Interplay between N-form and N-dose influences ecosystem effects of N addition to boreal forest

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Abstract

Aims Nitrogen (N) addition effects on boreal forest ecosystem are influenced by an interplay between N-form and N-dose. We hypothesize that trees take up organic N more efficiently than inorganic N and that unwanted side-effects of organic N are smaller. We predicted that 1) the tree growth response to arginine (ARG) addition is larger than to ammonium-nitrate (AN) and, 2) understory vegetation and ectomycorrhizal (EcM) changes following ARG addition are smaller than following AN addition.

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Methods We investigated the effects of AN and ARG addition (50 and 150 kg N ha⁻¹) during five years on tree growth, understory vegetation and EcM fungi in a *Pinus sylvestris* L. forest (c 50 years old) in northern Sweden.

Results N addition increased tree growth and changed understory vegetation composition with few significant differences between AN and ARG. Differences in responses mainly occurred for the bryophyte *Pleurozium schreberi* which decreased more from ARG, and for EcM sporocarps, which sharply declined from AN, but not from ARG.

Conclusions We found very few differences in responses between AN and ARG addition with the exception of EcM and bryophytes. These species groups have several key functions in boreal forests and the differences in responses merits further investigations.

Keywords Carbon sequestration · Forest fertilization · Forest productivity · Nitrogen leaching

Introduction

For more than a decade it has been known that conifer seedlings such as Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst) can be cultivated efficiently with nitrogen (N) supplied in the form of the amino acid arginine (ARG). (Öhlund and Näsholm 2001, 2002; Gruffman et al. 2012). These studies have documented lower leakage of N during nursery operations as well as enhanced root:shoot ratios and higher

incidence of mycorrhizal root tips. It has also been demonstrated that root uptake rates of ARG is substantially higher than that of ammonium or nitrate (Öhlund and Näsholm 2001; Gruffman et al. 2013; Oyewole et al. 2016).

Organic N in the form of amino acids naturally dominates the soluble soil N pool directly available to plants and ectomycorrhizal (EcM) fungi in coniferous nutrient poor boreal forests (Jones and Kielland 2002; Kielland et al. 2007; McFarland et al. 2010; Inselsbacher and Näsholm 2012). Numerous short-term uptake studies have confirmed a high capacity of boreal forest plants (Näsholm et al. 1998; Nordin et al. 2001; Persson et al. 2003) as well as temperate trees (Scott and Rothstein 2011) to take up different amino acids. Ammonium is the main inorganic N form in boreal forest soils and many boreal plant species prefer ammonium over nitrate (Wallander et al. 1997; Marschner et al. 1991; Nordin et al. 2001). Nitrate uptake may even be inhibited by the addition of ammonium (Marschner et al. 1991; Gruffman et al. 2014), although some understory species, like the grass *Avenella flexuosa* (L.) Drejer, have a particularly high capacity for nitrate uptake (Nordin et al. 2006).

The N dose applied to a forest ecosystem may influence the distribution of the added N between different ecosystem compartments. Total ecosystem N recovery is higher at lower ($\leq 50 \text{ kg N ha}^{-1}$) than higher ($> 50 \text{ kg N ha}^{-1}$) N doses, and a major proportion (c. 50%) of the added N is normally found in the soil (Melin and Nommik 1988; Templer et al. 2012). At high N doses ($\geq 150 \text{ kg N ha}^{-1}$) N may be lost through leakage (5–10% of the added N), mainly as nitrate which is mobile in the soil and there are concerns about eutrophication of receiving aquatic ecosystems (Binkley et al. 1999; Nohrstedt 2001; Saarsalmi and Mälkönen 2001). Plant N acquisition may increase disproportionately with increased N dose (Nordin et al. 1998; Nadelhoffer et al. 1999), although linear relations between increased plant growth and increased N dose have also been reported (Gundale et al. 2014; From et al. 2016).

