Arbuscular mycorrhizae vs. Ectomycorrhizae: limiting or stimulating tree growth with N deposition?

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Arbuscular vs Ectomycorrhizal fungi: limiting or stimulating tree growth with N deposition?

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Abstract

Plant species in N-limited forest are expected to increase growth with anthropogenic N deposition and increase the carbon (C) sink of the forest. However, growth enhancement is not always the result in practice and mycorrhizal fungi appears a factor that could limit or stimulate plant growth. Mycorrhizal fungi are living in and among roots and form a mutualistic relation with the host plant, receiving carbohydrates from the plant in return for nitrogen (N). This research analyses the tree growth of plant species associated with either arbuscular mycorrhizae (AM) or ectomycorrhizae (ECM), and how tree growth alters with N fertilization. The mycorrhizal fungi are integrated in the resource optimization model FUN to the influence of mycorrhizal fungi in the N uptake of the plant. The AM and ECM trees occurs in the same hardwood temperate forest in Indiana, USA. Stem increment, leaf production and fine root production is measured for C and N allocation differences between AM and ECM tree species and with or without N fertilization. The results show that AM trees are growing faster than ECM trees, due to more inorganic N availability in AM forest soils but also because N uptake through AM costs less C for the plant than with ECM fungi. Higher N availability leads to a positive response of tree growth in ECM trees on higher N availability while AM trees show a negative response. This indicates that the added N is not taken up by the ECM fungi but by the trees, while AM fungi are taking up the N and demand for more C from the tree. Since the C and N transfer between the fungi and the plant is not only dependent on the demand of N from the plant, the incorporation of the mycorrhizal fungi in the model as a decrease of the cost for N uptake is insufficient to model N uptake of AM and ECM trees.

Keywords: Arbuscular Mycorrhizae, Ectomycorrhizae, Nitrogen Fertilization, Tree Growth, FUN model

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AM	= Arbuscular Mycorrhizae
BA	= Basal Area
BAI	= Basal Area Increment
BNF	= Biological N Fixation
C	= Carbon
CO_2	= Carbon dioxide
DBH	= Diameter at Breast Height
ECM	= Ectomycorrhizae
F	= fertilized
FRP	= Fine Root Production
N	= Nitrogen
NPP	= Net Primary Productivity
P	= Phosphorous
RGR	= Relative Growth Rate
SOM	= Soil Organic Matter
UF	= unfertilized

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1. Introduction

Climate change is a threat for nature and human society. Droughts, floods, hurricanes, forest fires and many other natural disturbances are expected to occur more frequently and on a larger scale. Terrestrial ecosystems have the ability to mitigate climate change by removing carbon dioxide (CO_2) from the atmosphere. Currently, terrestrial biota act as a carbon (C) sink of around $2.8 \cdot 10^{15}$ gram C per year [Canadell et al., 2007] and mostly consists of the carbon sink of forests. The future role of forests in absorbing additional CO_2 from the atmosphere is however uncertain. Net primary productivity (NPP) of forest depends on light, water, CO_2 and nutrients in especially nitrogen (N) and phosphorus (P). N availability in forests, especially in Europe, North America and South East Asia, has been enhanced due to increasing anthropogenic N deposition, caused by intensive agriculture, traffic and industrialization [Galloway et al., 2004]. Total global NO_y and NH_x atmospheric deposition increased from $31.6*10^6$ tonnes N yr^{-1} in 1860 to $103*10^6$ tonnes N yr^{-1} in the early 1990's, towards estimates of $195*10^6$ tonnes yr^{-1} in 2050 [Galloway et al., 2004]. Since most ecosystems are N-limited, it is expected that higher N availability will increase forest productivity and thus potentially also increase the carbon (C) sink of these forests. This C and N interaction between climate and vegetation is incorporated in various models. However, there are a number of studies showing that a higher N availability only leads to little or even no enhanced growth. Forest inventory data from five US states reports over the past century very little growth enhancement that could be attributed to any kind of environmental change [Thomas et al., 2010]. Many factors may affect the productivity and the C sink of forests, such as water, CO_2, temperature, presence of micro-organisms and other nutrients such as phosphorous (P). For predicting the ability of forest to mitigate climate change, it is necessary to find the arguments for the positive or negative response of NPP to higher N availability.

1.1 Mycorrhizal fungi

One of the factors that might influence the response of tree growth to additional N are mycorrhizal fungi, which play a major role in N acquisition in most temperate tree taxa [Courty et al., 2010]. On a global scale, between 86% and 94% of plants are mycorrhizal [M. C. Brundrett, 2009] and the fungi can be found in a wide range of habitats, including aquatic ecosystems, deserts, lowland tropical forests, high altitudes, high latitudes and in canopy epiphytes [M. F. Allen, 1991]. Mycorrhizal fungi live on, in and among plant roots and form mutualistic relationships with the host plants. The mycorrhizae receive carbohydrates from their plant host and in return enhance the supply of critical nutrients, both N and P, to the plant [Smith and Read, 1997]. Plants with mycorrhizal associations have a larger absorbing area for nutrient uptake because they are connected with the hyphae network. Therefore they have a higher nutrient uptake than non-mycorrhizal plants and lower chance of local nutrient and/or soil water depletion [M. F. Allen, 1991; Johansen et al., 1992]. The two most abundant mycorrhizal associations are arbuscular mycorrhizae (AM) and ectomycorrhizae (ECM) [Smith and Read, 1997]. The type of mycorrhizal fungi can be influencing the response of tree growth on N deposition. A large tree growth analysis, 24 tree species, from Thomas et al. [2010] showed that all five AM tree species increased tree growth with N deposition, while the ECM species responded both positive and negative on the higher N availability.

1.2 AM versus ECM

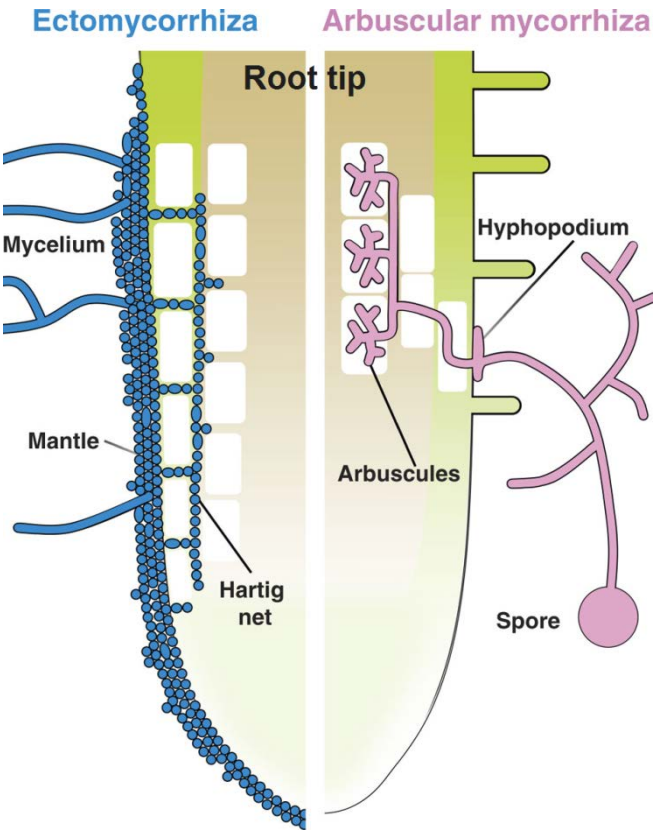

Figure 1-1 Root colonization structures of ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi. ECM fungi covers the root tip with a mantel of hyphae whereas AM fungi develop hyphae from a spore and produce arbuscules within root cortex cells (Bonfante and Genre 2010).

While both AM and ECM fungi transfer N and P to plants in exchange for C, they have different morphologies, growth patterns, biochemistry and nutrient capturing mechanisms [Smith and Read, 2008]. A large difference between the two fungi is that only ECM are able to break down complex organic substrates. The decomposition of soil organic matter (SOM) consists of two steps: (i) the breakdown of complex polymeric organic substrates into monomers or oligomers by extracellular enzymes, and (ii) the metabolism of these small compounds and the release of CO_2 by soil microbes. While both ECM and AM can take up amino acids or other simple organic compounds from the soil, only ECM can produce extracellular enzymes to execute the first step [Chalot and Brun, 1998; Read and Perez-Moreno, 2003]. Organic decomposition by ECM instead of other micro-organisms has the advantage that N losses are minimal because the amino acids and inorganic N released from decomposition are trapped in a matrix created by the ECM fungi [J. E. Hobbie and Hobbie, 2006]. Therefore N can be directly taken up by the fungi and transferred to the host plant. ECM associated plant species thus have more access to the organic N pool compared to AM plants.

Another contrasting difference is that AM hyphae are thinner and have a relative larger fungal network compared to ECM. Therefore have AM hyphae a larger absorbing area and access to a larger soil volume relative to ECM fungi [Smith and Read, 2008]

1.3 AM and ECM plant species

Not only have AM and ECM different characteristics, the plants associated with either AM or ECM fungi show differences as well, which lead to advantages towards nonmycorrhizal plants. In general, AM plant species depend mostly on inorganic N sources and ECM plant species are more specialised on organic N.

AM plant species occur in predominantly mineral mull soils with higher pH. These soils have greater availability of inorganic N but are often poor in inorganic P in comparison with soils occupied by ECM plant species [Read, 1991]. AM plant species produce leaf litter with low

C:N ratio [Cornelissen et al., 2001; E. A. Hobbie, 2006] which supply the micro-organisms with relatively high N and stimulates higher rates of decomposition. The high investment of N in the leaves results in higher N availability through the fast decomposition. Furthermore are AM hyphae thin and have a relative higher absorbing area compared to fine roots, and therefore it is more efficient for AM plants to allocate C to the fungi than investing C in the production of fine roots [Koide, 1991].

ECM plant species contribute to the organic N pool by producing litter with a relative high C:N ratio that decomposes slowly [Cornelissen et al., 2001]. In the forest soils where ECM plants occur, the slow decomposition rate results in the accumulation of nutrients in organic form, creating a thick organic layer in the upper part of the soil [Phillips et al., 2013]. Although the ability of ECM plant species to tap resources from the organic N pool is an enormous potential advantage compared to plants without ECM, the ECM plant species are not dominant in most terrestrial ecosystems. It has been suggested that the carbon cost for receiving N from organic matter through ECM is too high for ECM plant species to become dominant. The production of enzymes by ECM for decomposition requires a large amount of C from the host plant. The relative high C cost for N for ECM plant species may constrain the potential release of N from organic N and therefore total N uptake by the plant [Talbot and Treseder, 2010].

1.4 Mycorrhizal fungi response on N deposition

Increasing anthropogenic N deposition urges the understanding how mycorrhizal fungi influence the response in tree growth on N deposition. Although some studies propose that higher N availability makes the plant less dependent on the mycorrhizae, leading to a decrease of fungal biomass [Treseder, 2004; Van Diepen et al., 2007; van Diepen et al., 2011; Aponte et al., 2013], other studies show that the change of C and N transfer with N deposition depends on the type of mycorrhizae and initial nutrient availability [Treseder and Allen, 2000].

Studies on AM fungal growth response on N fertilization showed that AM fungi decreases with higher N availability [Treseder, 2004; Van Diepen et al., 2007]. When AM fungi are N-limited, however, the additional N is taken up by the fungi for its own growth, thus increasing AM biomass and retaining N from the plant while increasing the demand for C (figure 1-2). According to Johnson [2003] higher N availability in P-limited soils increases AM biomass, but decreases when P is sufficient.

ECM fungi on the other hand are not responding to N deposition according to Näsholm et al. [2013]. Their research used isotopic labelling for tracing ^{15}N and ^{13}C for studying the C and N transfer between fungi and host plant. They conclude that ECM are less efficient to immobilize N after fertilization as more of tracer N is found in the leaves in the fertilized tree species. As ECM are more specialized in taking up organic N, they may be less responsive to a sudden pulse of inorganic N. Thus, higher N availability in N limiting conditions can lead to more N uptake by ECM plant species, while this would not be the case for AM plant species.

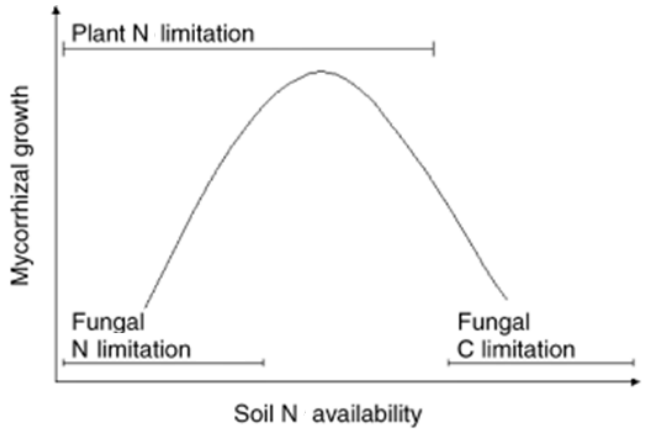

Figure 1-2 Effect of nutrient availability on mycorrhizal growth for AM. N-limited AM fungi increases biomass with higher nutrient availability and continue to increase as long as the plant is N limited and will allocate C to the fungi in return for N. In nutrient-rich soils, the plant is less depending on the fungi and allocate less C to the fungi, making the AM fungi C-limited and decreasing mycorrhizal growth. Adjusted from: Treseder & Allen, 2002

1.5 Scope of research

Even though mycorrhizal associations play a major role in N acquisition in most temperate tree taxa [Courty et al., 2010], the interaction between the fungi and the host and the differences between AM and ECM are still uncertain. Most studies have been focusing on plant species that are associated with either AM or ECM fungi, but to my knowledge only a few studies have examined the differences in plant growth between AM and ECM plant species in the field [Cornelissen et al., 2001]. Attempts have been made to compare studies on AM and ECM plant species but the research areas may vary in climate, ecosystem and/or type of plant species. In the research presented here I examine both AM and ECM tree species occurring in the same forest. Just as most temperate and boreal forests, this forest is N-limited [Nadelhoffer and Raich, 1992]. This temperate deciduous forest is located in Indiana, USA, and is a teaching preserve from the Indiana University in Bloomington. This study is part of a larger fertilization experiment, which started adding N fertilizer in May 2011. This research analyses the differences in tree growth between AM and ECM plant species and how tree growth alters with higher N availability. Tree growth is represented here by stem growth, leaf production and fine root production and indicates the C sink of the forest and the N uptake by trees (figure 1-3).

