lacbi	KOG0683	6.3.1.2	Glutamate--ammonia ligase
188	Lacqui1.1652450	Lacqui	KOG0683	6.3.1.2	Glutamate--ammonia ligase
189	Lacqui1.1772382	Lacqui	KOG0683	6.3.1.2	Glutamate--ammonia ligase
190	Lacqui1.1779066	Lacqui	KOG0683	6.3.1.2	Glutamate--ammonia ligase
191	Lacqui1.1787361	Lacqui	KOG0683	6.3.1.2	Glutamate--ammonia ligase
192	Lacqui1.1831108	Lacqui	KOG0683	6.3.1.2	Glutamate--ammonia ligase
193	Lacqui1.1877906	Lacqui	KOG0683	6.3.1.2	Glutamate--ammonia ligase
194	Lacvol1.1122829	Lacvol	KOG0683	6.3.1.2	Glutamate--ammonia ligase
195	Lacvol1.1161911	Lacvol	KOG0683	6.3.1.2	Glutamate--ammonia ligase
196	Lacvol1.1284330	Lacvol	KOG0683	6.3.1.2	Glutamate--ammonia ligase
197	Lacvol1.1302828	Lacvol	KOG0683	6.3.1.2	Glutamate--ammonia ligase
198	Lacvol1.1434703	Lacvol	KOG0683	6.3.1.2	Glutamate--ammonia ligase
199	Morel2.156454	Morel	KOG0683	6.3.1.2	Glutamate--ammonia ligase
200	Morel2.35934	Morel	KOG0683	6.3.1.2	Glutamate--ammonia ligase
201	Paxin1.137295	Paxin	KOG0683	6.3.1.2	Glutamate--ammonia ligase
202	Paxin1.166476	Paxin	KOG0683	6.3.1.2	Glutamate--ammonia ligase
203	Paxin1.171414	Paxin	KOG0683	6.3.1.2	Glutamate--ammonia ligase
204	Paxin1.74163	Paxin	KOG0683	6.3.1.2	Glutamate--ammonia ligase
205	Phisc1.366317	Phisc	KOG0683	6.3.1.2	Glutamate--ammonia ligase
206	Phisc1.684186	Phisc	KOG0683	6.3.1.2	Glutamate--ammonia ligase
207	Phisc1.693711	Phisc	KOG0683	6.3.1.2	Glutamate--ammonia ligase
208	Pilcr1.815449	Pilcr	KOG0683	6.3.1.2	Glutamate--ammonia ligase
209	Pilcr1.823397	Pilcr	KOG0683	6.3.1.2	Glutamate--ammonia ligase
210	Pilcr1.94641	Pilcr	KOG0683	6.3.1.2	Glutamate--ammonia ligase
211	Rhivi1.777127	Rhivi	KOG0683	6.3.1.2	Glutamate--ammonia ligase
212	Rhivi1.777798	Rhivi	KOG0683	6.3.1.2	Glutamate--ammonia ligase
213	Rhivi1.846623	Rhivi	KOG0683	6.3.1.2	Glutamate--ammonia ligase
214	Rhivi1.868145	Rhivi	KOG0683	6.3.1.2	Glutamate--ammonia ligase
215	Rusbre1.73501	Rusbre	KOG0683	6.3.1.2	Glutamate--ammonia ligase
216	Rusbre1.904848	Rusbre	KOG0683	6.3.1.2	Glutamate--ammonia ligase
217	Rusbre1.981683	Rusbre	KOG0683	6.3.1.2	Glutamate--ammonia ligase