There are numerous reports of effects on tree growth and species composition of the vegetation from additions of inorganic N. A single application of 150 kg N ha^{-1} in the form of ammoniumnitrate (AN) can enhance tree growth by 30% during a ten-year period (Nohrstedt 2001), while annually repeated AN applications may even double it (Bergh et al. 1999; Brockley 2010). A common N-addition effect on the boreal understory

vegetation is transition to a species composition indicative of more fertile sites. For example grasses (e.g. *A. flexuosa*) and forbs are commonly promoted by AN addition, possibly due to their high capacity to take up N in the form of nitrate (Nordin et al. 2001, 2006; Persson et al. 2003), while more N-conservative ericaceous dwarf-shrubs efficiently take up organic forms or ammonium (e.g. *Vaccinium* spp.) may decrease (Nordin et al. 2001; Strengbom and Nordin 2008; Hedwall et al. 2013). Another common effect is a shift in species composition of bryophytes with a decrease in the most abundant species and in total cover (Strengbom et al. 2001; Nordin et al. 2009). These effects may be transient after a single AN application (Nohrstedt 2001), but may return in connection with disturbance of the tree layer (Strengbom and Nordin 2008, 2012).

Nitrogen addition to forest may also affect above- and belowground occurrence of ectomycorrhizal fungi (EcM). Most studies of EcM responses to N addition have been done in experiments with yearly N additions over several consecutive years or in naturally occurring gradients of inorganic N deposition. These studies indicate that responses to inorganic N addition are genus-specific. Genera like *Cortinarius*, *Piloderma* and *Suillus* have been reported to decline in abundance from long-term AN addition, while genera like *Laccaria*, *Lactarius*, *Paxillus* and *Russula* have been found to increase their sporocarp production (Lilleskov et al. 2001, 2002; Strengbom et al. 2001; Avis et al. 2003). More recently it has also been reported that organic N addition may enhance EcM colonization of tree seedlings growing in the field or in nurseries (Avolio et al. 2009; Gruffman et al. 2012).

Hence, it is clear that the forest ecosystem effects of N addition are influenced by an interplay between N form and N dose. The indications lately that organic N in the form of amino acids may be the dominant soluble N source directly available to uptake by plants in boreal forest (Inselsbacher and Näsholm 2012) may open up new solutions for increasing boreal forest productivity and carbon sequestration. We report the results of the first large-scale experiment investigating and comparing forest ecosystem effects of amino acid and AN addition in the field. We choose the amino acid arginine (ARG) as it is the most N-rich amino acid with an N-to-C ratio of 4:6. In the pH range normal for boreal forest soils it is positively charged and as such efficiently retained in the soil. We added ARG and AN to separate plots in two doses (50 and 150 kg N ha^{-1}) to a c. 50-year-old Scots

pine forest in northern Sweden. We hypothesized: 1) that the addition of ARG would result in a larger tree growth response than the addition of AN, and 2) that the addition of AN would cause relatively higher concentrations of mobile N in the soil and more pronounced species shifts in vegetation and fungal communities than the addition of ARG. We recorded basal area growth of trees, species composition of the forest floor vegetation and of above- and belowground EcM communities, and soil mobile N retained on ion exchange capsules for five years following the N addition.

Material and methods

Study site

The experiment was conducted in an even-aged (48 years) stand of Scots pine (*P. sylvestris* L.) with only a small number of other tree species; *Picea abies* (L.) Karst (0.2% of the basal area of trees) and *Betula* spp. (0.1%). The stand was planted at the site (64°11'N, 19°33'E) in 1964, 70 km from the Gulf of Bothnia in the province of Västerbotten, northern Sweden. The site had a flat topography and the soil was a podzol developed from glacial till with an organic layer of 15 cm. The ground vegetation was dominated by the ericaceous dwarf-shrub *Vaccinium myrtillus* L. with *V. vitis-idaea* L. and the grass *A. flexuosa* (L.) Drejer also being rather frequent. The bottom layer was dominated by the bryophyte *Pleurozium schreberi* (Brid.) Mitt. The background atmospheric N deposition for the area is estimated to be $<2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and the mean annual temperature is 1 °C and mean annual precipitation is 600 mm (Swedish Meteorological and Hydrological Institute: www.smhi.se).