Besides measuring tree growth to study whether higher N availability is leading to more tree growth for AM and ECM tree species, a model is used to examine how the N uptake of AM and ECM trees is influenced by the mycorrhizae. The resource optimization model FUN (Fixation and Uptake of Nitrogen) simulates plant N acquisition based on the theoretical framework of C cost economics, where plants spend C as low as possible for N while maximizing its growth [Fisher et al., 2010]. As mycorrhizal fungi are not included in the original model, some adjustments are made to simulate the relation between the fungi and the plant. Data from field measurements were used as an input for the model to simulate how total N uptake and C available for growth is different for AM and ECM trees, with and without higher N availability. Model outputs compared with the results of the field experiments contribute to a better understanding of the interactions between AM and ECM with their host plants and how mycorrhizae can influence the C and N cycle of the plant.

Their role in the C and N interactions between plant and climate can be of influence in climate and vegetation models. With environmental conditions changing rapidly it is necessary to estimate future C storage in terrestrial ecosystems and their ability to mitigate climate change.

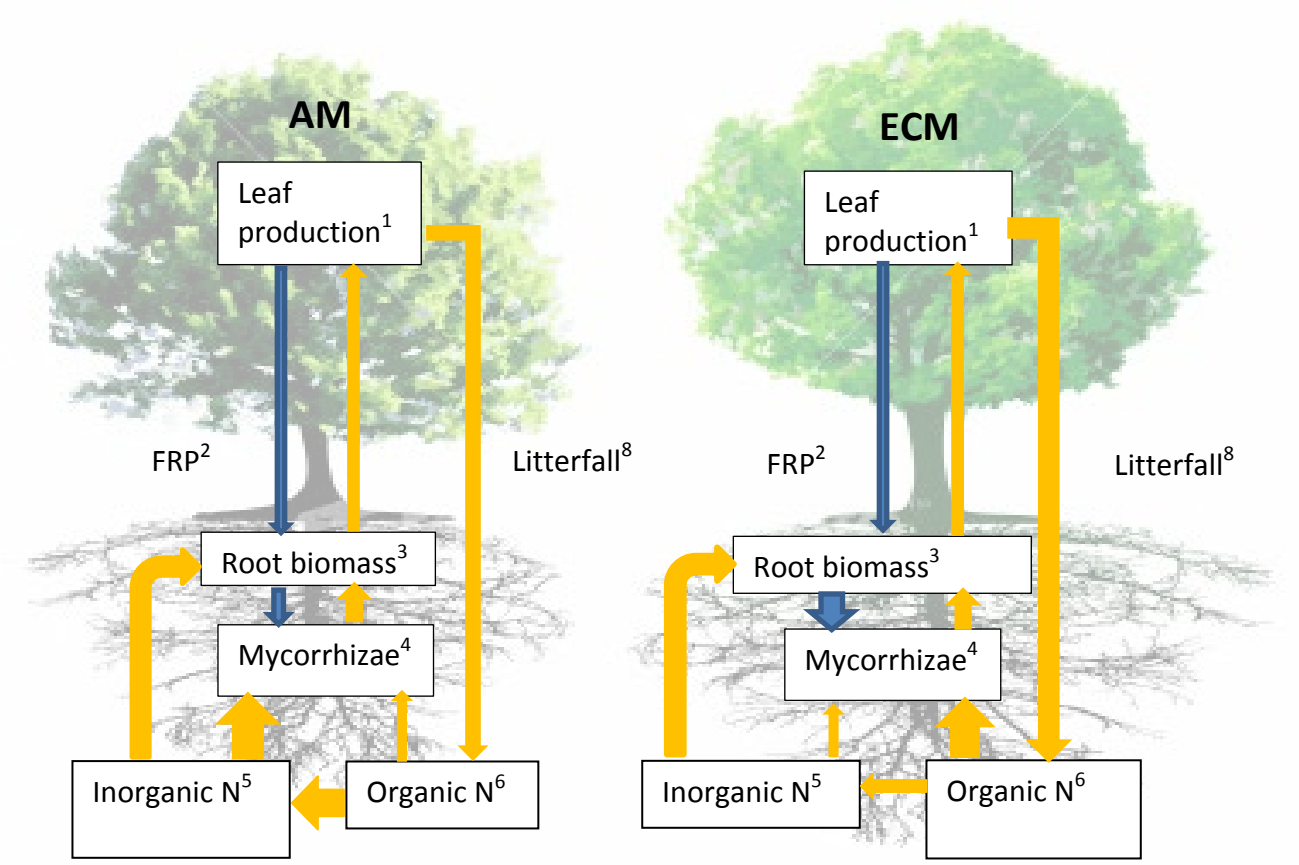

Figure 1-3 Carbon (C, blue arrows) and nitrogen (N, orange arrows) fluxes and pools in AM and ECM plots addressed in this research. Size of the arrow and boxes represent the relative size compared to the other mycorrhizal type. (1) Leaf production (g C $m^{-2}yr^{-1}$ and g N $m^{-2}yr^{-1}$) is a function of measured LAI, measured C & N concentrations and standard SLA values. (2) FRP (fine root production, g C $m^{-2}yr^{-1}$) is the carbon allocated to root biomass. (3) Root biomass and C & N concentrations are measured. (4) Mycorrhizal biomass, growth and turnover is unknown. (5) Inorganic N concentrations and mineralization rate (ammonium and nitrate) were measured by M. Midgley [2013 *unpublished*]. (6) Organic N is related to Inorganic N with ratio inorganicN:organicN relation with ECM basal area from Philips et al. [2013]. (7) C & N concentrations in litter, data available from 2011.

1.6 Research question

This research analyses the differences in tree growth between AM and ECM trees and how this alters under higher N availability, thereby providing an answer on the following research question:

Do AM and ECM trees differ in tree growth under increased N availability, located in a Northern American hardwood forest?

The general hypothesis is that **AM trees grow relatively more than ECM trees** due to the higher inorganic N availability in soils with AM species and relative high C cost for N acquisition for ECM trees. However, in an N-limited forest I expect a different responds in tree growth to N fertilization between AM and ECM trees. Therefore I state a second hypothesis that **ECM trees increases tree growth with higher N availability whereas AM trees decreases tree growth**, because AM fungi take up the added N and increases demand

for C from the plant for its own growth, while ECM fungi do not respond on the added N and the tree takes up up the added N and allocate less C to the fungi.

Besides tree growth I also studied differences in N allocation and C:N ratios between AM and ECM trees. With this I attempted to get more insight in the N uptake of the trees, the role of the mycorrhizal fungi in the N uptake of the tree and how this alters under higher N availability.

2. Material and Methods

2.1 Study area

The research area is located in a central hardwood forest in Indiana, USA. The experiments are carried out in Moores Creek, a Research and Teaching Preserve of Indiana University. The Preserve is a temperate deciduous forest, about 80 year-old, located near Bloomington, IN (39°09'N 86°31'W), and with elevation ranging from 165 to 230 m AMSL. The climate is humid continental, with mean annual precipitation of 1139 mm (yrs 2008-2010) and mean annual temperature of 11 °C (average from 2008-2010) [AmeriFlux, 2013]. Soils are thin, unglaciated inceptisoils, derived from siltstone, shale and to a lesser extent limestone [AmeriFlux MMSF, 2013a].

ECM	AM
American Beech *(Fagus grandifolia Ehrh.)*	Sassafras *(Sassafras albidu)*
Black Oak *(Quercus velutina Lam.)*	Sugar Maple *(Acer saccharum Marshall)*
Pignut Hickory *(Carya glabra P. Mill.)*	Tulip Poplar *(Liriodendron tulipifera L.)*
Red Maple *(Acer rubrum L.)*	
Red Oak *(Quercus rubra L.)*	
White Oak *(Quercus alba L.)*	

Table 2-1 List of common AM and ECM tree species in Moores Creek, Indiana, USA

Trees in Moores Creek are associated with either AM or ECM fungi according to previously described mycorrhizal designations [M. Brundrett et al., 1990; Wang and Qiu, 2006]. The most common AM and ECM tree species within the research area are listed in table 2-1. Plots of 20 m x 20 m have been established with greater than 80% of the basal area containing either AM or ECM trees (figure 2-1).

This study is part of a larger fertilizer experiment carried out by PhD student M. Midgley. The fertilized plots have been treated with NH_4SO_4 and $NaNO_3$ granular fertilizer at a rate of 50 kg N $ha^{-2}{\cdot}yr^{-1}$ and the unfertilized plots are untreated. Since May 2011 through August 2013 fertilizer has been added to the plots in the months May through October, once every four weeks. The rate of 50 kg N ha^{-2} yr^{-1} is equivalent to the deposition rate projected to occur across large areas of the Midwest and Northeast by 2050 [Galloway et al., 2004]. Currently, Moores Creek is exposed to low levels (~5 kg $N{\cdot}ha^{-2}{\cdot}yr^{-1}$) of ambient N deposition [Holland et al., 2005]. Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations and net mineralization rates in the AM and ECM plots, unfertilized and fertilized, are measured by M. Midgley and the results are listed in table II of Appendix C.

In the experiments a distinction is made in mycorrhizal type and fertilization treatment, creating four different groups: ectomycorrhizae unfertilized (ECM UF), ectomycorrhizae fertilized (ECM F), arbuscular mycorrhizae unfertilized (AM UF) and arbuscular mycorrhizae fertilized (AM F). In the research area 7 pairs of each mycorrhizal type are present; where each pair consists of an unfertilized and fertilized plot, see figure 2-1.

Figure 2-1 Map of the research area in Moores Creek, Indiana, USA. Research area consists of 7 AM pairs and 7 ECM pairs, where each pair consists of two 20x20 m plots: unfertilized (UF) and fertilized (F). Pair AM 1 and pair ECM 3 are left out of the study due to extreme slope and relative low total basal area of trees

2.2 Field measurements

Tree growth in this research is represented by stem growth, fine root production (FRP) and leaf production, and indicate the relative carbon allocation of AM and ECM trees. Although fine roots are only a small part of total tree biomass, they are an important regulator of the complex nutrient cycling belowground. FRP indicates the C allocated to the roots, but the root biomass indicates the size of the root network for N uptake. Therefore root biomass is also measured in this research.

The ratio of C allocated to roots versus C allocated to the leaves, also called root-shoot ratio, is an important indicator for the resource demand of the plant. Investing more carbon in the production of leaves makes the plant more able to intercept photosynthetic active radiation and increases photosynthetic rate. When a plant allocates more carbon to the roots to extend the root network, it can take up more N. How root-shoot ratio changes with higher N availability indicates whether C is invested to gain more N or more C. How FRP, leaf production and stem increment was measured is described below.

Fine root production & root biomass

Fine root production (FRP, g C·m^{-2}·yr^{-1}) was measured by using the ingrowth core method [Mancuso, 2012]. The ingrowth cores can be seen as a bag with 2 mm mesh that allows the growth of new fine roots inside the core. The ingrowth cores were 15 cm deep and 5 cm in diameter and contained root-free soil: 50% soil originating from the plots and 50% sand. Four ingrowth cores per plot were placed randomly in the end of April. The mesh permits the ingrowth of fine roots smaller than 2 mm. After three months, the ingrowth cores were taken out of the soil and brought to the lab for analyses.

Fine root biomass (g C·m^{-2}) was measured by taking multiple soil core samples directly from the plot. Using a soil corer, four soil core samples of 15 cm deep and 5 cm in diameter were taken from each plot. Soil core samples were taken three times during the growing season, in the months April, June and August. The soil core samples were brought to the lab for analyses.

The soil core samples collected for measuring root biomass and the ingrowth cores for measuring fine root production were analysed with the same method. First, the soil samples were divided into two soil layers: 0-5 and 5-15 cm. The upper soil layer contains more nutrients and fine roots than the lower soil layer and is in terms of N uptake relatively more important than the deeper soil layer, and therefore the soil layers were analysed separately. After dividing the two soil layers, roots were separated from the soil, washed and cleaned. Roots were separated into life versus death roots and fine (<2 mm) versus coarse (>2 mm) roots. The samples were oven-dried at 60 ^{o}C, weighted and grinded. These samples were analysed for C and N concentrations using a Costech Elemental Combustion System.

The measured dry root biomass from the soil core samples were converted to a measure of g C per m^2 by dividing the fine roots (g dry biomass) by the surface area of the core and multiplied by the C concentration of the fine roots:

$$C_{root} = \frac{M_{root}}{A} * p_R \quad (1)$$

where C_{root} (g C·m^{-2}) is the root biomass, M_{root} is the dry biomass of fine roots (g), A is the surface area (m^2) and p_R is the C concentration in the fine roots.

FRP (g C·m^{-2}·yr^{-1}) from the ingrowth cores was calculated similar as root biomass, but divided by dt as FRP is a production over time:

$$FRP = \frac{\frac{M_{root}}{A} * p_r}{dt} \quad (2)$$

The increment cores were left in the soil for 3 months and the C_{root} from these cores is the production of fine roots over these 3 months. Assuming fine roots are only produced during the growing season of 6 months, from April through September, dt is then 3/6= 0.5.

Leaf production

Leaf production (g C·m^{-2}·yr^{-1}) was calculated by measuring the Leaf Area Index (LAI) and leaf C and N concentrations, and using standard tree specific SLA values. LAI is a quantity of green leaf area per unit ground surface area (m^2/m^2) and also an indicator for photo-synthetic capacity. The optical instrument Li-Cor LAI-2000 measured the amounts of diffuse light penetrating the canopy, and was used at the end of July when canopy density reached a maximum. The software FV2000 converted the measured solar radiation into LAI-values.
C and N concentrations of the leaves were measured from fresh leaves collected in July. These leaves were dried in the oven at 60 ^{o}C, grinded and analysed for C and N concentrations with the Costech Elemental Combustion System.
SLA is the ratio of leaf area by the dry weight and is a widely accepted key leaf characteristic. SLA values of the dominant tree species in the plots were available from previous measurements in the Morgan Monroe State Forest (AmeriFlux MMSF, 2013b).
Because SLA and C and N concentrations are values per tree species, the relative basal area (RBA) of tree species per plot was used to calculate the weighted average SLA value and C and N concentrations per plot. Leaf production was calculated with the following formula:

$$C_{leaf} = \frac{\sum \frac{LAI}{SLA} * p_L}{dt} \quad (3)$$

where C_{leaf} is the leaf production (g C·m^{-2}), *LAI* the leaf area per area (m^2·m^{-2}), *SLA* is the specific leaf area per plot (m^2 g^{-1}), p_L is the average carbon concentration of the leaves per plot and *dt* the time of production, which is 1 since the leaves are produced once a year.