218	Rusdis1.1181134	Rusdis	KOG0683	6.3.1.2	Glutamate--ammonia ligase
219	Rusdis1.1221793	Rusdis	KOG0683	6.3.1.2	Glutamate--ammonia ligase
220	Rusdis1.1310680	Rusdis	KOG0683	6.3.1.2	Glutamate--ammonia ligase
221	Rusrug1.1088663	Rusrug	KOG0683	6.3.1.2	Glutamate--ammonia ligase
222	Rusrug1.910820	Rusrug	KOG0683	6.3.1.2	Glutamate--ammonia ligase
223	Rusrug1.948692	Rusrug	KOG0683	6.3.1.2	Glutamate--ammonia ligase
224	Sclci1.106076	Sclci	KOG0683	6.3.1.2	Glutamate--ammonia ligase
225	Sclci1.1209188	Sclci	KOG0683	6.3.1.2	Glutamate--ammonia ligase
226	Sclci1.1212711	Sclci	KOG0683	6.3.1.2	Glutamate--ammonia ligase
227	Sclci1.1224577	Sclci	KOG0683	6.3.1.2	Glutamate--ammonia ligase
228	Sclci1.15356	Sclci	KOG0683	6.3.1.2	Glutamate--ammonia ligase
229	Sebve1.331445	Sebve	KOG0683	6.3.1.2	Glutamate--ammonia ligase
230	Sebve1.334244	Sebve	KOG0683	6.3.1.2	Glutamate--ammonia ligase
231	Sebve1.68708	Sebve	KOG0683	6.3.1.2	Glutamate--ammonia ligase
232	Suibr2.789626	Suibr	KOG0683	6.3.1.2	Glutamate--ammonia ligase
233	Suibr2.838210	Suibr	KOG0683	6.3.1.2	Glutamate--ammonia ligase
234	Suilu3.797877	Suilu	KOG0683	6.3.1.2	Glutamate--ammonia ligase
235	Suilu3.806253	Suilu	KOG0683	6.3.1.2	Glutamate--ammonia ligase
236	Trima3.1252879	Trima	KOG0683	6.3.1.2	Glutamate--ammonia ligase
237	Trima3.1297256	Trima	KOG0683	6.3.1.2	Glutamate--ammonia ligase
238	Trima3.1421346	Trima	KOG0683	6.3.1.2	Glutamate--ammonia ligase
239	Tubbor1.1070453	Tubbor	KOG0683	6.3.1.2	Glutamate--ammonia ligase
240	Tubbor1.1078486	Tubbor	KOG0683	6.3.1.2	Glutamate--ammonia ligase
241	Tubbor1.1098919	Tubbor	KOG0683	6.3.1.2	Glutamate--ammonia ligase
242	Wilmi1.588673	Wilmi	KOG0683	6.3.1.2	Glutamate--ammonia ligase
243	Wilmi1.640447	Wilmi	KOG0683	6.3.1.2	Glutamate--ammonia ligase
244	Xerba1.1453015	Xerba	KOG0683	6.3.1.2	Glutamate--ammonia ligase
245	Xerba1.1512811	Xerba	KOG0683	6.3.1.2	Glutamate--ammonia ligase
246	Xerba1.1606984	Xerba	KOG0683	6.3.1.2	Glutamate--ammonia ligase
247	Xerba1.706248	Xerba	KOG0683	6.3.1.2	Glutamate--ammonia ligase
248	Acema1.603847	Acema	KOG0805	3.5.5.1	Nitrilase
249	Acema1.618307	Acema	KOG0805	3.5.5.1	Nitrilase
250	Acema1.680622	Acema	KOG0805	3.5.5.1	Nitrilase
251	Acema1.795915	Acema	KOG0805	3.5.5.1	Nitrilase
252	Boled1.936652	Boled	KOG0805	3.5.5.1	Nitrilase
253	Cenge3.659460	Cenge	KOG0805	3.5.5.1	Nitrilase
254	Cenge3.679398	Cenge	KOG0805	3.5.5.1	Nitrilase
255	Cenge3.691736	Cenge	KOG0805	3.5.5.1	Nitrilase
256	ClaPMI390.2055397	ClaPMI390	KOG0805	3.5.5.1	Nitrilase
257	Hymvar1.368325	Hymvar	KOG0805	3.5.5.1	Nitrilase
258	Hymvar1.440437	Hymvar	KOG0805	3.5.5.1	Nitrilase
259	Paxin1.174001	Paxin	KOG0805	3.5.5.1	Nitrilase
260	Phisc1.574080	Phisc	KOG0805	3.5.5.1	Nitrilase
261	Phisc1.661486	Phisc	KOG0805	3.5.5.1	Nitrilase
262	Phisc1.678774	Phisc	KOG0805	3.5.5.1	Nitrilase
263	Phisc1.686126	Phisc	KOG0805	3.5.5.1	Nitrilase
264	Phisc1.687202	Phisc	KOG0805	3.5.5.1	Nitrilase
265	Pilcr1.827391	Pilcr	KOG0805	3.5.5.1	Nitrilase
266	Rhivi1.498787	Rhivi	KOG0805	3.5.5.1	Nitrilase
267	Suibr2.844573	Suibr	KOG0805	3.5.5.1	Nitrilase
268	Suilu3.800566	Suilu	KOG0805	3.5.5.1	Nitrilase
269	Tubbor1.79324	Tubbor	KOG0805	3.5.5.1	Nitrilase
270	Tubbor1.977331	Tubbor	KOG1231	3.5.5.1	Nitrilase
271	Wilmi1.660384	Wilmi	KOG0805	3.5.5.1	Nitrilase
272	Xerba1.1401665	Xerba	KOG0805	3.5.5.1	Nitrilase
273	Acema1.394767	Acema	KOG0682		Ammonia permease
274	Acema1.491295	Acema	KOG0682		Ammonia permease
275	Acema1.577293	Acema	KOG0682		Ammonia permease
276	Acema1.578938	Acema	KOG0682		Ammonia permease
277	Acema1.598633	Acema	KOG0682		Ammonia permease
278	Acema1.601410	Acema	KOG0682		Ammonia permease
279	Acema1.673973	Acema	KOG0682		Ammonia permease
280	Acema1.681371	Acema	KOG0682		Ammonia permease
281	Acema1.731370	Acema	KOG0682		Ammonia permease
282	Amamu1.1040015	Amamu	KOG0682		Ammonia permease
283	Amamu1.178797	Amamu	KOG0682		Ammonia permease
284	Boled1.1055711	Boled	KOG0682		Ammonia permease

