THE ROLE AND DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN *ACER SACCHARUM* DOMINATED FOREST ECOSYSTEMS UNDER NATURAL AND N-AMENDED CONDITIONS

By
Linda van Diepen

A DISSERTATION
Submitted in partial fulfillment of the requirements
for the degree of
DOCTOR OF PHILOSOPHY
(Forest Science)

MICHIGAN TECHNOLOGICAL UNIVERSITY
2008

Copyright © Linda T.A. van Diepen 2008
UMI Number: 3339442

INFORMATION TO USERS

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleed-through, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.
Summary

Human activities have altered global nitrogen (N) deposition and fixation through fossil fuel combustion, the use of fertilizers, and increases in N-fixing agricultural crops. Mycorrhizal fungi make up a large part of the microbial biomass in terrestrial ecosystems and play a pivotal role in plant carbon and nutrient balance, supplying nutrients, including nitrogen, to host plants in exchange for carbon. This mycorrhizal symbiosis could therefore be influenced by changes in nitrogen deposition, which in turn could alter not only nutrient cycling, but also carbon cycling within an ecosystem.

The main goal of my study was to assess changes in the functioning of arbuscular mycorrhizal fungi (AMF) in an ecosystem exposed to elevated levels of nitrogen. This was addressed using four established long term research sites looking at the effects of altered N-availability in northern hardwood forests dominated by sugar maple (Acer saccharum). Sugar maple belongs to one of the few dominant north temperate tree genera that form a symbiotic relationship with arbuscular mycorrhizal fungi (AMF), and forms extensive stands in northern temperate biomes.

The first objective was to gain basic knowledge of changes in AMF presence with N addition in northern hardwood forests. This was addressed by estimating the AMF abundance within the roots of the dominant tree species, sugar maple. The abundance was measured using a traditional root staining technique with ocular estimation, as well as analyzing roots for the AMF indicator fatty acid 16:1ω5c in phospholipid (biomass indicator) and neutral lipid (lipid storage indicator) fractions. AM fungal biomass, storage structures and lipid storage declined in response to N addition measured by both methods. This pattern was found when AM response was characterized as colonization intensity, on an areal basis and in proportion to maple aboveground biomass. The phospholipid fraction of the fatty acid 16:1ω5c was positively correlated with total AMF colonization and the neutral lipid fraction with vesicle colonization. The fatty acid (phospho- and neutral lipid fraction) 16:1ω5c was found to be a good indicator for AMF active biomass and stored energy, respectively.
The observed decrease in AMF abundance in roots could be associated with 1) a decrease in all species present, 2) a change in relative abundance of the associated species, or 3) a complete change in community composition. My second objective therefore was to identify the AMF species present within the roots and their diversity among treatments. I performed molecular DNA-based AMF community analyses on maple roots targeting the 18S rDNA region using fungal specific primers. Changes in the AMF community composition were observed with N-amendment, and effects on AMF species diversity differed among sites. Over 80% of all the AMF clones present in the roots were represented by seven dominant OTU’s (Operational Taxonomic Units). Some of the OTU’s declined in response to N-amendment, some increased and a few were unaffected by N-amendment. Other studies on AMF species have found that some AMF taxa that are abundant at high N-levels are less beneficial or even detrimental to the host plant, which is reflected through decreased nutrient uptake efficiency or an increased carbon cost for the host plant.

The actual nutrient uptake in a mycorrhizal system takes place in the extraradical mycelium of AMF. Hence, when studying nutrient cycling within ecosystems, the function of both the intra- and extraradical AMF are of importance. My third objective therefore was to study the effects of N-amendment on both intra- and extraradical AMF biomass simultaneously including the rest of the microbial biomass within the soil. Root and soil samples, taken as paired samples, were analyzed for phospho- and neutral lipid fatty acids (PLFA and NLFA). Intra- and extraradical AMF biomass were decreased equally by N-amendment as measured by AMF indicator fatty acid 16:1ω5c. Furthermore, total microbial biomass decreased and a change in microbial community composition was found under N-amendment. The composition change was dominated by a decrease in fungal to bacterial biomass ratios. The largest portion of AMF biomass within our system was represented by extraradical mycelium (ERM). Other studies have estimated ERM provides up to 30% of the total microbial biomass. ERM have a substantial amount of photosynthetically derived carbon flowing through them, thus the observed changes in ERM biomass could greatly influence soil respiration.
Therefore my fourth and last objective was to estimate the production and respiration rate of AMF extraradical mycelium biomass. Hyphal in-growth bags were designed that would allow ERM but no root colonization and minimize ERM of saprotrophic fungi, by using mesh of 50µm and sand without organic matter content, respectively. Bags were buried in the soil for one growing season to allow colonization of ERM and measured for CO$_2$ flux at harvest at the end of the growing season. A similar mean reduction of AMF extra-radical biomass with N-amendment was found using the in-growth bags compared to the soil PLFA 16:1ω5c measurements. Hyphal in-growth bag CO$_2$ flux was not significantly decreased by N-amendment, but a trend was seen at two sites, and an average decrease of 7% was found. However, hyphal in-growth bags CO$_2$ flux was positively related to hyphal biomass, which suggests that AMF hyphal CO$_2$ flux is mainly controlled by the biomass of AMF mycelium.

In conclusion, increased chronic nitrogen deposition as simulated by long term N-amendment had a negative effect on both intra- and extraradical AMF biomass, total microbial biomass, fungal to bacterial biomass ratio, and AMF CO$_2$ efflux in a northern hardwood forest dominated by sugar maple. The observed decrease in AMF abundance correlated with a decrease in AMF CO$_2$ flux suggests reduced carbon (C) allocation to these fungi or a direct soil N-mediated decline. The decrease in fungal to bacterial biomass ratio could have been caused by a change in organic matter quality, specifically a decrease in C:N ratio, which has been observed in the litter at our sites. The decrease in overall microbial biomass could have negative effects on soil organic matter decomposition. Consistent with this, a decreased rate of organic matter decomposition has been observed at our study sites. Furthermore N-amendment changed intraradical AMF community composition, with some taxa increasing while others decreased in abundance with N-amendment. Given that functional diversity exists among AMF species, this change in community composition could have implications for the functioning of this type of ecosystem. Our observed decrease in AMF and microbial biomass together with changes in intraradical AMF and soil microbial community composition with N-amendment thus has the potential to substantially change both nutrient and carbon cycling within northern hardwood forests. Further investigations
should focus on effects of additional environmental variables on the AMF biomass and community composition, such as foliar nutrient content. These analyses could reveal some indirect effects of increased N-amendment and could be useful for the creation of models predicting changes in fungal biomass and communities and plant-fungal relationships.