Stem growth

Stem growth is the increment of dry biomass at the stem and is reported in three ways: (1) the width of the tree ring (mm), (2) the relative growth rate (RGR) which is the increase of basal area (BA) of the stem in terms of percentage, and (3) the total stem increment per plot (kg C·m^{-2}·yr^{-1}).
The width of the tree rings were measured by taking increment cores in July. Due to time restrictions, it was not possible to take increment cores from each tree. Therefore from each plot, five trees with a minimum of 10 cm in DBH (Diameter at Breast Height) were selected which represented the dominant tree species in the plot. Since trees are not growing symmetrical, increment cores were taken from two sides: uphill and downhill. From the increment cores, the width of the last 6 growth rings were measured, representing the years from 2008 to 2013, to include both years with (2011-2013) and without (2008-2010) fertilization. The cores were sanded to make the growth rings better visible. WinDENDRO Image Analyse System is used to scan the increment cores and measure the width of the tree rings. Stem increment of the year 2013 was not possible to measure since the tree rings were not fully grown in July.

Because tree species have different growth characteristics and the initial size of a tree determines the width of the tree ring, the RGR is calculated as a relative measure for tree growth:

$$RGR = \frac{BAI}{BA_{13}} \quad (4)$$

where *BAI* stands for basal area increment and BA_{13} is the basal area calculated from the DBH of the tree.

Total stem increment per plot is calculated using the DBH of all trees, tree specific allometric equations and C concentrations of woody tissue. From all trees in the plots, DBH was measured to calculate their BA_{13}. Stem increment of the unsampled trees were estimated by multiplying average RGR for each tree species, both unfertilized and fertilized, by the BA_{13} (Eq. 4). The BAI of the trees were converted into dry biomass using tree specific allometric equations (see Appendix A). C and N concentrations of the trees were measured only from tree core samples from unfertilized plots due to time restrictions. C and N concentrations of the cores were measured by drying (60 °C) and grinding up the tree cores, using a Thomas Wiley mill with a 0.5 mm^2 screen. The samples were analysed with a Costech Elemental Combustion System. Total stem increment per plot in terms of [kg C m^{-2} yr^{-1}] is the sum of C increment of all trees in the plot divided by the size of the plot (20 m^2).

2.3 The FUN model

The influence of mycorrhizal type on the N uptake of trees is examined by an adjusted version of the Fixation and Uptake of Nitrogen (FUN) model. FUN is a resource optimization model that simulates plant N acquisition based on the theoretical framework of C cost economics [Fisher et al., 2010]. Mycorrhizal fungi was not incorporated in this model yet, and adjustments to the model were made to simulate the role of mycorrhizae on the total N uptake by the plant. First, the most important parts of the original FUN model are described, followed by the explanation of the adjustments in the model.

Model description

In the original model, N can be acquired through four pathways: (1) through advection, also called passive uptake ($N_{passive}$), (2) resorbtion of N from the leaves (N_{resorb}), (3) active uptake (N_{active}) and (4) uptake by biological N fixation (N_{fix}) (figure 2-2). $N_{passive}$ is the N taken up through the transpiration stream and therefore has no explicit carbon cost for the plant. N_{resorb} is the resorption of N from leaves before senescence or leaf fall. Resorption requires C to synthesize the enzymes and regulatory elements that degrade and remobilize leaf nutrients, and to drive the translocation stream in which the nutrients are suspended [Fisher et al., 2010]. Active uptake is an ion-specific enzyme catalysed process that uses a large amount of respiratory C to move N against concentration gradients [Fisher et al., 2010]. The fourth pathway is biological N fixation (BNF), performed by bacteria living in symbiosis within root nodules on certain types of plants. Atmospheric nitrogen is converted into ammonia (NH_3) and further into ammonium (NH_4^+) by enzymes. The plant takes up the available N and in return they give carbohydrates to support the bacteria and the process.

First, the model calculates the demand for N (N_{demand}, kg N·m^{-2}·yr^{-1}) for the plant, based on the available C from net primary productivity (C_{NPP}, kg C m^{-2}·yr^{-1}) and the C:N ratio ($r_{C:N}$) of the plant:

$$N_{demand} = \frac{C_{NPP}}{r_{C:N}} \quad (5)$$

Passive nitrogen uptake ($N_{passive}$, kg N·m^{-2}·yr^{-1}) does not require any carbon costs and is the first source of N for the plant. $N_{passive}$ is dependent on the transpiration stream and the N availability in the soil:

$$N_{passive} = \min(N_{soil} * \frac{ET}{s_d}, N_{soil}) \quad (6)$$

The transpiration stream is a function of the transpiration rate *ET* (m·yr^{-1}) and the available water in the soil (s_d, m). N_{soil} (kg N·m^{-2}) is the sum of nitrate and ammonium available in the soil. As $N_{passive}$ is restricted to the N available in the soil it can never exceed N_{soil}. The function *min(-,-)* sets the maximum of $N_{passive}$ to N_{soil} by returning only the smallest value of the two elements between the brackets.

When N_{demand} of the plant is not met by passive uptake alone, the plant uses resorption, active uptake, and/or BNF to increase N uptake. First, the carbon cost for active uptake, resorption and BNF is calculated. Active uptake ($Cost_{active}$, kg C·kg N^{-1}) is a function of N availability in the soil and root biomass:

$$Cost_{active} = \left(\frac{k_N}{N_{soil}}\right) * \left(\frac{k_C}{C_{root}}\right) \quad (7)$$

where k_N and k_C are both 1 (kg C·m^{-2}), N_{soil} is the nitrogen content in the soil (kg N·m^{-2}) and C_{root} is the fine root biomass (kg C·m^{-2}). It is a simplistic equation where low N availability in the soil and/or low root biomass leads to a high cost for N uptake. In contrast, when there is a high N availability and/or high root biomass, the cost are lower.

The carbon cost for resorption of N from the leaves, $Cost_{resorb}$ (kg C·kg N^{-1}), is dependent on the N available in the leaves (N_{leaf}, kg N·m^{-2}):

$$Cost_{resorb} = \frac{k_R}{N_{leaf}} \quad (8)$$

$Cost_{resorb}$ is negatively related to the N availability in the leaves: higher N_{leaf} results in lower carbon costs. The parameter k_R is set to 0.01 and is based on global observations [Fisher et al., 2010].

For the cost of biological fixation, $Cost_{fix}$ (kg C·kg N^{-1}), is the equation of [Houlton et al., 2008] for normalized nitrogenase activity as a function of the temperature of the soil (T_{soil}) combined with the observed C cost range as constrained by Gutschick [1981]:

$$Cost_{fix} = s\left(\exp\left(a + b * T_{soil}\left(1 - 0.5\frac{T_{soil}}{c}\right)\right) - 2\right) \quad (9)$$

where *a, b,* and *c* (-3.62, 0.27 and 25.15, respectively) are empirical curve-fitting parameters given by Houlton et al. [2008]; *s* is -5 times the Houlton et al. [2008] scaling factor of 1.25 (= -6.25), which inverts the Houlton et al. [2008] equation and constrains it between 7.5 and 12.5 kg C·kg N^{-1} [Fisher et al., 2010].

FUN compares the different cost for N acquisition and uses the cheapest source for N uptake until N demand is met. C from photosynthesis (C_{NPP}) is used as efficient as possible, with the optimum balance of C allocation for N acquisition (C_{acq}, kg C·m^{-2}), and C allocation to tree growth ($C_{growth,}$ kg C·m^{-2}). The optimization of resource C is simulated by calculating the following three equations simultaneously:

$$C_{growth} = C_{NPP} - C_{acq} \quad (10)$$

$$N_{acq} = \frac{C_{acq}}{Cost_{acq}} \quad (11)$$

$$r_{C:N} = \frac{C_{growth}}{N_{passive} + N_{acq}} \quad (12)$$

The C available for tree growth is the difference between plant NPP and the C cost for N acquisition (Eq. 10). The amount of N acquired in Eq. 11 (N_{acq}) is the C available for C acquisition divided by the C cost for N acquisition. The C:N ratio of the plant is the C available for tree growth divided by the total uptake of N, thus N acquisition plus passive N uptake (Eq. 12).

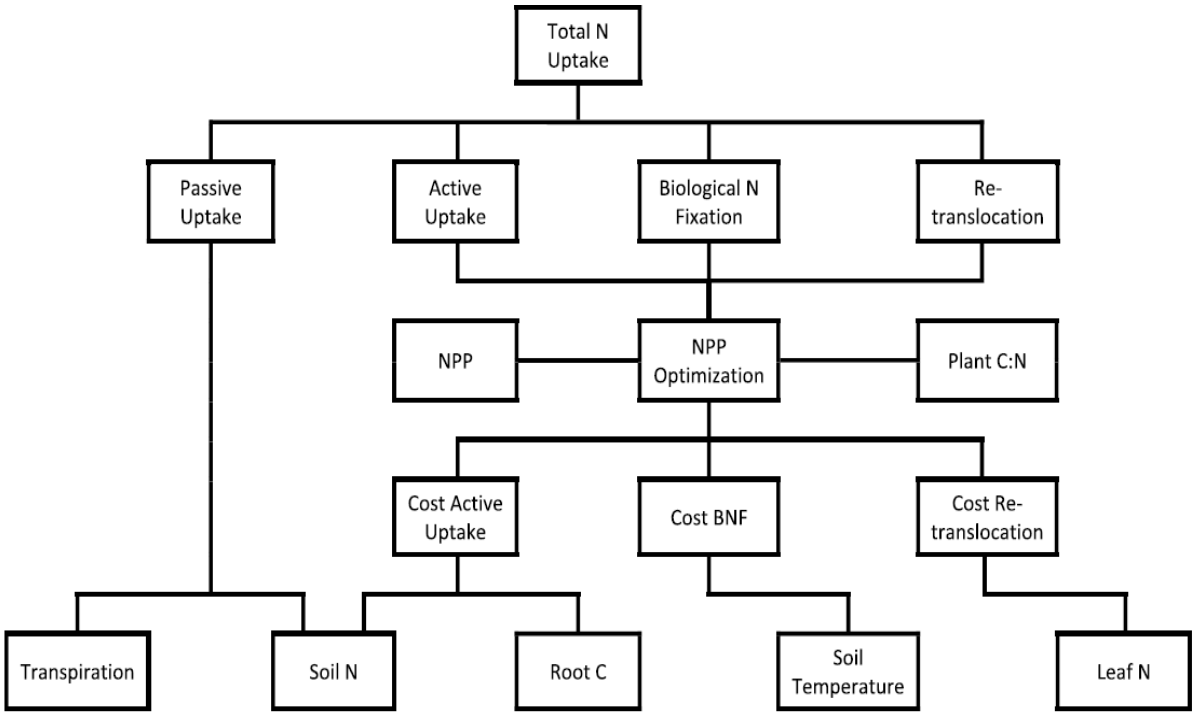

Figure 2-2 Structure of the original FUN model. Total N uptake is the sum of passive N uptake via the transpiration stream, active N uptake through respiratory expenditure, biological N fixation by microbes and retranslocation of N from the leaves. The model optimizes C allocation for N acquisition and tree growth. Source: Fisher et al., 2010.

Model adjustments

There is no unifying framework that accurately predicts the amount of N given in exchange for C [Fisher et al., 2010]. An attempt is made to incorporate mycorrhizal fungi in the model through adjustments in the cost functions for N acquisition. Because fungal hyphae are connected to plant roots and form a pipeline that transfers nutrients directly to the plant, the hyphae act more as a root extension network than as a part of the microbial biomass [M. F. Allen, 1991]. Therefore AM and ECM are incorporated in the model by adjusting the k_N and k_C parameters in the cost function for active N uptake (Eq. 7). AM fungi mostly benefits plant N uptake by increasing the absorbing area and this lowers the costs for AM plants active uptake. Therefore is k_C lower than k_N for AM trees in the function of $cost_{active}$ (Eq. 7). ECM fungi benefits plant uptake N mainly by mobilizing N from complex organic substrates, and thus lower the costs associated with soil inorganic N availability for ECM trees. Therefore is k_N higher than k_C for ECM fungi in Eq. 7 [Brzostek, 2013 *unpublished*]. Furthermore, the cost for active N uptake was made more sensitive for root biomass and soil N availability by taking the square number from N_{soil} and C_{root}:

$$Cost_{active} = \left(\frac{k_N}{{N_{soil}}^2}\right) * \left(\frac{k_C}{{C_{root}}^2}\right) \quad (13)$$

In the original model, the pathway with the lowest C cost for N acquisition is chosen first, and when N_{demand} of the plant is not met, the next cheapest source of N is used until N_{demand} is met. This results in either a high amount of active uptake, resorption or biological fixation, with unrealistic sudden shifts towards the pathway with the lowest C cost. To simulate N uptake through active uptake, biological fixation and retranslocation simultaneously, a parallel resistance network based on Ohm's law is included in the model (figure 2-3). The combined resistor in the parallel network is in the model represented by the combined cost for N uptake ($cost_{acq}$), where the combined cost is always lower than the pathway with lowest C cost for N acquisition [Brzostek, 2013 *unpublished*]:

$$\frac{1}{Cost_{acq}} = \frac{1}{Cost_{active}} + \frac{1}{Cost_{resorb}} + \frac{1}{Cost_{fix}} \quad (14)$$

$Cost_{acq}$ (kg C·kg N^{-1}) is used in Eq. 11 to determine the optimum balance for C allocated for N acquisition and growth.