285	Boled1.908555	Boled	KOG0682	Ammonia permease
286	Cenge3.560732	Cenge	KOG0682	Ammonia permease
287	Cenge3.641073	Cenge	KOG0682	Ammonia permease
288	Cenge3.649203	Cenge	KOG0682	Ammonia permease
289	Cenge3.659613	Cenge	KOG0682	Ammonia permease
290	Cenge3.679722	Cenge	KOG0682	Ammonia permease
291	Cenge3.699790	Cenge	KOG0682	Ammonia permease
292	ClaPMI390.2057426	ClaPMI390	KOG0682	Ammonia permease
293	ClaPMI390.2099573	ClaPMI390	KOG0682	Ammonia permease
294	ClaPMI390.2136878	ClaPMI390	KOG0682	Ammonia permease
295	ClaPMI390.2214031	ClaPMI390	KOG0682	Ammonia permease
296	Hebcy2.22652	Hebcy	KOG0682	Ammonia permease
297	Hebcy2.249585	Hebcy	KOG0682	Ammonia permease
298	Hebcy2.30318	Hebcy	KOG0682	Ammonia permease
299	Hebcy2.76606	Hebcy	KOG0682	Ammonia permease
300	Hymvar1.257858	Hymvar	KOG0682	Ammonia permease
301	Hymvar1.376049	Hymvar	KOG0682	Ammonia permease
302	Hymvar1.385003	Hymvar	KOG0682	Ammonia permease
303	Hymvar1.390120	Hymvar	KOG0682	Ammonia permease
304	Hymvar1.396501	Hymvar	KOG0682	Ammonia permease
305	Hymvar1.433026	Hymvar	KOG0682	Ammonia permease
306	Lacam2.108948	Lacam	KOG0682	Ammonia permease
307	Lacam2.11646	Lacam	KOG0682	Ammonia permease
308	Lacam2.1625650	Lacam	KOG0682	Ammonia permease
309	Lacam2.182240	Lacam	KOG0682	Ammonia permease
310	Lacam2.640142	Lacam	KOG0682	Ammonia permease
311	Lacam2.677229	Lacam	KOG0682	Ammonia permease
312	Lacam2.681776	Lacam	KOG0682	Ammonia permease
313	Lacbi2.188643	Lacbi	KOG0682	Ammonia permease
314	Lacbi2.190906	Lacbi	KOG0682	Ammonia permease
315	Lacbi2.255304	Lacbi	KOG0682	Ammonia permease
316	Lacbi2.300932	Lacbi	KOG0682	Ammonia permease
317	Lacbi2.313221	Lacbi	KOG0682	Ammonia permease
318	Lacbi2.331747	Lacbi	KOG0682	Ammonia permease
319	Lacbi2.484007	Lacbi	KOG0682	Ammonia permease
320	Lacqui1.1719837	Lacqui	KOG0682	Ammonia permease
321	Lacvol1.1305322	Lacvol	KOG0682	Ammonia permease
322	Lacvol1.1362652	Lacvol	KOG0682	Ammonia permease
323	Morel2.104564	Morel	KOG0682	Ammonia permease
324	Morel2.116310	Morel	KOG0682	Ammonia permease
325	Morel2.93236	Morel	KOG0682	Ammonia permease
326	Paxin1.16099	Paxin	KOG0682	Ammonia permease
327	Paxin1.164643	Paxin	KOG0682	Ammonia permease
328	Phisc1.198700	Phisc	KOG0682	Ammonia permease
329	Phisc1.457340	Phisc	KOG0682	Ammonia permease
330	Phisc1.55504	Phisc	KOG0682	Ammonia permease
331	Phisc1.574918	Phisc	KOG0682	Ammonia permease
332	Phisc1.618579	Phisc	KOG0682	Ammonia permease
333	Phisc1.680050	Phisc	KOG0682	Ammonia permease
334	Phisc1.682250	Phisc	KOG0682	Ammonia permease
335	Phisc1.789950	Phisc	KOG0682	Ammonia permease
336	Phisc1.84323	Phisc	KOG0682	Ammonia permease
337	Pilcr1.65373	Pilcr	KOG0682	Ammonia permease
338	Pilcr1.693189	Pilcr	KOG0682	Ammonia permease
339	Pilcr1.71308	Pilcr	KOG0682	Ammonia permease
340	Pilcr1.773636	Pilcr	KOG0682	Ammonia permease
341	Pilcr1.815462	Pilcr	KOG0682	Ammonia permease
342	Pilcr1.818299	Pilcr	KOG0682	Ammonia permease
343	Pilcr1.826791	Pilcr	KOG0682	Ammonia permease
344	Rhivi1.680885	Rhivi	KOG0682	Ammonia permease
345	Rhivi1.801313	Rhivi	KOG0682	Ammonia permease
346	Rhivi1.814607	Rhivi	KOG0682	Ammonia permease
347	Rhivi1.865633	Rhivi	KOG0682	Ammonia permease
348	Rusbre1.969632	Rusbre	KOG0682	Ammonia permease
349	Rusdis1.1171474	Rusdis	KOG0682	Ammonia permease
350	Rusrug1.1185715	Rusrug	KOG0682	Ammonia permease
351	Sclci1.114719	Sclci	KOG0682	Ammonia permease