The experimental plots were distributed over an area of c. four hectares (ha). We set up 30 plots each sized 625 m² (25 m × 25 m) with a 10 m buffer zone between adjacent plots in a complete randomized block design with five plots within each of the six blocks. The five treatments were untreated control, 50 and 150 kg N ha⁻¹ as ARG and AN, and each treatment were replicated six times. A single application of 150 kg N ha⁻¹ AN is used in practical forest fertilization in Scandinavia and the effects on the ecosystem are well-documented (Nohrstedt 2001) and thus suitable for a comparison with ARG. The lower dose was chosen to test if ARG could generate an increased tree growth at a lower dose than AN. Hence, there were two low-dose treatments

(abbreviated to LARG and LAN) and two high-dose treatments (HARG and HAN). The powdery ARG and granulated AN was spread by hand in mid-June 2008 after the start of the vegetation period to promote uptake by trees and other vegetation.

Tree growth measurements

The diameter at breast height was measured on all trees in early autumn following the end of the growth season in 2008, 2011 and 2013. The growth response of Scots pine to N addition develops over a few years and mainly the immediate response is an increase of needle N concentrations as shoot growth is pre-determined already in the previous year. Thus, total basal area growth within each plot over the years 2009–2013 was used to assess treatment effects on tree growth in the statistical analyses.

Soil mobile N estimated with ion exchange capsules

Ion exchange capsules (PST-1 resin capsule; UNIBEST International, Walla Walla, Washington, USA From et al. 2015) were inserted between the organic and mineral soil layers immediately before N addition in June 2008 to get an in situ estimate of mineralization of the added ARG and levels of ammonium and nitrate in the soil solution. These capsules were recovered in late October 2008 and new capsules were inserted. This was repeated so that capsules were kept in the soil from late May to October in 2009, from October 2009 to October 2010 and finally from October 2010 to October 2013. In each period, four to five capsules were put in each main plot. The capsules were extracted in 1 mol L⁻¹ KCl, and the extracts were analyzed for concentrations of ammonium-N and nitrate-N with an AutoAnalyzer 3 (SEAL Analytical). Ammonium-N and nitrate-N per capsule (mg capsule⁻¹) were calculated and the mean values of these within plot and sampling occasion were used as response variables.

Monitoring of sporocarps

Fungal sporocarps were counted every two weeks (six occasions) from mid-August to mid-October in 2008, 2009 and 2013. Two persons walked across each plot 7 times per person and counted sporocarps. To avoid double counting, counted sporocarps were cut off. Fungal species were identified mainly in situ. The

sporocarps were summed over inventories within year and plot for further analyzes. The total number of sporocarps and the number of sporocarps from the four most common species/genera in the control treatment were used as response variables.