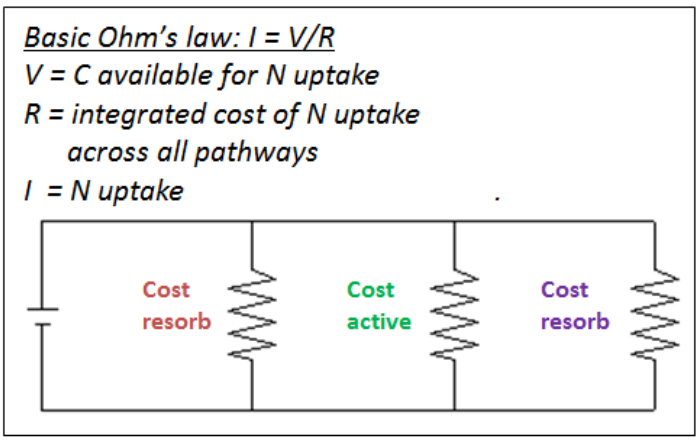

Figure 2-3 Parallel resistance network integrated in the model based on Ohm's law. The calculation of integrated carbon cost for N acquisition through retranslocation, active uptake and biological fixation is equal to the calculation of the combined resistance from Ohm's law. Adjusted from: Brzostek, 2013 *unpublished*

Input variables

Most input variables for the model are measured variables from the plots (table 2-2) while others are estimated. NPP (C_{NPP}) is estimated from the tree growth in the plots. Average tree growth was 4.5 kg C·m^{-2}· yr^{-1} and because plants invest an additional amount of NPP in N acquisition, C_{NPP} was estimated on 5.5 kg C·m^{-2}· yr^{-1}. C_{NPP} is for each plot the same for comparison of C allocation and N uptake between the plots. Also, LAI and leaf production, two indicators for photosynthetic rate, do not show variation between AM and ECM plots, with and without fertilization and thus indicate an equal amount of C available.

C_{root} (kg C·m^{-2}) is the root biomass measured from soil core samples, as described earlier. N_{leaf} is the leaf production as from Eq. 3 but with the C concentration replaced by the N concentrations measured in the leaves. Plant C:N ratio ($r_{C:N}$) is the total C measured in tree growth per plot divided by the total N measured in tree growth per plot. N_{soil} (kg N·m^{-2}) is the inorganic N availability and is equal to the net mineralization rate because it represents the amount of N that becomes available for the plant. Because net mineralization rate is a flux, N_{soil} is the amount of N that becomes available per year since the model runs on a yearly timescale.

	Definition	Unit	Source
Input variable			
C_{NPP}	net primary production	kg C·m^{-2}· yr^{-1}	Estimated from data 2013
C_{root}	root biomass	kg C·m^{-2}	Data 2013
ET	transpiration	m yr^{-1}	Fisher et al. [2010]
N_{leaf}	N content in leafs	kg N·m^{-2}	Data 2013
N_{soil}	Inorganic N available in soil	kg N·m^{-2}	Midgley, 2013, *unpublished*
$r_{C:N}$	C:N ratio of plant	kg C·kg N^{-1}	Data 2013
sd	soil water depth	m	Fisher et al. [2010]
T_{soil}	Temperature of the soil	°C	Brzostek, 2013, *unpublished*
Output variable			
$Cost_{acq}$	Carbon cost for N acquisition	kg C·kg N^{-1}	
$Cost_{active}$	Carbon cost for active N uptake	kg C·kg N^{-1}	
$Cost_{resorb}$	Carbon cost for N resorption	kg C·kg N^{-1}	
$Cost_{organic}$	Carbon cost for organic N uptake		
C_{acq}	Carbon used for N acquisition	kg C·m^{-2}	
C_{growht}	Carbon available for growth	kg C·m^{-2}	
N_{acq}	Total N acquired	kg N·m^{-2}	
N_{active}	N from active uptake	kg N·m^{-2}	
N_{demand}	N demand	kg N·m^{-2}	
$N_{passive}$	Passive N uptake	kg N·m^{-2}	
N_{resorb}	N from resorption	kg N·m^{-2}	

Table 2-2 Variables and parameters in the model.

T_{soil} is estimated based on the average soil temperature measured in Morgan Monroe State Forest (12.031 °C) which is located 50 km North from the research area, and is similar for all

plots. *ET* and *sd* are similar to the values used by Fisher et al. [2010], with *ET* = 1 (m s^{-1}) and *sd* is 50 (m) in each plot.

Time scale

The adjusted FUN model runs on a yearly time step because most input variables are only available on a yearly base and estimations of monthly or daily input variables will increase the error and uncertainty of the model. However, an iterative cost function of resportion and active uptake is used to allow the cost of resorption and active uptake to increase when more N is taken out of each pool with each step. N_{soil} and N_{leaf} are updated after each step with N_{active} and N_{resorb} respectively, and the model recalculates the cost for resoprtion and active uptake. The amount of steps was set to ten.

Parameter calibration

For the calibration and parameterization of the model, measurements of the field and values obtained from other studies were used. k_r (Eq. 8) was calibrated by resorption values estimated from N concentrations in litter from 2011 [Midgley, 2013 *unpublished*] and N concentrations from the fresh leaves.

k_n *and* k_c in the function $cost_{active}$ were calibrated to fit C_{acq} as a percentage from C_{NPP}. According to Hobbie & Hobbie [2006], between 8 and 17% of plant NPP is supplied to mycorrhizal fungi to maintain the symbiosis. k_n *and* k_c where calibrated to fit C_{acq} in this range of NPP while keeping in AM plots $k_n < k_c$ and in ECM plots $k_n > k_c$ as described earlier. The parameters for BNF was kept similar to the original FUN model. All parameters with their values are listed in Appendix B, table II.

N deposition

Although net mineralization rate is influenced by higher N availability, the sudden pulse of inorganic N is not included in the yearly value of the net mineralization rate. N deposition in the model is simulated by adding the fertilization rate of 5 g $N \cdot m^{-2} \cdot yr^{-1}$ to the mineralization rate measured in the fertilized plots.

2.4 Statistical analysis

Statistical analysis was conducted with the software package IBM SPSS Statistics 20. Statistical significances between response variables were tested with a linear mixed model. Differences between AM UF, AM F, ECM UF and ECM F (n=7) are tested pairwise with the repeated variable 'treatment', unfertilized and fertilized, and Compound Symmetry as repeated covariance type. Variables were tested on fixed effects of 'mycorrhizae', 'treatment', and 'mycorrhizae*treatment'. Mean difference are significant when $p<0.05$. The Pearson correlation coefficient was used to test significant correlation between variables.

3. Results

3.1 Tree growth: AM versus ECM

Total tree growth is mostly represented by stem increment (g C·m^{-2}·yr^{-1}), see figure 3-1a. Unfertilized AM plots have higher stem increment than ECM unfertilized plots ($p<0.001$). The sum of stem increment, leaf production and fine root production as total tree growth is therefore in AM unfertilized plots also higher than ECM unfertilized plots ($p=0.001$). Stem increment per plot is the sum of all the stem growth per plot divided by the area of the plot, and thus depends on the amount of trees in the plots. Total basal area (BA) is however similar between AM and ECM plots, where AM plots have on average a total BA of $13.3*10^3$ m^2 per plot and ECM plots have on average a total BA of $13.2*10^3$ m^2 per plot.

Other reported measurements of stem increment also show that AM trees grow relatively more than ECM trees. Average annual tree ring width is larger for AM unfertilized trees than in ECM unfertilized trees ($p=0.026$, table 3-1). RGR of unfertilized AM trees is on average 0.017, meaning that the basal area of the trees have increased by 1.7 % in 2012. RGR in AM plots is almost twice as high than the average RGR of ECM unfertilized trees (RGR=0.009; $p=0.004$, table 3-1).

Stem increment in unfertilized AM and ECM plots is positively correlated with inorganic N availability (N_{soil}, g N·m^{-2}) but within each mycorrhizal group there is no significant correlation (figure 3-2).

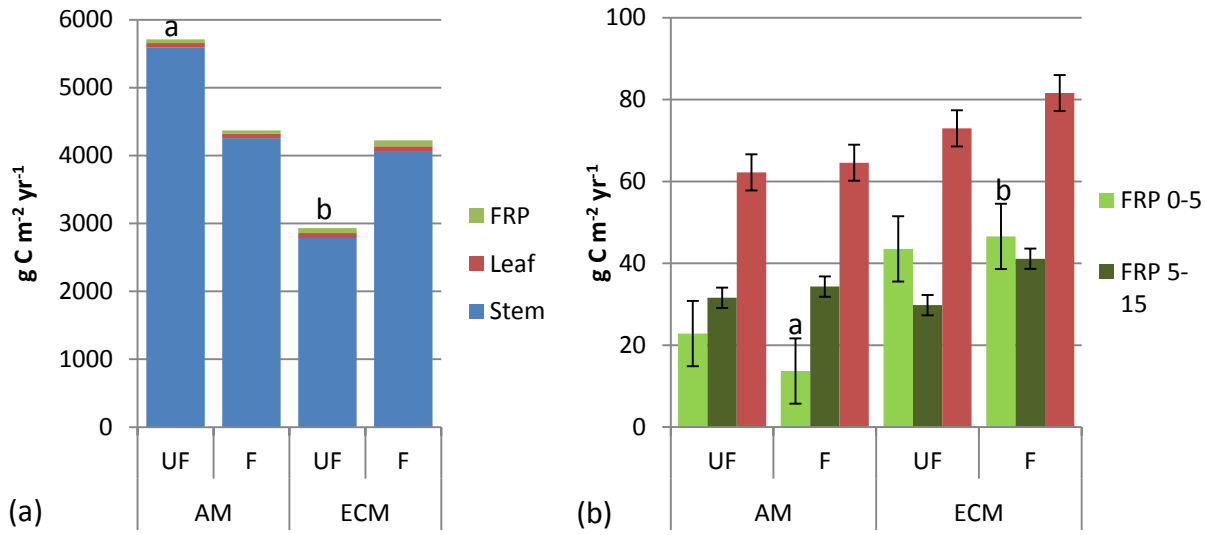

Figure 3-1 Mean total tree growth based on stem increment, leaf production and fine root production in AM and ECM plots, with and without fertilization. Left panel (a) shows total tree growth as the sum of fine root production (FRP), leaf production and stem increment. Stem increment is the largest contribution to total tree growth and for clarification shows figure (b) leaf production and FRP for both soil layers 0-5 and 5-15. Means which share dissimilar letters are significantly different ($p<0.05$).

The fraction of FRP from total tree growth is very small and does not influence the total tree growth. However, fine root production (FRP) is an important indicator for C allocation for N uptake through the roots, and is in ECM plots 3 times higher than in AM plots in the upper soil layer ($p=0.001$, figure 3-1b). FRP in the whole soil layer is higher in ECM plots than in AM plots, but only significant between the AM and ECM fertilized plots ($p=0.017$, table 3-1) and not between the unfertilized plots ($p=0.076$). A similar trend is visible for root biomass, where ECM plots, unfertilized and fertilized plots combined, have on average 2,5 times

higher root biomass than AM plots for the upper soil layer ($p<0.001$, table 3-1). This indicates that root turnover rates are relatively similar between AM and ECM plots.

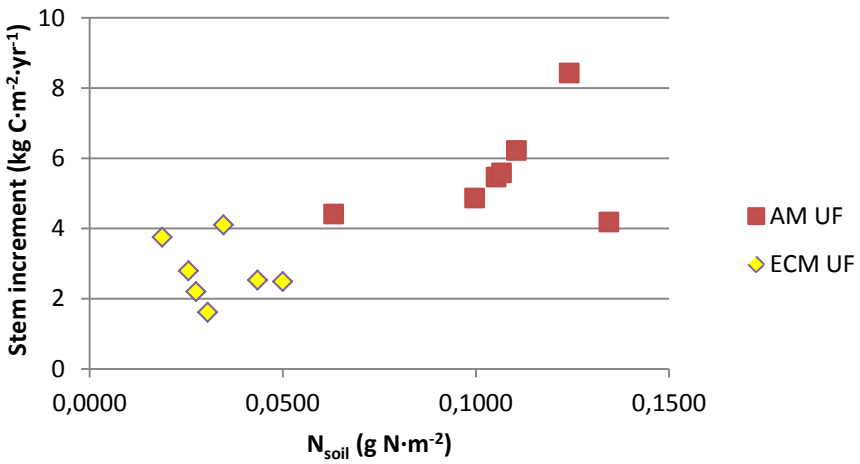

Figure 3-2 Correlation of stem increment with inorganic N availability in the soil for unfertilized AM and ECM plots. N_{soil} (g $N \cdot m^{-2}$) is positively correlated with stem increment (kg $C \cdot m^{-2} \cdot yr^{-1}$) taking AM UF and ECM UF as one group ($r=0.776$, $p=0.001$, $n=14$). Within AM UF and ECM UF is stem increment not significantly correlated with N_{soil}.

3.2 N allocation

The N taken up by a tree can be allocated to the roots, the stem or the leaves. The largest fraction of total N is allocated to the stem since there is the largest growth in biomass (figure 3-3). N in stem increment is in AM unfertilized plots significantly higher than in ECM unfertilized plots ($p=0.013$). N in fine root production and leaf production does not differ significantly between AM and ECM plots (figure 3-3 and table 3-1).

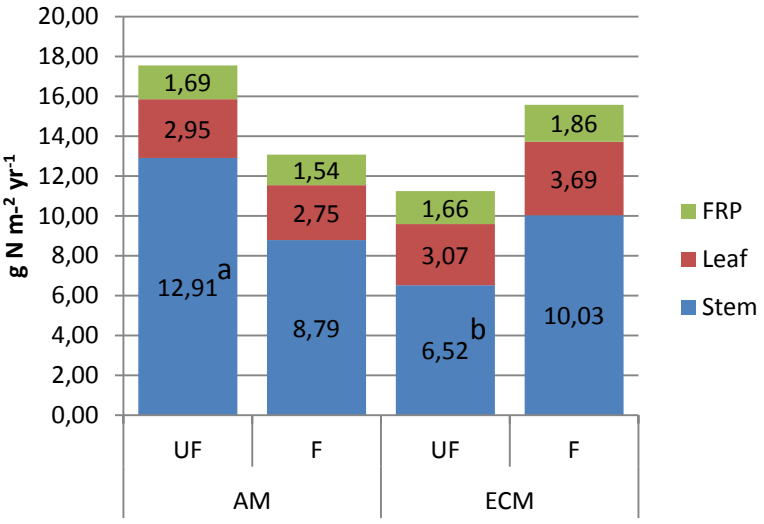

Figure 3-3 Average N allocation to FRP, leaf production and stem increment. Largest N allocation (g $N \cdot m^{-2} \cdot yr^{-1}$) is in stem increment. Means which share dissimilar letters are significantly different ($p<0.05$).