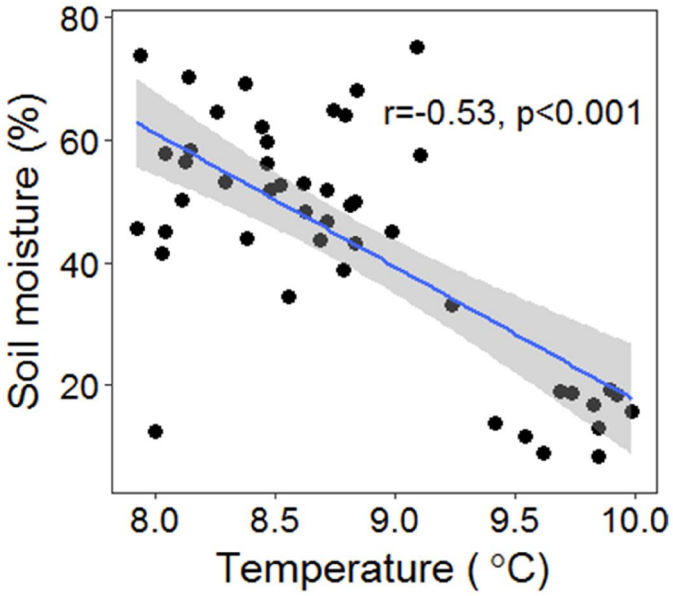
352	Sclci1.1218182	Sclci	KOG0682	Ammonia permease
353	Sclci1.14567	Sclci	KOG0682	Ammonia permease
354	Sclci1.852123	Sclci	KOG0682	Ammonia permease
355	Sebve1.325740	Sebve	KOG0682	Ammonia permease
356	Sebve1.327041	Sebve	KOG0682	Ammonia permease
357	Suibr2.771835	Suibr	KOG0682	Ammonia permease
358	Suibr2.786410	Suibr	KOG0682	Ammonia permease
359	Suibr2.845958	Suibr	KOG0682	Ammonia permease
360	Suilu3.758484	Suilu	KOG0682	Ammonia permease
361	Suilu3.801647	Suilu	KOG0682	Ammonia permease
362	Suilu3.811475	Suilu	KOG0682	Ammonia permease
363	Trima3.1377724	Trima	KOG0682	Ammonia permease
364	Trima3.1426836	Trima	KOG0682	Ammonia permease
365	Tubbor1.1100749	Tubbor	KOG0682	Ammonia permease
366	Tubbor1.1122825	Tubbor	KOG0682	Ammonia permease
367	Tubbor1.979267	Tubbor	KOG0682	Ammonia permease
368	Wilmi1.536895	Wilmi	KOG0682	Ammonia permease
369	Wilmi1.593102	Wilmi	KOG0682	Ammonia permease
370	Wilmi1.634883	Wilmi	KOG0682	Ammonia permease
371	Wilmi1.663800	Wilmi	KOG0682	Ammonia permease
372	Xerba1.1057860	Xerba	KOG0682	Ammonia permease
373	Xerba1.1489382	Xerba	KOG0682	Ammonia permease
374	Xerba1.1505423	Xerba	KOG0682	Ammonia permease