Identification of belowground ectomycorrhizal fungi

In mid to late August 2009, fine root systems of *P. sylvestris* in the humus layer were collected from five to seven locations in each plot. The roots from each location were put in plastic bags and kept in 4 °C for a maximum of two weeks before further analyses. The roots were washed carefully with tap water, and EcM root tips were assigned to morphotypes under a dissecting microscope. From each sampling location, a representative root tip from each morphotype was washed in an ultrasonicator and thereafter subjected to molecular identification. Extraction of DNA was performed using the CTAB method described by Ishida et al. (2007). The rDNA internal transcribed spacer (ITS) regions were amplified using ITS1F (Gardes and Bruns 1993) primer with a 5' D2 fluorescent labeled (Sigma-Aldrich, France) and ITS4 primer (White et al. 1990) with a 5' D3 fluorescent labeled. We also amplified the same ITS region using a 5' D4 fluorescent labeled ITS1F and non-labeled ITS4 primers. For the polymerase chain reaction (PCR), KOD Plus DNA polymerase (Toyobo, Osaka, Japan) was mainly used; Qiagen Multiplex PCR kit was used when the product was faint or absent using KOD Plus DNA polymerase. We incubated 2 µl 5' labeled PCR product with 1 U of either *HinfI* (Takara Shuzo, Shiga, Japan, or Fermentas, St. Leon-Rat, Germany) or *TaqI* (Takara Shuzo or Fermentas) in the reaction mixtures. The digested products and undigested both 5' labeled PCR product were diluted with MilliQ water to standardize the fluorescent strength. The diluted products were added in 30 µl of formamide (Beckman Coulter, Fullerton, CA, USA) containing 0.31 µl of CEQ 600 size standard (Beckman Coulter). Capillary gel electrophoresis was performed using a CEQ 8000 (Beckman Coulter). The terminal restriction fragment lengths were determined using FRAGMENTS implemented in a CEQ 8800 genetic analysis system (Beckman Coulter). Four fragments (ITS1F with *HinfI* digested (labeled with D2), ITS4 with *HinfI* digested (D3), ITS1F with *TaqI* digested (D4) and undigested PCR product (D2 and D3)) were obtained for each sample, but small (<60 bp) and large (>700 bp)

fragments were neglected due to the difficulty in determining the size. When samples had fragments within ± 2 bp they were considered to be the same terminal restriction fragment length polymorphism (T-RFLP) type. Morphotype was also considered to determine the T-RFLP types.

The ITS region of each T-RFLP type was amplified again using non-labeled ITS1F and ITS4 primers, purified with a PCR product presequencing kit (USB Co., Cleveland, OH, USA), then its ITS sequence was determined by the commercially available DNA sequencing service (Eurofins MWG Operon, Ebersberg, Germany) or using Beckman 2000 (Beckman Coulter). Some T-RFLP types were sequenced using the samples from the EMF root tips (either on spruce or pine), or EMF sporocarps and *Vaccinium* spp. roots, which were collected in northern Sweden. When two or more sequences showed high identity (>98%) in ITS region (excluding the 18S and 28S rDNA region), these T-RFLP types were regarded as the same taxon. We mainly used ITS1F primer to determine the ITS sequence but ITS4 was used when necessary. The obtained sequences were compared with the sequences of known species in the UNITE database (Kõljalg et al. 2005) or in the GenBank by blast to infer the taxonomic position of the species. EMF morphotypes were assigned to T-RFLP types in each sampling location. The same T-RFLP types were pooled within plots and the relative abundance of each fungus was also calculated.

A total of 17,384 EcM root tips were morphotyped from 184 sampling locations 15 months following the N additions. Among 470 samples that were subjected to molecular identification, 410 samples were categorized as 49 T-RFLP types. ITS sequences were successfully determined for 40 T-RFLP types, in which six T-RFLP types were pooled to the other types due to the high sequence identity (>98%). The most dominant T-RFLP type (identified as *Piloderma fallax*) was observed in two morphotypes, each of which showed distinct color (yellow or silver) under the stereotyped microscope. Because these morphotypes have different ITS sequences (data not shown), they were considered as different species in this study. Species of three T-RFLP types were determined according to the identified sporocarps that had matching T-RFs with root tips. Finally, 29 basidiomycota, 9 ascomycota and 6 unknown species were obtained and regarded as putative species in this study (ESM 1). Among the remaining 60 samples, 44 were assigned to a known species based on their

morphological characteristics (*Cenococcum geophilum* or *Piloderma fallax* (yellow type); ESM 1). T-RFLP types of the remaining 16 samples were unknown, corresponding to 3.7% of the examined root tips, and were removed from further analyses.

Monitoring of forest floor vegetation

Forest floor vegetation was monitored in seven permanent subplots in each main plot. We used the point-intercept method with a frame sized 0.2×0.6 m with 30 random points. At each point a stick (4 mm diameter) was inserted to the vegetation and the number of hits of individual plant species was counted. Monitoring was conducted in August 2007 (a year before the N addition treatments), in August each year 2008, 2009 and 2013. The number of hits per m^2 was calculated and the mean values within plot and year for the five most common species/species groups were used as response variables.