Average total N measured in tree growth per plot is higher in AM unfertilized plots than ECM unfertilized plots ($p=0.013$). The C:N ratio of total tree growth per plot is the total C in tree growth per plot (g $C \cdot m^{-2} \cdot yr^{-1}$) divided by total N in tree growth per plot (g $N \cdot m^{-2} \cdot yr^{-1}$) and is

higher in AM unfertilized plots than in ECM unfertilized plots (p=0.006, table 3-1). AM trees invest relatively more in stem increment than in fine root production compared to ECM trees, and since stem increment has a higher C:N ratio than fine roots it results in higher C:N ratios in AM plots.

FRP and root biomass have significant lower C:N ratios in AM plots than in ECM plots for both the upper and lower soil layer (both p<0.001, table 3-1). Although AM trees have relatively higher N in their fine roots, total N in FRP is not significant different between AM and ECM and N in root biomass is even higher in ECM plots (table 3-1) due to higher fine root production in ECM plots. Leaf C:N ratio does not significantly differ between AM and ECM plots.

3.3 Tree growth and higher N availability

Table 3-1 shows that fertilization does not lead to significant difference in stem growth, FRP and stem increment, for both AM and ECM plots. Fertilization did lead to higher N availability in both AM and ECM plots since inorganic N concentrations (NH_4^+, NO_3^-) in the soil were higher in the fertilized plots compared to the unfertilized plots [Midgley, 2013 *unpublished*]. Although higher N availability does not lead to significant differences between unfertilized and fertilized plots, the response of higher N availability on stem increment differ significantly between AM and ECM plots. Stem increment in AM fertilized plots is on average 1.34 kg $C \cdot m^{-2} \cdot yr^{-1}$ lower than in AM unfertilized plots, thus AM trees show a decrease in stem increment with higher N availability. For ECM plots, stem increment in fertilized plots is 1.27 kg $C \cdot m^{-2} \cdot yr^{-1}$ higher than in unfertilized plots, showing a positive response on higher N availability. Although the stem increment is not significantly different between AM unfertilized and fertilized plots (p=0.054) and between ECM unfertilized and fertilized plots (p=0.066), the differences between the paired unfertilized and fertilized plots are significantly different between AM and ECM plots (p=0.012). Similar results is found for the total tree growth as it is mostly represented by stem growth. Total tree growth in AM plots in kg $C \cdot m^{-2} \cdot yr^{-1}$ respond negative to fertilization (p=0.062) and ECM positive (p=0.072), and this response is significantly different between AM and ECM plots (p=0.015).
There is no significant difference in annual tree ring width and RGR between fertilized and unfertilized trees in AM and ECM plots (table 3-1).
Where stem increment showed a positive correlation with inorganic N availability in unfertilized AM and ECM plots, there is no significant correlation between the stem increment and N_{soil} in the fertilized plots (figure 3-4). AM fertilized plots seem to respond negatively to inorganic N availability while ECM plots show a slightly positive response to N availability, although not significant.

		Unit	AM UF	AM F	ECM UF	ECM F	
FRP 0-15	**C**	g C m^{-2}·yr^{-1}	52.24 ±8.11	45.75 ±10.30^{a}	69.37 ±9.19	86.8 ±18.90^{b}	
	N	g N m^{-2}·yr^{-1}	1.65 ±0.31	1.5 ±0.29	1.62 ±0.30	1.78 ±0.34	
	C:N	g C g N^{-1}	33.95 ±2.35^{a}	29.93 ±1.37^{a}	45.6 ±3.92^{b}	47.72 ±1.77^{b}	
Root biomass	**C**	g C m^{-2}	47.61 ±6.14^{a}	57.41 ±6.36^{a}	106.17 ±2.18^{b}	103.1 ±10.33^{b}	
	N	g N m^{-2}	1.52 ±0.18^{a}	1.84 ±0.17^{b}	2.37 ±0.15^{c}	2.44 ±0.17^{c}	
	C:N	g C g N^{-1}	33.11 ±2.06^{a}	29.65 ±0.70^{a}	44.26 ±2.01^{b}	44.30 ±1.34^{b}	
Leaf production	**LAI**	m^{2}·m^{-2}	3.21 ±0.29	3.29 ±0.32	2.81 ±0.10	3.15 ±0.14	
	C	g C m^{-2}·yr^{-1}	62.22 ±7.49	64.57 ±7.23	72.96 ±3.17	81.6 ±3.34	
	N	g N m^{-2}·yr^{-1}	2.95 ±0.49	2.75 ±0.29	3.07 ±0.17	3.69 ±0.15	
	C:N	g C g N^{-1}	22.03 ±1.08	23.4 ±0.24	23.88 ±0.65	22.13 ±0.35	
Stem increment	**Tree ring**	mm	1.21 ±0.11^{a}	1.00 ±0.12	0.82 ±0.12^{b}	0.87 ±0.12	
	RGR		0.017 ±0.003^{a}	0.013 ±0.002	0.009 ±0.001^{b}	0.011 ±0.001	
	C	kg C m^{-2}·yr^{-1}	5.6 ±0.54^{a}	4.26 ±0.57	2.79 ±0.33^{b}	4.05 ±0.30	
	N	g N m^{-2}·yr^{-1}	12.91 ±1.61^{a}	8.8 ±1.12^{b}	6.52 ±1.04^{b}	10.03 ±1.33	
	C:N	g C g N^{-1}	444.65 ±21.34	489.94 ±21.98	451.01 ±30.31	418.99 ±22.27	
Root-shoot	**C**	g C g C^{-1}	0.9 ±0.18	0.71 ±0.12	0.94 ±0.11	1.06 ±0.26	
	N	g N g N^{-1}	0.62 ±0.15	0.55 ±0.08	0.53 ±0.09	0.49 ±0.11	
Total tree	**C**	kg C m^{-2}·yr^{-1}	5.71 ±0.55^{a}	4.37 ±0.56	2.93 ±0.39^{b}	4.32 ±0.34	
	N	g N m^{-2}·yr^{-1}	17.51 ±2.19^{a}	13.04 ±1.19^{a}	11.3 ±1.11^{b}	15.95 ±1.53^{b}	
	C:N	g C g N^{-1}	334.03 ±13.74^{a}	332.71 ±23.09^{a}	256.8 ±17.97^{b}	273.55 ±11.03^{b}	

Table 3-1 Mean values of fine root production (FRP), root biomass, leaf production, stem increment, root-shoot ratio and total tree growth for unfertilized and fertilized AM and ECM plots. Means (± SE) with dissimilar letters show significant different mean values ($p<0.05$, n=7). The column sparklines on the far left summarize the values presented in the previous columns in qualitative way and the red column represents the highest value.

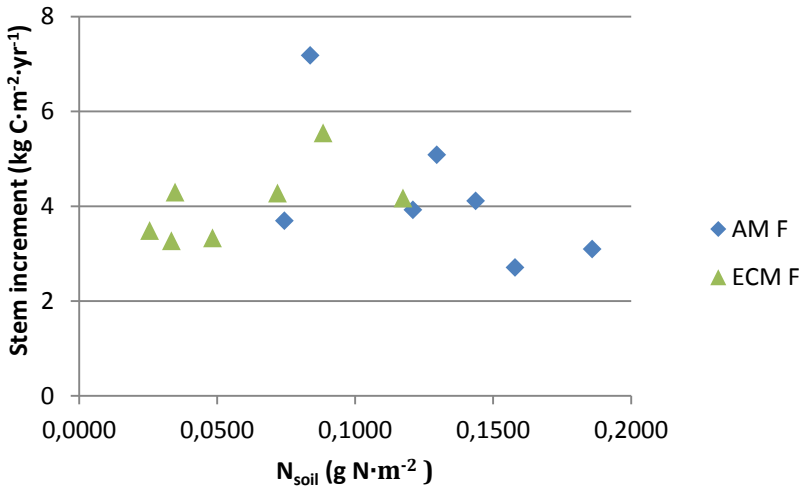

Figure 3-4 Stem increment plotted against inorganic N availability in fertilized AM and ECM plots. No significant correlation is found between stem increment (kg C·m^{-2}·yr^{-1}) and N_{soil} (g N·m^{-2}) in both AM and ECM fertilized plots.

Although FRP is not significantly different between unfertilized and fertilized plots, it does have a negative correlation with N availability in AM plots but not in ECM plots (figure 3-5).

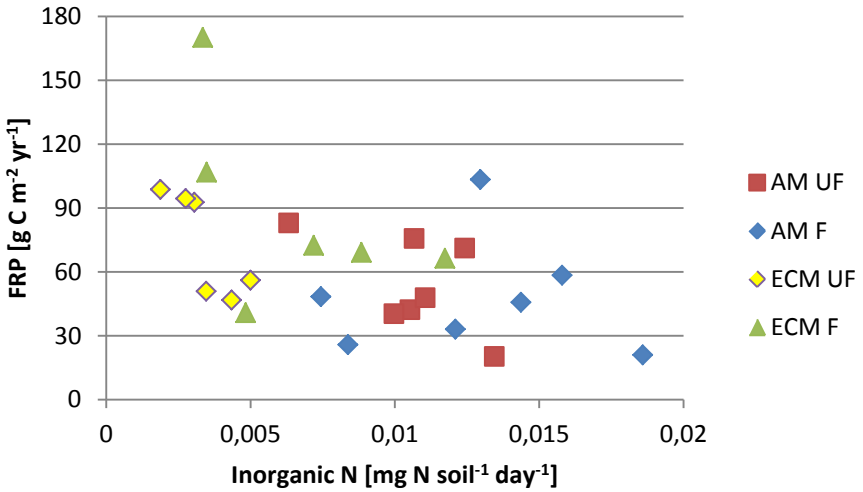

Figure 3-5 Relationship between inorganic N availability and fine root production (FRP) in 0-15 cm soil layer in unfertilized and fertilized AM and ECM plots. FRP (g C·m^{-2}·yr^{-1}) correlates negatively with inorganic N availability (mg N·soil^{-1}·day^{-1}) in all AM plots (R^2=-0.449, p=0.017) but no correlation is found in ECM plots.

3.4 Fertilization and N allocation

Higher N availability lead to differences in N allocation in stem increment in AM plots but not for ECM plots (table 3-1). N in stem increment (g N·m^{-2}·yr^{-1}) is lower in fertilized than in unfertilized AM plots (p=0.027). This is the result of lower stem growth in dry biomass since C and N concentrations in stem increment is only measured from trees in unfertilized plots. Also, the tree species composition in a plot influences the average C:N ratio of total tree growth per plot because tree species have different C:N ratios.

Although the N allocation to stem increment per plot is not significant different between ECM unfertilized and fertilized plots, the response of fertilization on N allocation to stem

increment is significantly different from the response in AM plots. N in stem increment in ECM plots responds positive on fertilization while AM plots decreases N allocation to stem increment with fertilization (p=0.007).

Since tree growth is mostly represented by stem increment, total N allocation in AM plots show a similar negative response on fertilization while total N allocation in ECM plots increases with fertilization. Although the values between the groups are not significant, the different response on fertilization between AM and ECm plots is significant (p=0.005).

N and C:N ratio in FRP do not respond on higher N availability. N in root biomass increases with fertilization in AM plots (p=0.029, table 3-1).

Fungi	Treatment	Tree species	SLA	N_{leaves} [%]	N_{litter} [%]	Resorption
AM	UF	Sassafrass	168	2,86	0,82	71,4%
		Sugar Maple	206	1,78	0,91	48,9%
		Tulip Poplar	189	2,53	0,87	65,6%
		Average	188	2,39	0,87	63,8%
	F	Sassafrass		3,61	0,66	81,7%
		Sugar Maple		2,00	0,98	51,2%
		Tulip Poplar		1,43	0,80	44,1%
		Average		2,35	0,81	65,4%
ECM	UF	Beech	227	2,12	0,82	61,3%
		Black Oak/Red Oak	260	1,77	0,65	63,5%
		Pignut Hickory	140	1,98	0,81	59,1%
		White Oak	168	2,26	0,69	69,6%
		Average	199	2,03	0,74	63,6%
	F	Beech		1,95	0,84	56,9%
		Black Oak/Red Oak		2,05	0,66	68,0%
		Pignut Hickory		2,19	0,79	63,9%
		White Oak		2,16	0,70	67,6%
		Average		2,09	0,75	64,2%

Table 3-2 Mean SLA values, leaf and litter N concentrations for AM and ECM tree species, with and without fertilization. Litter N concentrations are significant higher in AM plots than in ECM plots (p=0,042). SLA, leaf N, and resorption are not significantly different between the plots. SLA values are leaf specific and are similar for fertilized and unfertilized trees. Litter N concentrations are from 2011 and leaf N data is from 2013. Sources for SLA values: Lee et al., 2005; AmeriFlux MMSF, 2013b. Source for litter N concentrations: Midgley, 2013, *unpublished*.

Resorption values of leaf N before leaf senescence and leaf fall, estimated from leaf N concentrations and litter data from 2011, are listed in table 3-2. Estimations of resorption ranges between 60 and 65% of the N concentrations measured in the fresh leaves in 2013, and show no differences between AM and ECM, with and without fertilization. However, litter N concentrations are higher in AM plots than in ECM plots, while leaf N concentrations do not show any significant differences between AM and ECM plots.

3.5 Modelled results from unfertilized plots

Simulated output variables from the model are presented in table 3-3 and the used input variables are listed in Appendix B, table I.

C allocation

Simulated C_{growth} in the unfertilized plots is in the same range as observed C in tree growth, but shows less variation within each group (figure 3-6). Simulated C_{growth} is higher in AM unfertilized plots than ECM unfertilized plots (table 3-3), which corresponds to the higher observed tree growth in AM unfertilized plots compared to unfertilized ECM plots (table 3-1). C allocation for N acquisition (C_{acq}) is in AM unfertilized plots on average 21% of NPP and for ECM unfertilized plots 41% of NPP. ECM trees spend almost the double amount of C for N acquisition but total N uptake is in unfertilized ECM plots lower than in unfertilized AM plots.