Appendix Table 3.6 Common genes to A and H among differentially expressed genes

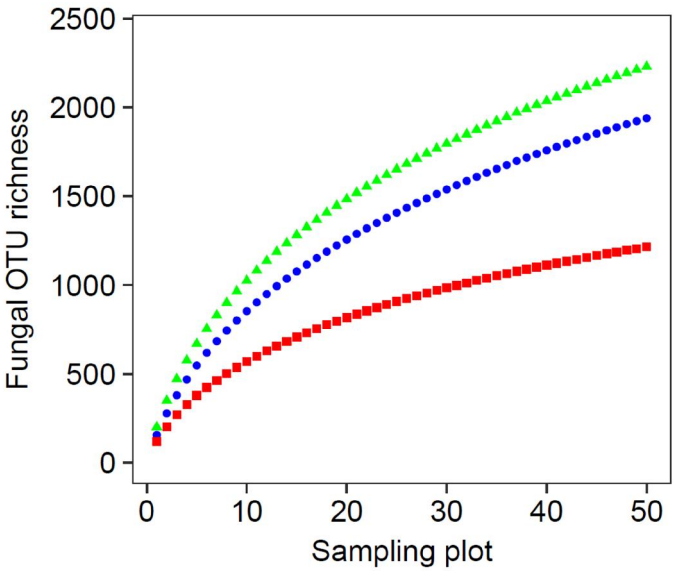
	Fasyl ID	AGI ID	Putative function	H-S		H-A		S-A	
				Log2fc	p value*	Log2fc	p value*	Log2fc	p value*
1	Fasyl ID	AGI ID	Putative function	log2fc1.2	padj1.2	log2fc1.3	padj1.3	log2fc2.3	padj2.3
2	fasyl_1232754	AT1G78780	pathogenesis-related family protein	-0.95	0.04	0.43	1.00	1.37	0.00
3	fasyl_1905152	AT1G78780	pathogenesis-related family protein	-1.07	0.01	0.47	1.00	1.54	0.00
4	fasyl_2556709	AT5G35450	Disease resistance protein (CC-NBS-LRR class) family	1.64	0.00	0.17	1.00	-1.47	0.03
5	fasyl_0654506	AT2G46150	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	1.76	0.00	0.48	1.00	-1.28	0.04
6	fasyl_2568183	AT1G69870	nitrate transporter 1.7	-1.45	0.00	0.38	1.00	1.83	0.00
7	fasyl_1051267	AT2G38940	phosphate transporter 1;4	0.91	0.01	0.02	1.00	-0.89	0.04
8	fasyl_2568106	AT3G54700	phosphate transporter 1;7	0.89	0.01	-0.17	1.00	-1.06	0.01
9	fasyl_1656484	AT2G01770	vacuolar iron transporter 1	-2.98	0.00	0.06	1.00	3.04	0.00
10	fasyl_0020415	AT1G23090	sulfate transporter 91	1.28	0.01	0.02	1.00	-1.27	0.04
11	fasyl_1187318	AT1G07750	RmlC-like cupins superfamily protein	0.94	0.01	-0.03	1.00	-0.97	0.03
12	fasyl_1007580	AT3G63060	EID1-like 3	1.92	0.00	0.48	1.00	-1.44	0.02
13	fasyl_0279451	AT2G37570	HSP20-like chaperones superfamily protein	-0.50	0.02	0.18	1.00	0.67	0.00
14	fasyl_0096454	AT3G59410	protein kinase family protein	0.74	0.05	-0.15	1.00	-0.89	0.04
15	fasyl_1122409	AT3G21420	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	-1.09	0.02	0.07	1.00	1.16	0.05
16	fasyl_2214162	AT5G08380	alpha-galactosidase 1	1.35	0.00	0.23	1.00	-1.12	0.03
17	fasyl_0405854	AT4G27450	Aluminium induced protein with YGL and LRDR motifs	1.51	0.00	0.33	1.00	-1.18	0.03
18	fasyl_0720251	AT2G37570	HSP20-like chaperones superfamily protein	-0.56	0.01	0.07	1.00	0.62	0.02
19	fasyl_2564988	AT4G25433	peptidoglycan-binding LysM domain-containing protein	-1.15	0.02	0.10	1.00	1.25	0.04
20	fasyl_2380254	AT3G23640	heteroglycan glucosidase 1	-0.62	0.00	-0.04	1.00	0.58	0.02
21	fasyl_1295410	AT5G06080	LOB domain-containing protein 33	-1.77	0.00	-0.10	1.00	1.67	0.00
22	fasyl_2562105	AT1G07150	mitogen-activated protein kinase kinase kinase 13	1.30	0.00	-0.10	1.00	-1.40	0.01
23	fasyl_0957303	AT2G38830	Ubiquitin-conjugating enzyme/RWD-like protein	1.95	0.00	0.51	1.00	-1.44	0.05