Statistical analyzes

All statistical analyzes were done in R 3.1.1 (R Core Team 2014). Generalized Linear Mixed Models (GLMM) with block as a random variable, and treatment and year/sampling occasion as fixed factors, were used to model the effects of N addition and time, as well as the interaction between N addition and time. When a significant treatment effect was identified in a single year, the GLMM was followed by a Generalized Linear Models (GLM) for that certain year with block as a fixed factor. If the block-effect was non-significant the block factor was removed from the models. Additionally, a factorial variable, indicating the location of a plot in relation to the stand border, was included in the full models of belowground EMF. The GLMs was followed by planned contrasts of the following pairs of treatments; LAN-LARG, HAN-HARG, LAN-HAN, LARG-HARG, with a correction of the P -values by the fdr -method (Benjamini and Hochberg 1995). All response variables were modelled with a Gamma error distribution and log-link. The models were evaluated and checked for heteroscedasticity and other remaining patterns in the residuals by plotting the residuals against the predicted values. A threshold of $P = 0.05$ was used to define significant differences between treatments. The GLMMs were done by applying the `glmmPQL` function in the MASS package (Venables and Ripley 2002) while

the pair-wise comparisons were done by the `glht` function in the multcomp package (Hothorn and Westfall 2008).

Results

Tree growth

During the five-year period following the N additions the stand basal area had increased by $2.3 \text{ m}^2 \text{ ha}^{-1}$ in the control treatment. The basal area growth was significantly higher in three of the N addition treatments (LAN, HAN and HARG) than in the control (Fig. 1). The highest growth was observed in the high AN treatment (HAN) (+40%), but there were no significant differences between the three different N treatments showing a significant growth response (LAN, HAN and HARG) (Fig. 1).

Mobile soil N retained on ion capsules

Nitrogen addition caused, independently of N-form or dose, elevated amounts of ammonium retained on the ion exchange capsules during the growth season in the year of N addition (2008) (Fig. 2). The highest amount of ammonium was observed in the HAN treatment with more than 90 times the amount retained on the capsules

Fig. 1 The basal area (BA) change between 2009 and 2013 in the five treatments: LAN = 50 kg N ha^{-1} AN, LARG = 50 kg N ha^{-1} ARG, HAN = 150 kg N ha^{-1} AN, HARG = 150 kg N ha^{-1} ARG. Treatments with rastered bars were significantly different from the control ($P < 0.05$) in the GLM. There were no significant differences in basal area growth between the three significant N addition treatments according to the planned contrasts analysis

than in the control (Fig. 2). There was a significant effect of N-form on ammonium-N in the low-dose treatments. According to the planned contrasts low addition of AN (LAN) caused significantly larger ($P = 0.004$) amounts of ammonium-N on the capsules than low addition of ARG (LARG) (Fig. 2). There was no significant difference between the doses of AN ($P = 0.091$) but the capsules in HARG retained significantly larger amounts of ammonium-N than the ones in LARG ($P < 0.001$) (Fig. 2).

The GLMM also showed elevated amounts of nitrate-N retained on the capsules in LAN ($P < 0.001$) and HAN ($P < 0.001$) compared to the control treatment (Fig. 2). Significant contrasts were found between N-forms within dose in which capsules in LAN retained larger amounts than LARG ($P < 0.001$) and HAN higher than HARG ($P < 0.001$), while there was no significant difference between LAN and HAN, and between LARG and HARG (Fig. 2). From the winter 2008/2009 and on, both the amounts of ammonium-N and of nitrate-N returned to levels equal to those on control plots and there were no further significant effects of any of the N addition treatments (data not shown).