	Unit	AM UF	AM F	ECM UF	ECM F
$Cost_{active}$	kg C·kg N^{-1}	1,57E+03	2,22E+02	3,79E+03	4,61E+02
$Cost_{fix}$	kg C·kg N^{-1}	6,73E+03	6,73E+03	6,73E+03	6,73E+03
$Cost_{resorb}$	kg C·kg N^{-1}	1,00E+04	8,30E+03	1,18E+04	6,29E+03
C_{acq}	kg C·m^{-2}·yr^{-1}	1,19E+00	2,87E-01	2,24E+00	6,29E-01
C_{growth}	kg C·m^{-2}·yr^{-1}	4,31E+00	5,21E+00	3,26E+00	4,87E+00
$N_{passvie}$	kg N·m^{-2}·yr^{-1}	9,71E-04	2,02E-03	6,02E-04	1,72E-03
N_{active}	kg N·m^{-2}·yr^{-1}	8,78E-03	1,32E-02	6,79E-03	1,43E-02
N_{fix}	kg N·m^{-2}·yr^{-1}	1,77E-03	4,27E-04	3,33E-03	9,34E-04
N_{resorb}	kg N·m^{-2}·yr^{-1}	1,44E-03	4,05E-04	2,12E-03	1,01E-03
N_{total}	kg N·m^{-2}·yr^{-1}	1,30E-02	1,60E-02	1,28E-02	1,80E-02

Table 3-3 Mean simulated variables for unfertilized (UF) and fertilized (F) AM and ECM plots.

N uptake

Simulated total N uptake is higher in unfertilized AM plots than unfertilized ECM plots and corresponds with higher observed N in tree growth in AM unfertilized plots (table 3-1).

Active uptake (N_{active}) has the lowest C cost for N acquisition, both in unfertilized AM and ECM plots, with the exception of the passive N uptake which does not require C. Therefore more N is taken up through active uptake in unfertilized plots than through BNF and resorption from the leaves. $Cost_{active}$ is lower in AM UF plots than in ECM UF plots because of the higher N availability in the soil, even when root biomass is higher in ECM plots than in AM plots (table 3-1). The lower cost results in higher N_{active} in AM UF plots and because active uptake is the largest source of N, total N uptake is higher in AM UF than in ECM UF.

Resorption of N from the leaves (N_{resorb}) is in the unfertilized plots on average 58% of N_{leaf}, which is comparable to the resorption values estimated from the leaf N concentrations in 2013 and litter N concentrations in 2011 (table 3-2). Simulated N_{resorb} is in AM plots lower than in ECM plots because AM plots take up N mostly through active uptake since they have lower $cost_{active}$ than in ECM plots (table 3-3). Although estimated resorption of N_{leaf} do not significantly differ between AM and ECM plots, the lower percentage of N found in the litter of ECM trees corresponds with the higher simulated N_{resorb} (table 3-2).

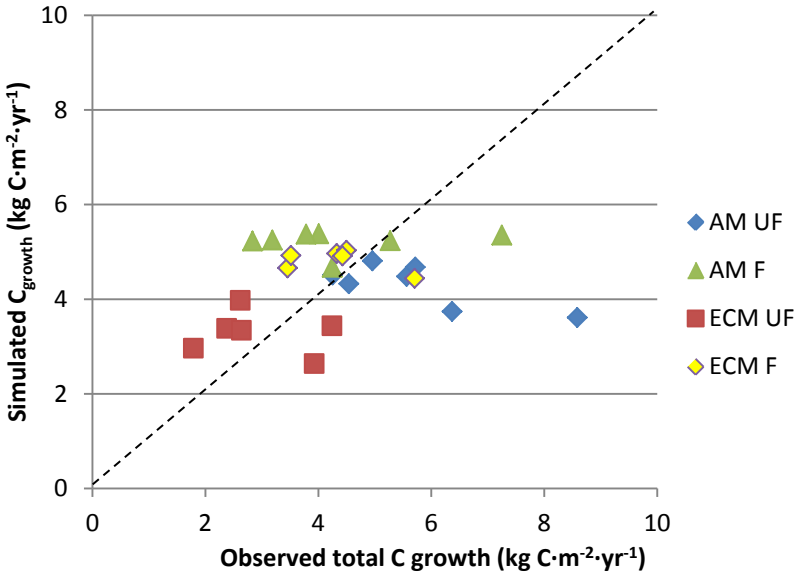

Figure 3-6 Observed C in tree growth plotted against simulated C_{growth} for AM and ECM plots, with and without fertilization. Observed C growth (kg C·m^{-2}·yr^{-1}) is the sum of stem increment, fine root production and leaf production.

3.6 Modelled N deposition

Modelled C_{growth} and N_{total} are both higher in fertilized plots than unfertilized plots (table 3-3), which does not corresponds with the negative stem increment seen in AM plots. However, C_{growth} increases relatively more in ECM plots and the difference in simulated C_{growth} between AM and ECM plots decreases.

C_{acq} is lower in fertilized plots than in unfertilized plots, and the N deposition results in an even larger difference in C_{acq} between AM and ECM plots. N_{total} increases stronger in ECM than AM, resulting in larger total N uptake in ECM plots compared to AM plots. C_{growth} is still higher in AM plots than ECM plots because AM plots have lower C:N ratios.

3.7 Sensitivity analysis

The sensitivity of simulated total N uptake to each input parameter and driver while holding all other inputs constant, is presented in figure 3-7. The default drivers were set as annual averaged constants with C_{NPP} = 5.5 kg C m^{-2}·yr^{-1}; C_{root} =0.1 kg C·m^{-2}; ET = 1 m s^{-1}; N_{leaf} =0.003 kg N·m^{-2}; N_{soil} = 0.05 kg N m$^{-2;}$ $r_{C:N}$ = 300 kg C·kg N^{-1}; sd = 50 m; T_{soil} = 12 °C.

Total N uptake is most sensitive to C_{NPP} and $r_{C:N}$, medium sensitive to C_{root} and N_{soil}, and almost insensitive to N_{leaf}, T_{soil}, sd and ET. N_{total} is sensitive for C_{NPP} because C_{NPP} determines how much C is available for N acquisition. When there is more C available, and there is still N available for uptake, the plant will increase N uptake although the costs are increasing. $r_{C:N}$ has a large influence on N_{total} because it determines the balance between N uptake and C growth (Eq. 12). With a lower $r_{C:N}$ there is relatively less C needed for growth and thus more C can be allocated for N acquisition. Higher N uptake with lower $r_{C:N}$ should not be correlated with higher C_{growth}, because the higher C_{acq} for higher N_{total} is at the expense of C available for growth.

C_{root} and N_{soil} regulates $cost_{active}$ (Eq. 13) and since N_{active} is the largest source of N for trees, N_{total} is more sensitive to C_{root} and N_{soil} than the parameters from the other cost functions. Increasing N_{soil} from 0.02 kg $N \cdot m^{-2}$ to 0.06 kg $N \cdot m^{-2}$, tripling N_{soil} in the range where total N uptake is most sensitive for $N_{soil,}$ results in an increase of total N uptake with 0.005 kg $N \cdot m^{-2}$. In general, AM plots have more N uptake than ECM plots because of their lower $cost_{active}$. When C_{root} and N_{soil} increases, N_{active} increases more in AM plots than in ECM plots. AM plots reaches the maximum of N_{active} faster, when N_{soil} becomes depleted, and the difference in N_{total} between AM and ECM plots becomes less.

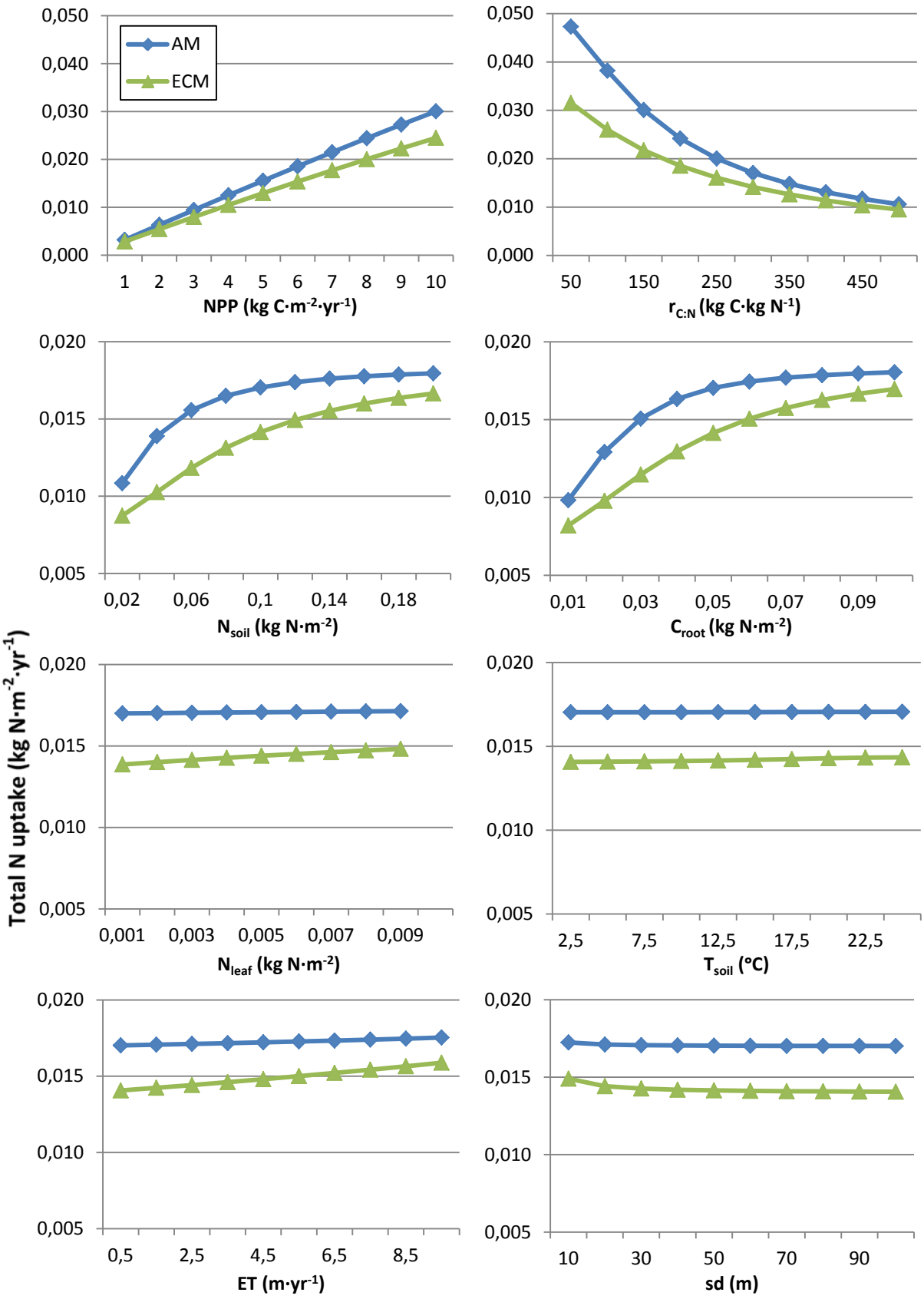

Figure 3-7 Sensitivity to simulated total N uptake (y axis) to variation in each input parameter (x axis) while holding other parameters constant. With C_{NPP} = 5.5 kg C m^{-2}·yr^{-1}; C_{root} =0.1 kg C m^{-2}; ET = 1 m s^{-1}; N_{leaf} =0.003 kg N m^{-2}; N_{soil} = 0.05 kg N $m^{-2;}$ $r_{C:N}$ = 300 kg N^{-1}; sd = 50; T_{soil} = 12 °C. Blue line corresponds to total N uptake in AM plots and green line corresponds to total N uptake in ECM plots.

4. Discussion

The objectives of this study were to analyze the difference in tree growth between AM and ECM tree species and how N availability alters tree growth. Measurements of stem increment, leaf production and fine root production (FRP) in plots with either AM or ECM tree species, with and without fertilization, gave insight in tree growth differences between AM and ECM tree species and their response on higher N availability. Besides field measurements, the resource optimization model FUN was used to simulate plant N acquisition based on the theoretical framework of C cost economics. The relation between the mycorrhizal fungi and the tree was incorporation in the FUN model and simulations of C growth and N uptake were compared with observed values from the field experiments. First, the difference in tree growth between AM and ECM will be discussed, followed by a discussion of the results from the fertilization experiment. Then, the limitation and uncertainties of the research will be discussed followed by suggestions for further research.

4.1 Tree growth

The largest fraction of carbon (C) in tree growth is allocated to the stem (>95%) and therefore is stem increment, without leaf production and fine root production, a good indicator for the total tree growth. This has the consequence that uncertainties in tree growth is increasing by limiting the tree growth to one source: stem growth. These uncertainties will be addressed later.

Stem growth in terms of annual tree ring width, relative growth rate (RGR) and total stem increment per plot all show significant higher stem growth in AM plots than in ECM plots. Similar results were found by a meta-analysis with 78 plant species by Cornelissen et al. [2001] which showed significant higher RGR for AM tree species than ECM trees species. Higher stem growth indicate that AM tree species grow relatively more than ECM tree species. This supports the first hypothesis which predicted that AM trees grow relatively more than ECM trees. Because the stem C:N ratio is indifferent between AM and ECM trees (table 3-1), higher stem increment indicates more N uptake by AM trees compared to ECM trees.

Inorganic N availability is positively correlated with stem increment from unfertilized plots (figure 3-2) and the higher inorganic N availability in AM plots can be the cause of the larger stem growth of AM trees compared to ECM trees. High inorganic N availability is typical for AM soils and is assumed to be caused by low C:N ratios of litter that results in fast decomposition [Cornelissen et al., 2001; Phillips et al., 2013]. This is supported by the higher litter N concentrations of AM trees compared to ECM trees (table 3-2) and the higher mineralization rates found in AM plots compared to ECM plots [Midgley, 2013 *unpublished*].