H, Hainich-Dün; S, Schorfheide-Chorin; A, Schwäbische Alb

Log2fc: $\log_2$ fold change, * Adjusted p value



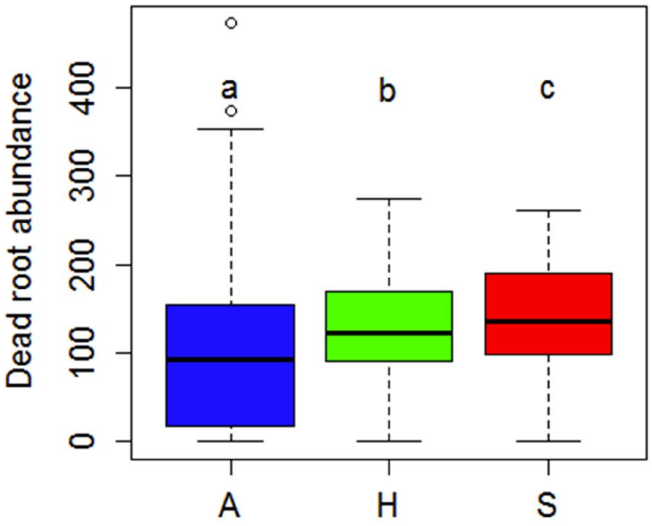
Appendix Fig. 4.1 Correlation between soil moisture and annual mean temperature in forest plots across Germany in 2014. Pearson correlation coefficient (r) with the associated P value is shown.



Appendix Fig. 4.2 Rarefaction curves depicting the fungal operational taxonomic unit (OTU) richness in 50 forest plots for each study region: Schwäbische Alb (blue), Hainich-Dün (green) and Schorfheide-Chorin (red).

	A			H			S		
	SYM	SAP	PAT	SYM	SAP	PAT	SYM	SAP	PAT
Soil N				0.023		0.036		0.024	
Soil NH4									
Soil NO3							0.003		0.005
Soil water							0.016		
Root C				0.006					
Root glucose			0.011						
Root starch						0.007		0.003	
Root N				0.003		0.011			
Root NH4	0.000	0.005	0.007						
Root NO3	0.016	0.002				0.034	0.005	0.000	

Appendix Fig. 4.3 Significant relationships between soil and root chemistry and the read abundance of root-associated trophic groups in the three study regions. SYM=symbiotroph, SAP=saprotroph and PAT=pathotroph. A = Schwäbische Alb, H = Hainich-Dün and S = Schorfheide-Chorin. Blue colors indicate negative correlations while red colors stand for positive correlations. P values were represented numerically where significant effects were found.



Appendix Fig. 4.4 Mean abundance of dead root tips per plot (n=30) in the three biogeographical regions. A = Schwäbische Alb, H = Hainich-Dün and S = Schorfheide-Chorin. Different small letters indicate significant difference among the means of the three regions at the 1% level.

Appendix Table 4.1 Mean read abundance and OTU richness of root-associated fungal trophic groups in three biogeographic regions. A = Schwäbische Alb, H = Hainich-Dün, and S = Schorfheide-Chorin. SYM = symbiotroph, SAP = saprotroph, PAT = pathotroph and UNK = unknown fungi. Data were analyzed with generalized linear model (Poisson regression). Significant differences at $p < 0.05$ are indicated with bold letters. Data are means of n = 50 plots per region ± SE.

Trophic mode	Study region			P value			
	A	H	S	A-H-S	A-H	A-S	H-S
Mean reads per plot							
SYM	4827 ± 202	5031 ± 125	3573 ± 203	**<0.001**	**<0.001**	**<0.001**	**<0.001**
SAP	821 ± 149	746 ± 71	1150 ± 137	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PAT	89 ± 17	145 ± 26	523 ± 79	**<0.001**	**<0.001**	**<0.001**	**<0.001**
UNK	2663 ± 159	2479 ± 139	3154 ± 166	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Mean numbers of OTUs per plot							
SYM	47 ± 2	62 ± 3	27 ± 1	**<0.001**	**<.001**	**<0.001**	**<0.001**
SAP	30 ± 2	34 ± 2	26 ± 1	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PAT	9 ± 1	15 ± 1	10 ± 1	**<0.001**	**<0.001**	0.251	**<0.001**
UNK	70 ± 3	89 ± 3	56 ± 2	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Appendix Table 4.2 Characterization and statistical analyses of mean relative abundance of fungal orders per plot in three biogeographical regions. A = Schwäbische Alb, H = Hainich-Dün and S = Schorfheide-Chorin. Orders with a relative abundance < 0.1% are grouped in Others. Significant differences at $p < 0.05$ are indicated with bold letters. Data are means of n = 50 plots per region ± SE.

Order	Study region			p value			
	A	H	S	A-H-S	A-H	A-S	H-S
Basidiomycota							
Agaricales	2732 ±197	2213 ±190	1669 ±122	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Russulales	1448 ± 206	2507 ± 256	2570 ± 211	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Sebacinales	727 ± 137	456 ± 92	1 ± 0.5	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Atheliales	517 ± 117	242 ± 73	93 ± 30	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Thelephorales	482 ± 73	507 ± 66	187 ± 61	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Trechisporales	68 ± 25	107 ± 30	435 ± 71	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Boletales	28 ± 9	182 ± 57	305 ± 58	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Cantharellales	146 ± 55	192 ± 46	24 ± 12	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Polyporales	34 ± 18	58 ± 29	23 ± 15	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Auriculariales	4 ± 1	7 ± 2	39 ± 30	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Ascomycota							
Helotiales	565 ± 59	455 ± 39	1698 ± 88	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Pezizales	413 ± 75	574 ± 64	290 ± 64	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Archaeorhizomycetales	31 ± 14	3 ± 1	301 ± 90	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Chaetothyriales	64 ± 11	79 ± 15	34 ± 5	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Pleosporales	48 ± 12	27 ± 5	18 ± 8	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Xylariales	47 ± 21	9 ± 2	11 ± 3	**< 0.001**	**< 0.001**	**< 0.001**	**0.038**
Capnodiales	2 ± 1	15 ± 6	33 ± 6	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Rhytismatales	1.2 ± 0.8	0.2 ± 0.1	28 ± 10	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Hysteriales	18 ± 8	24 ± 6	25 ± 5	**< 0.001**	**< 0.001**	**< 0.001**	0.500
Hypocreales	19 ± 7	15 ± 4	4 ± 1	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Glomeromycota							
Glomerales	325 ± 84	176 ± 42	27 ± 12	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Zygomycota							
Mortierellales	15 ± 2	15 ± 1	13 ± 1	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Chytridiomycota							
Chytridiales	30 ± 29	1 ± 0	1 ± 0	**< 0.001**	**< 0.001**	**< 0.001**	0.626
Others	148 ± 21	129 ± 19	168 ± 28	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Unidentified	485 ± 58	407 ± 57	402 ± 48	**< 0.001**	**< 0.001**	**< 0.001**	0.407

Declarations of the author's contributions

Chapter 2

Quang Dung Nguyen conducted the measurements of beech performance, characterized and identified ectomycorrhizal communities, conducted ^{15}N labeling experiment, analyzed data and wrote the manuscript draft. Rodica Pena planned and set up the experiment. Nitrogen isotope analysis was conducted at Centre for Stable Isotope Research and Analysis, University of Göttingen with the support of Lars Szwec. Andrea Polle designed the study, supervised data analyses and contributed to the writing of the manuscript.

Chapter 3

Quang Dung Nguyen participated in field harvests and collected fine root samples in the three study regions, extracted RNA from the roots, analyzed the RNA-Seq data, wrote this chapter. RNA sequencing was done by Chronix Biomedical GmbH, Göttingen, Germany with the support of Julia Beck. Dennis Janz did the bioinformatic analysis of RNA-Seq data. Andrea Polle designed the study, supervised the data analyses and writing of the chapter

Chapter 4

Quang Dung Nguyen sampled fine roots in Schorfheide-Chorin, conducted the root measurements (root N and C, root carbohydrates), extracted DNA and prepared DNA amplicon, analyzed data and wrote the chapter. Root NH_4^+ and NO_3^- were measured by Jan-Lukas Barke. Rodica Pena and Silke Ammerschubert samped fine roots in Hanich-Dün and Schwäbische Alb, respectively. Illumina amplicon sequencing, DNA sequence processing and bioinformatic analysis were conducted at Department of Genomic and Applied Microbiology, University of Göttingen with the support of Andrea Thürmer, Dominik Schneider and Rolf Daniel. Bin Song and Rodica Pena measured soil NH_{4+} and NO_3^- variables. Ingo Schöning provided soil carbon and nitrogen, soil pH and soil water content. N deposition data were provided by Sophia Leimer, Martin T. Schwarz and Wolfgang Wilcke. Andrea Polle designed the study, supervised data analyses and the writing of this chapter.