N uptake is not positively correlated with root biomass, since root biomass is lower in AM plots compared to ECM plots. Fine root production (FRP) is also lower in AM plots compared to ECM plots, indicating that AM trees allocate less C to the roots than ECM trees. Although ECM plants have more access to organic nutrients and have up to 2.5 times larger root biomass in the upper soil layer, it has lower total N uptake than AM trees. Thus, AM trees require a smaller root network for higher N uptake.

The relative low root biomass for AM trees while taking up more N than ECM trees could be explained by the higher inorganic N availability in AM soils, but also by a higher C and N transfer between the fungi and the plant. According to Koide [1991] it is more efficient for AM plant species to invest C in AM fungi than in roots since the absorbing area of the AM hyphae is relatively larger than plant roots. For ECM trees on the other hand it is relative costly to allocate C to the fungi in return for N, since the enzymes produced by ECM to decompose complex organic substrates requires a high amount of C [Talbot and Treseder, 2010]. Furthermore, studies on plant growth show that mycorrhization is not always beneficial to the plant. A meta-analysis carried out by Corrêa et al. [2012] found that C allocation from plant to fungi is a result of excess C produced by the plant and not a cost. When the tree is N-limited and allocates the excess C to the ECM fungi, it stimulates the growth of mycorrhizae and induces the immobilization of N by the fungi, thereby limiting the nutrient availability even further [Näsholm et al., 2013]. The relative high FRP in ECM plots also indicates that it is more efficient for ECM trees to invest C in the production of fine roots than to allocate C to the fungi.

The higher tree growth and N uptake in AM plots is also simulated by the adjusted FUN model. This is mainly the result of lower cost for active uptake in AM plots. AM trees allocates less C to the fungi and get more N in return than ECM trees, which results in higher growth.

4.2 Tree growth with higher N availability

Higher N availability does not lead to significant differences in stem increment, FRP, leaf production, root biomass or root:shoot ratios between unfertilized and fertilized plots. This indicates that the plots are either not limited by N, that fertilization is not sufficient to increase N availability, or that the group size (n=7) is too small to prove significance.

Inorganic N concentrations were higher in fertilized plots than in unfertilized plots, which indicates that fertilization led to higher N availability. The sensitivity analysis of the model showed that total N uptake was medium sensitive to N availability (figure 3-7) and thus should the increase of inorganic N availability be sufficient to increase N uptake.

Higher N availability however did not increased the net mineralization rates in the fertilized plots in 2013 [Midgley, 2013 *unpublished*]. In general, net mineralization rates increases in N-limited systems, but decrease as those systems become N saturated [Aber et al., 1998; Wallenstein et al., 2006]. The unaffected mineralization rates indicates that the fertilized plots became saturated as a result of three years of N fertilization. However, the measured stem growth is from the year 2012, and in 2011 and 2012 the net mineralization rates were higher in fertilized plots than in unfertilized plots [Midgley, 2013 *unpublished*]. This indicates that the plots were not N saturated in 2012.

Other factors that could limit growth is temperature and water availability. In the summer of 2012, North-America was hit by an intense drought where 81% of the surface was designated as abnormally dry (D0) conditions and from this 81%, 64% suffered from at least moderate drought (D1) conditions [US Droughtmonitor, 2013]. Carbon flux measurements

from the AmeriFlux tower in Morgan-Monroe State Forest, approximately 50 km from the research area, showed that photosynthetic rates declined during this severe drought [Brzostek et al., 2013]. Therefore it is assumable that tree growth in 2012 in the plots is limited by the drought and could explain the indifferences in tree growth between fertilized and unfertilized plots. Another factor that could have limited growth was phosphorous (P), but P measurements in 2011 and 2012 indicated that there was no sign of P limitation [Midgley, 2013 *unpublished*].

Although there is no significant difference in tree growth variables between the unfertilized and fertilized plots, the AM plots are responding significantly different on fertilization than ECM plots. AM trees are responding negative to fertilization while ECM trees are increasing growth with fertilization. This is line with our second hypothesis, which stated that AM trees decrease growth with higher N availability and ECM trees increase growth.

AM trees do not take up more N and increase growth with fertilization, although inorganic N concentrations in the soil were higher [Midgley, 2013 *unpublished*]. Although there are no measurements of mycorrhizal biomass, it is likely that the extra N available is immobilized by the fungi. AM fungi increases in biomass with higher N availability as long as the plant is N limited [Treseder and Allen, 2002]. N can also be leached to the groundwater, however this would limit an increase of stem increment rather than result in a negative growth response. The negative response of AM tree growth on higher N availability does not correspond with the findings of the model, where higher inorganic N leads to lower cost for active uptake and therefore more N uptake. Since there is no mycorrhizal biomass included in the model, the N demand of the fungi is not simulated and it is assumed that the C and N transfer between the plant and the fungi is only dependent on the demand for N from the plant.

ECM trees responds positive in stem increment on fertilization, which indicates that the added N is taken up by the tree and increases tree growth. This is also shown in the model results, where ECM fertilized plots are taking up more N than in unfertilized ECM plots. These results correspond with the finding of Näsholm [2013] that ECM fungi does not respond to fertilization and the added N is taken up by the plant. The increase in N availability is in ECM plots relative higher than in AM plots, and assuming that N acquisition for ECM trees requires relatively more C allocation to the fungi, ECM trees are likely to benefit more from N fertilization than AM trees. Also, in the case that ECM fungi is N-limited and not beneficial to the plant, the higher N availability leads to less excessive C towards the fungi, which decreases mycorrhizal biomass and the immobilization of N for its own growth [Näsholm et al., 2013].

The negative response of tree growth to N deposition for AM trees and the positive tree growth response of ECM trees does not correspond with the results of the large tree growth analysis carried out by Thomas et al. [2010]. The study showed that for all 5 AM tree species, N deposition resulted in an increase in tree growth, while ECM tree species responded both positive and negative to higher N availability. All ECM tree species that responded negative to tree growth are not included in this research, thus the positive tree growth of ECM tree species in this research also showed positive growth response in the study of Thomas et al. [2010]. The different outcome for AM tree species can be a result of different initial N concentrations in the soil, as the N-limitation of the fungi and the plant determines the

growth response of AM fungi to N deposition. However, initial inorganic N concentrations are not explicitly mentioned in the study of Thomas et al. [2010].

4.3 Uncertainties and limitations of the research

Field experiments

Stem increment is the largest contributor to tree growth and an important representative of tree productivity. Stem increment is based on tree ring width, DBH, allometric equations and C and N concentrations of stem increment. Uncertainties in each of these parameters could result in high variations in stem increment and thus total tree growth. Estimations of average stem increment per plot ranges between 1.6 and 8.4 kg $C \cdot m^{-2} \cdot yr^{-1}$ and are likely overestimated since net primary productivity (NPP) of temperate deciduous forests usually ranges between 0.5 and 2 kg C $m^{-2}yr^{-1}$ [Kicklighter et al., 1999; Curtis et al., 2002; Newman and Matthews, 2006]. The average stem increment per plot is calculated with relative growth rates (RGR) which relate stem increment to the size of the tree. This technique is based on theory that tree growth occurs at a constant percentage of initials size, also called the compound interest law [South, 1995]. However, percentage of growth is usually not constant but declining with size. When RGR is applied to relative large trees, the dry biomass increment can be overestimated. Looking at the basal area (BA) of the trees, AM and ECM plots have the same amount of large trees: 24 trees with DBH > 50 cm. The sum of BA of all AM trees in the plots are equal to the total BA of ECM trees (0.186 and 0.185 km^2 respectively). Therefore it is likely that the RGR method is likely overestimating stem increment per plot, but is similar for AM and ECM plots. Since we analyse the stem increment of AM and ECM trees in a comparative way, the RGR method unlikely results in large uncertainties in calculating average stem increment per plot.

There are controverting hypotheses how FRP could be under- or overestimated with the ingrowth core method. On the one hand, FRP can be overestimated since (1) the damage of the roots could result in the proliferation of adventitious roots and (2) lack of root competition in the core [Hendricks et al., 2006]. On the other hand, FRP could be underestimated since the roots first have to recover from the damage before they can grow, leading to a shorter timespan for fine root production [Lukac and Godbold, 2001]. However, the uncertainties would be similar in AM and ECM plots and in this study we use the FRP in a comparative way. Furthermore, the contribution of FRP to total tree growth is very small and uncertainties in FRP is unlikely to affect the total tree growth.

Overestimated of FRP in ECM plots is possible because the ECM hyphae are covering the root tips with a thick mantel (figure 1.1). Ouimette et al., [2013] studied the root chemistry of ECM roots and estimates that 25% of the fine root production is actually fungal sheet. In the case that the ECM hyphae is included in the measurements of FRP, the hyphae alone cannot explain the 2.5 times higher FRP found in ECM plots than in AM plots in the upper soil layer. Corrected FRP would still be higher in ECM than AM plots. The ECM fungal sheet can also underestimate the C:N ratio found in the ECM roots, because ECM fungi have on average C:N ratio of 10 [M. Allen et al., 2003] while the measured C:N ratio of ECM roots is ~45. The C:N ratio of ECM roots was significant higher than AM roots (44.3 and 31.4) and a correction should only increase the C:N ratio of ECM roots and increase the difference in C:N ratio of AM and ECM roots even further.

Average annual leaf production did not show any variation between AM and ECM plots, with and without fertilization. There are a few uncertainties in estimating annual leaf production which could have flattened the results. Annual leaf production is calculated with LAI, SLA and C and N concentrations. LAI is measured with the optical instrument Li-Cor LAI-2000 and it measures diffuse light penetrating the canopy. Because the light is not only reflected by the leaves but also by the branches, the LAI value should be corrected with a LAI-0 value. This LAI-0 should be measured before growing season starts when there are no leaves on the branches. Because the research started after the trees started to produce their first leaves, it was not possible to measure LAI-0. I assumed that all plots had the same LAI-0 value of 1, the average value of LAI-0 measured in Morgan Monroe State Forest, <50 km from the research area, with similar tree species distribution. This assumption could have flattened the LAI values between the plots.
Furthermore, SLA values and leaf C and N concentrations were not available for all the tree species in the plots. The missing tree species represent less than 10% of the basal area of the plots and missing values were replaced by average values from AM and ECM trees, differentiating between unfertilized and fertilized plots.
Uncertainties in the variables for SLA, LAI and C and N concentrations lead to uncertainties in leaf production, which had similar values for both mycorrhizal type and did not response on fertilization. AM plant species have in general higher N concentrations in leaves than ECM plant species [Cornelissen et al., 2001; E. A. Hobbie, 2006] which was found in our measurement but it was not a significant result. Uncertainties in leaf production is unlikely to affect total tree growth as leaf production is only a small part of total tree growth.

Model

Simulated C tree growth and N uptake is similar to the average observed values and shows the same differences between AM and ECM unfertilized plots (figure 3-6). Also, C_{acq} in AM plots is matching the predicted range of 7-18% of NPP to mycorrhizal fungi [Hobbie & Hobbie, 2006]. Simulated C_{acq} in ECM plots is higher, but C_{acq} represents besides the C allocated to the fungi also the C allocation for BNF and resorption, which is higher in ECM plots than AM plots.

The model does not accurately predict the total C growth (figure 3-6), which can be addressed to the $cost_{active}$ function (Eq. 13). Plots with high observed N in tree growth have high inorganic N availability and in general low root biomass, especially in AM plots, since FRP is negatively related with inorganic N availability (figure 3-5). According to the model, lower root biomass increases the cost for active uptake because it would decrease the absorbing area of the root network (Eq. 13). The decrease of $cost_{active}$ due to high inorganic N availability is overturned by the low root biomass.

It also seems that the variation within the plots is not visible in the simulated output variables. The input variables N_{leaf}, N_{soil}, $r_{C:N}$ and C_{root} are measured in each plot, but not all variables were possible to measure due to time restrictions. Cost for passive uptake and biological N fixation are therefore the same for all plots. Furthermore, other environmental variables that influence tree growth are not incorporated in the model. However, this research is interested in the comparison of AM and ECM tree growth and the model does reflect the average differences between AM and ECM plots.

In the fertilized plots, the simulated tree growth is not corresponding with the observed tree growth in AM plots. A limitation of the model is that mycorrhizae are only included in the model through changing the $cost_{active}$ function. The model does not include a pool with mycorrhizal biomass, since this requires measurements of mycorrhizal biomass and C:N ratio to simulate N demand of the fungi. With the fertilized plots, the model does not simulate the response of mycorrhizal fungi on N deposition and how this can affect the C and N transfer between the fungi and the plant.

4.4 Future research

This approach aimed to find a relation between type of mycorrhizal fungi and tree growth and how this relation alters with N availability. It is only another step in the process to study the role of mycorrhizae in the carbon C sink of the forest, how this alters with climate change and how it can be integrated in climate models and land surface models. As the C and N transfer between the host and the plant is not only dependent on N limitation of the plant but also on the N and C limitation of the mycorrhizae, the next step should be to incorporate a pool of mycorrhizal fungi in the model and make the C and N transfer dependent on the nutrient demand of both fungi and plant.

Furthermore, the research can be extended by measuring the mycorrhizal biomass and how this alters with higher N availability. AM fungi would be expected to increase in biomass with higher N availability while ECM fungi are expected to decrease in biomass. Direct transfer of N from the mycorrhizae and the tree should be measured as well and can be done by using isotopic N [J. E. Hobbie and Hobbie, 2006; Näsholm et al., 2013].

Another step would be to examine the long term effects of N fertilization on tree growth of AM and ECM tree species. This could indicate how AM and ECM trees will respond on N availability as they will become less N-limited each year. Not only tree growth should be measured but other indicators of plant fitness as well, for example the survival rate. Short term increase of growth followed by a decrease in survival rate will not increase the C sink of the forest and mitigate climate change.

5. Conclusion

The objectives of this study were to analyze the difference in tree growth between AM and ECM tree species and how N availability alters the tree growth. The first hypothesis stating that AM trees grow more than ECM trees is accepted by the results of higher stem increment in AM plots than in ECM plots. Higher inorganic N availability in AM plots is partly responsible, but the relative lower C cost for N acquisition through AM fungi compared to ECM fungi plays a role in the higher stem growth and N uptake in AM plots.