Scientific activities during the PhD studies

Publications

Nguyen DQ, Pena R, Polle A (2017). Impact of ectomycorrhizal community composition and soil treatment on inorganic nitrogen nutrition and performance of beech (*Fagus sylvatica* L.) provenances. Trees, 31: 1891-1904 doi: https://doi.org/10.1007/s00468-017-1594-7

Presentations

Nguyen DQ (2018) Diversity and function of root-associated fungal communities in relation to nitrogen nutrition in temperate forests. Forstbotanisches Seminar, Department of Forest Botany and Tree Physiology, University of Göttingen, Germany (14 May 2018)

Nguyen DQ, Song B, Pena R, Janz D, Brinkmann N, Schneider D, Daniel R, Polle A (2017) Nitrogen and root mycobiome in temperate forests along a geographical gradient. 9th International Conference on Mycorrhiza. Prague, Czech Republic (30 Jul-4 Aug 2017)

Nguyen DQ (2017) Root fungal communities and nutrient profile in temperate forests across Germany. Forstbotanisches Seminar, Department of Forest Botany and Tree Physiology, University of Göttingen, Germany (3 Jul 2017)

Nguyen DQ, Ammerschubert S, Song B, Pena R, Müller M, Polle A (2016) Functional diversity of mycorrhiza in relation to land-use changes and ecosystem functions. 14th Assembly of the Biodiversity Exploratories, Wernigerode, Germany (21-24 Feb 2017)

Nguyen DQ, Ammerschubert S, Schröter K, Polle A (2016) Diversity of ectomycorrhizal fungi in relation to land-use changes and ecosystem functions. 13th Assembly of the Biodiversity Exploratories, Wernigerode, Germany (16-19 Feb 2016)

Nguyen DQ (2015) Soil organism-beech interaction in relation to nitrogen uptake. Forstbotanisches Seminar, Department of Forest Botany and Tree Physiology, University of Göttingen, Germany (2 Feb 2015)

Posters

Nguyen DQ, Schneider D, Song B, Brinkmann N, Janz D, Schöning I, Pena R, Daniel R, Polle A (2018) Root-associated fungal communities and their relationships with root and soil carbon and nitrogen in temperate forests. 15th Assembly of the Biodiversity Exploratories, Wernigerode, Germany (20-23 Feb 2018)

Nguyen DQ, Pena R, Song B, Polle A (2017) Carbon and nitrogen concentrations of fine roots in forests across the Exploratories as affected by forest management. 14th Assembly of the Biodiversity Exploratories, Wernigerode, Germany (21-24 Feb 2017)

Nguyen DQ, Pena R, Polle A (2016) Ammonium and nitrate uptake of beech progenies from the Exploratories as affected by ectomycorrhizal diversity. 13th Assembly of the Biodiversity Exploratories, Wernigerode, Germany (16-19 Feb 2016)

Nguyen DQ, Pena R, Polle A (2015) Beech performance and nitrogen uptake in response to soil fungal inoculation. GFÖ 2015: Ecology for a sustainable future, Göttingen, Germany (31 Aug-4 Sep 2015)

Nguyen DQ, Ammerschubert S, Pena R, Polle A (2015) Performance of beech as affected by soil fungi. 12th Assembly of the Biodiversity Exploratories, Wernigerode, Germany (17-20 Feb 2015)

Curriculum vitae

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Surname	Nguyen
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Education and Work Experience

Mar 2014 - present	Doctoral student in Molecular Sciences and Biotechnology of Crops and Trees at Forest Botany and Tree Physiology, University of Göttingen, Germany. Title of thesis: Diversity and function of root-associated fungal communities in relation to nitrogen nutrition in temperate forests.
Jun 2012-Mar 2014	Work as a researcher at Vietnamese Academy of Forest Sciences, Hanoi, Vietnam
Mar 2010-Jun 2012	Master student in Biological Sciences and Biotechnology at Murdoch University, Western Australia, Australia. Title of the thesis: *Hysterangium* mats and associated bacteria under *Eucalyptus gomphocephala* in south-western Australia
Sept 2005-Mar 2010	Work as researcher at Vietnamese Academy of Forest Sciences, Hanoi, Vietnam
July 1999-June 2003	Bachelor student in Forestry at Vietnam National University of Forestry, Hanoi, Vietnam

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Declaration of originality and certificate of authorship

I, Quang Dung Nguyen, hereby declare that I am the sole author of this dissertation entitled “Diversity and function of root-associated fungal communities in relation to nitrogen nutrition in temperate forests”. This work has not previously been submitted for a degree at any tertiary education institutions. To the best of my knowledge, all references and data sources that were used in this dissertation have been appropriately acknowledged.

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Place/Date Quang Dung Nguyen