The second hypothesis that AM trees decrease growth with higher N availability while ECM trees increase growth is supported by our results. AM trees decreased growth with N fertilization while ECM trees increased growth, however the results are not significant. The negative response of AM trees on fertilization is significantly different from the positive tree growth in fertilized ECM plots, which is in line with the second hypothesis. AM fungi is restraining the added N from the plant while ECM fungi do not respond to the fertilization. The severe drought of 2012 probably limited the tree growth and weakened the effect of N fertilization on tree growth.

Simulated tree growth showed that the simple adjustments of the FUN model to incorporate mycorrhizal fungi can explain the nutrient exchange between the fungi and the plant. However, with N deposition the model is lacking since it does not incorporate the effect of higher N availability on the growth of the mycorrhizal fungi, how this alters the C and N demand of the fungi and how this affects the C and N transfer between the fungi and the plant.

While the approach of this study has not exposed the direct causality of mycorrhizal fungi on tree growth, the linkages between mycorrhizal type, inorganic N availability and tree growth show that classification of plant by mycorrhizal association can distinguish the functioning of plants in relation to the N and C cycling. Further research is needed to incorporate mycorrhizal associations in dynamic vegetation and climate models since it influences the C sink of terrestrial ecosystems through N availability.

Appendix A

Table I List of allometric equations specified per tree species to convert DBH into dry biomass. d = DBH in [cm] and biomass is in [kg]. Source: Brzostek, 2013 *unpublished*

Tree species	Allometric equation
American Beech	=0,0842*(d^2,5715)-0,025*(d^1,83)
Black Cherry	=0,1225*(d^2,4253)-(exp(-3,498+(1,695*ln(d))))
Black Oak	=0,0945*(d^2,503)-(exp (-3,498+(1,695*ln(d))))
Black Walnut	=sum((exp(-2,437+(2,418*ln(d))))+(exp(-3,188+(2,226*ln(d)))))
Pignut Hickory	=0,0763*(d^2,6209)-(exp(-3,498+(1,695*ln(d))))
Red Maple	=0,1789*(d^2,334)-0,0373*(d^1,54)
Red Oak	=0,1335*(d^2,422)-0,048*(d^1,455)
Sassefras	=sum((exp(-2,437+(2,418*ln(d))))+(exp(-3,188+(2,226*ln(d)))))
Sugar Maple	=0,1008*(d^2,5735)-0,037*(d^1,695)
Tulip Poplar	=0,0687*(d^2,5153)-(exp(-3,498+(1,695*ln(d))))
White Oak	=0,0472*(d^2,701)-(exp(-3,498+(1,695*ln(d))))
White/Green Ash	=0,1063*(d^2,4798)-0,0026*(d^2,416)

Appendix B

Table I. Input variables of the model for each plot

Plot	N_{soil} [kg N m^{-2} yr^{-1}]	C_{root} [kg C m^{-2}]	sd [m]	N_{leaf} [kg N m^{-2} yr^{-1}]	ET [m]	C_{NPP} [kg C m^{-2} yr^{-1}]	$r_{C:N}$ [kg C kg N^{-1}]
AM2f	0,0517	0,0542	50	0,003049	1	5,5	289,0
AM3f	0,0790	0,0606	50	0,002354	1	5,5	319,2
AM4f	0,0477	0,0288	50	0,003688	1	5,5	278,7
AM5f	0,0593	0,0493	50	0,003144	1	5,5	313,9
AM6f	0,0389	0,0626	50	0,003545	1	5,5	301,1
AM7f	0,0363	0,0848	50	0,001806	1	5,5	367,6
AM8f	0,0448	0,0609	50	0,001821	1	5,5	454,1
AM2uf	0,0597	0,0391	50	0,00214	1	5,5	340,3
AM3uf	0,0453	0,0534	50	0,002759	1	5,5	328,6
AM4uf	0,0563	0,0269	50	0,005033	1	5,5	261,1
AM5uf	0,0588	0,0443	50	0,002513	1	5,5	352,4
AM6uf	0,0340	0,0353	50	0,004493	1	5,5	319,8
AM7uf	0,0285	0,0782	50	0,001811	1	5,5	360,4
AM8uf	0,0572	0,0504	50	0,001877	1	5,5	371,6
ECM1f	0,0348	0,1463	50	0,00455	1	5,5	272,4
ECM2f	0,0372	0,1073	50	0,003641	1	5,5	266,8
ECM4f	0,0319	0,1157	50	0,003073	1	5,5	298,7
ECM5f	0,0465	0,0834	50	0,004027	1	5,5	298,0
ECM6f	0,0203	0,0895	50	0,00355	1	5,5	247,8
ECM7f	0,0498	0,0631	50	0,003827	1	5,5	290,2
ECM8f	0,0325	0,1217	50	0,003225	1	5,5	232,7
ECM1uf	0,0241	0,1112	50	0,002882	1	5,5	256,2
ECM2uf	0,0292	0,1140	50	0,003251	1	5,5	197,4
ECM4uf	0,0341	0,1047	50	0,003119	1	5,5	236,8
ECM5uf	0,0426	0,0992	50	0,0034	1	5,5	301,3
ECM6uf	0,0339	0,1075	50	0,002983	1	5,5	239,5
ECM7uf	0,0288	0,0991	50	0,00302	1	5,5	308,9
ECM8uf	0,0180	0,1136	50	0,002699	1	5,5	253,2

Table II. Parameters used in the model

Parameter	**In function**	**Value**
K_c AM	$cost_{active}$	0.015
K_c ECM	$cost_{active}$	0.1
K_n AM	$cost_{active}$	0.03
K_n ECM	$cost_{active}$	0.025
k_r	$cost_{resorb}$	2
s	$cost_{fix}$	-400
a	$cost_{fix}$	-3.62
b	$cost_{fix}$	0.27
c	$cost_{fix}$	25.15

Appendix C

Table I. Measured leaf production, LAI, stem growth and root-shoot ratios for each plot

Plot	Leaf C g C m^{-2} yr^{-1}	Leaf N g N m^{-2} yr^{-1}	Leaf C:N g C· g N^{-1}	LAI m^2·m^{-2}	Stem C kg C m^{-2} yr^{-1}	Stem N g N m^{-2} yr^{-1}	Stem C:N g C· g N^{-1}	Root-shoot C g C· g C^{-1}	Root-shoot N g N· g N^{-1}
AM2f	69,49	3,01	23,05	3,58	2,71	4,73	572,92	0,78	0,62
AM3f	53,63	2,34	22,93	2,89	3,92	9,10	431,28	1,13	0,91
AM4f	88,45	3,66	24,18	4,51	4,11	9,97	412,41	0,33	0,29
AM5f	71,40	3,12	22,90	3,69	3,09	6,29	491,81	0,69	0,56
AM6f	85,01	3,53	24,11	3,89	5,08	10,99	462,71	0,74	0,56
AM7f	41,11	1,81	22,76	2,35	3,69	7,09	521,21	2,06	1,45
AM8f	42,90	1,80	23,87	2,15	7,18	13,37	537,27	1,42	1,11
AM2uf	48,90	2,14	22,85	3,23	4,19	9,88	423,59	0,80	0,43
AM3uf	56,35	2,76	20,38	2,74	5,47	13,04	419,25	0,95	0,55
AM4uf	97,46	5,06	19,27	4,6	6,22	17,55	354,55	0,28	0,20
AM5uf	56,85	2,52	22,58	2,9	5,58	11,15	500,98	0,78	0,62
AM6uf	82,19	4,49	18,29	3,83	8,43	19,88	424,19	0,43	0,27
AM7uf	44,97	1,81	24,84	2,58	4,41	8,49	520,15	1,74	1,29
AM8uf	48,79	1,88	25,99	2,56	4,87	10,36	469,83	1,03	0,73
ECM1f	67,75	3,12	21,69	2,56	3,49	7,89	441,71	2,16	1,15
ECM2f	86,37	4,02	21,47	3,37	4,17	10,84	385,02	1,24	0,58
ECM4f	93,57	4,01	23,34	3,34	4,30	8,81	487,62	1,24	0,60
ECM5f	78,08	3,55	22,00	2,97	4,28	9,75	438,49	1,07	0,53
ECM6f	88,74	3,96	22,38	3,73	5,55	17,48	317,41	1,01	0,59
ECM7f	81,86	3,93	20,83	3,05	3,33	7,05	472,85	0,77	0,35
ECM8f	74,81	3,22	23,20	3,05	3,27	8,39	389,81	1,63	0,79
ECM1uf	70,97	3,18	22,29	2,63	2,80	5,89	474,78	1,57	0,68
ECM2uf	79,71	3,56	22,37	3,19	1,61	3,01	535,80	1,43	0,86
ECM4uf	78,33	3,57	21,94	3,01	2,20	4,54	485,52	1,34	0,62
ECM5uf	70,22	2,87	24,43	2,69	2,49	4,64	536,17	1,41	0,80
ECM6uf	57,66	2,36	24,45	2,38	2,53	7,58	333,95	1,86	1,16
ECM7uf	82,82	3,27	25,35	2,91	4,11	9,53	430,78	1,20	0,61
ECM8uf	71,04	2,70	26,32	2,84	3,76	10,43	360,05	1,60	1,01

Table II. Measured fine root production (FRP), root biomass, inorganic N availability and net mineralization rates for each plot, separated by soil layer.

Plot	Soil layer	FRP C g C m^{-2} yr^{-1}	FRP N g N m^{-2} yr^{-1}	Root biomass C g C m^{-2}	Root biomass N g N m^{-2}	N inorganic g N m^{-2}	N mineralization g N m^{-2} yr^{-1}
AM2f	0-5	17,99	0,69	7,72	0,30	0,53	18,62
AM3f	0-5	10,27	0,39	28,83	1,10	0,51	26,62
AM4f	0-5	7,94	0,31	13,97	0,54	0,50	22,13
AM5f	0-5	10,03	0,36	29,22	1,05	0,29	28,52
AM6f	0-5	28,64	1,13	28,80	1,14	0,36	24,21
AM7f	0-5	14,15	0,53	38,39	1,45	0,43	27,97
AM8f	0-5	6,76	0,26	26,49	1,01	0,21	13,48
AM2f	5-15	40,51	1,38	46,46	1,58	0,52	33,12
AM3f	5-15	22,89	0,73	31,77	1,02	0,42	21,04
AM4f	5-15	37,78	1,30	14,80	0,51	0,29	16,81
AM5f	5-15	11,01	0,38	20,10	0,69	0,27	16,32
AM6f	5-15	74,79	1,86	33,77	0,84	0,33	21,05
AM7f	5-15	34,24	0,86	46,45	1,17	0,22	30,80
AM8f	5-15	19,09	0,55	34,43	0,99	0,22	15,05
AM2uf	0-5	5,04	0,17	10,21	0,34	0,46	17,36
AM3uf	0-5	13,56	0,43	28,99	0,92	0,71	29,88
AM4uf	0-5	18,66	0,83	12,36	0,55	0,27	22,38
AM5uf	0-5	36,11	1,41	27,63	1,08	0,42	17,74
AM6uf	0-5	42,06	1,69	18,43	0,74	0,39	30,44
AM7uf	0-5	25,36	0,87	40,45	1,39	0,43	12,26
AM8uf	0-5	19,17	0,56	23,92	0,70	0,35	27,35
AM2uf	5-15	15,34	0,31	28,86	0,59	0,29	61,67
AM3uf	5-15	28,72	0,70	24,38	0,59	0,44	29,45
AM4uf	5-15	29,28	0,95	14,49	0,47	0,21	13,88
AM5uf	5-15	39,64	1,15	16,66	0,48	0,51	41,98
AM6uf	5-15	29,20	0,79	16,82	0,45	0,33	25,87
AM7uf	5-15	57,66	1,44	37,76	0,94	0,39	21,70
AM8uf	5-15	21,27	0,54	26,48	0,67	0,30	29,85
ECM1f	0-5	-	-	115,91	3,14	0,09	12,68
ECM2f	0-5	31,47	0,80	67,97	1,73	0,11	13,89
ECM4f	0-5	71,71	1,78	77,58	1,93	0,31	4,22
ECM5f	0-5	31,56	0,97	40,86	1,26	0,12	19,82
ECM6f	0-5	25,18	0,74	61,33	1,80	0,10	15,22
ECM7f	0-5	26,43	0,67	35,17	0,90	0,18	24,16
ECM8f	0-5	93,04	2,36	72,44	1,84	0,12	18,57
ECM1f	5-15	-	-	30,42	0,46	0,08	22,10
ECM2f	5-15	34,95	0,55	39,36	0,62	0,13	18,04
ECM4f	5-15	35,18	0,45	38,15	0,49	0,27	16,04
ECM5f	5-15	40,94	0,58	42,50	0,61	0,09	12,66

ECM6f	5-15	44,00	0,84	28,14	0,54	0,11	14,01
ECM7f	5-15	14,40	0,25	27,97	0,49	0,15	18,46
ECM8f	5-15	77,16	1,13	49,27	0,72	0,11	10,18
ECM1uf	0-5	-	-	78,39	1,74	0,50	24,63
ECM2uf	0-5	55,16	1,53	76,15	2,11	0,25	27,14
ECM4uf	0-5	58,18	1,34	74,98	1,73	0,16	17,49
ECM5uf	0-5	27,53	0,77	66,12	1,85	0,09	11,56
ECM6uf	0-5	24,75	0,71	79,29	2,28	0,09	15,63
ECM7uf	0-5	24,76	0,57	67,55	1,55	0,13	21,23
ECM8uf	0-5	70,77	1,89	80,08	2,13	0,06	6,24
ECM1uf	5-15	-	-	32,86	0,44	0,17	12,60
ECM2uf	5-15	37,60	0,95	37,85	0,95	0,22	19,34
ECM4uf	5-15	36,30	0,58	29,76	0,48	0,16	32,28
ECM5uf	5-15	28,59	0,39	33,04	0,46	0,08	12,55
ECM6uf	5-15	22,07	0,36	28,25	0,46	0,10	18,46
ECM7uf	5-15	26,16	0,36	31,56	0,44	0,17	12,71
ECM8uf	5-15	27,97	0,49	33,55	0,59	0,07	11,73

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