Production of fungal sporocarps

The total number of fungal sporocarps was significantly lower in plots treated with AN than in the control plots (Fig. 3) while there was no such effect of ARG. This

Fig. 2 The amounts of ammonium-N and nitrate-N retained on ion exchange capsules during the growth season in the year of the N addition in the five treatments. Treatments with rastered bars were significantly different from the control ($P < 0.05$) in the

Fig. 3 Number of sporocarps found in 2009 divided on the four most abundant species/species groups in the five treatments. Combinations of species and treatments with rastered bars were significantly different from the control ($P < 0.05$) in the GLMM. Parts of bars with different letters indicate significant pair-wise contrasts based on a GLM ($P < 0.05$). Observe that the following contrasts were included in the analyzes: LAN-LARG, HAN-HARG, LAN-HAN and LARG-HARG

effect was, however, only discernable in 2009, the year following the N addition, but this year also had a generally higher abundance of sporocarps than the other

GLMM. Bars with different letters indicate significant pair-wise contrasts based on a GLM ($P < 0.05$). Observe that the following contrasts were included in the analyzes: LAN-LARG, HAN-HARG, LAN-HAN and LARG-HARG

studied years ($P = 0.027$). Significant contrasts between treatments in 2009 were found between HAN and HARG ($P < 0.001$) and close to significant between LAN and LARG ($P = 0.057$), while there were no differences between the doses within N-form (Fig. 3).

Both *C. semisanguinus* and *Cortinarius* spp. had lower number of sporocarps in LAN, HAN and HARG than in the control in 2009. On the other hand, there was no significant difference between the control and LARG (Fig. 3) indicating an effect of dose in ARG with larger effects at the higher dose, something that was absent in the AN treatments. There were significant contrasts between LAN and LARG ($P = 0.043$), HAN and HARG ($P < 0.001$) and between LAN and HAN ($P < 0.001$) in number of *C. semisanguinus* sporocarps (Fig. 3). Significant differences between the control and LAN, and the control and HARG, were found for the number of *Laccaria bicolor* and *Lactaria rufus* sporocarps, respectively. Both these species were significantly more abundant in the HARG than in the LARG and HAN treatments. Hence, although there was no significant effect of ARG on the total number of sporocarps, there was a considerable shift in species dominance in the ARG treatment with the highest dose (HARG) (Fig. 3).

Belowground EcM

Root tip EcM community was dominated by *C. geophilum* (in average over all plots on 21% of the root tips) and three *Piloderma* species (15, 15 and 11% of the root tips, respectively). All the other species were found on less than 3% of the root tips and observed on less than 13 of the plots. Among these four dominant species *C. geophilum* was found on a significantly higher share of the root tips in HAN than in the control according to the GLM ($P = 0.045$) and a higher share in LAN than in HAN in the planned contrasts ($P = 0.014$). Additionally, there was a larger share of *Piloderma* sp. in LAN than in the control ($P = 0.002$). A complete species list can be found in ESM 1.

Species composition of forest floor vegetation

There were no pre-N-addition differences between the treatments in abundance of any of the analyzed ground vegetation species (Fig. 4). Already in late summer 2008 (two months after N addition), the abundance of the

narrow-leaved grass *A. flexuosa* had responded with an increased abundance in all N addition treatments (Fig. 4). The general increase in this species due to fertilization remained in 2009 and 2013, although with a smaller effect in 2013 (Fig. 4). There were no effects of N-form on *A. flexuosa* in any of the years but significant differences between the AN doses in 2009 ($P = 0.039$) and between the ARG doses in 2008 ($P = 0.003$), 2009 ($P = 0.039$) and 2013 ($P = 0.016$) (Fig. 4). There were no effects of N addition on the abundance of *V. myrtillus* or forbs in any of the analyzed years (Fig. 4). Similarly, *V. vitis-idaea* did not show any response to N addition during the two first inventories. In 2013, however, the abundance of this species was significantly lower in all N treatments than in the control, while none of the analyzed contrasts were significant. The abundance of the bottom layer bryophyte *Pleurozium schreberi* was significantly lower in all N treatments other than LAN in 2009, and in all N treatments in 2013. HARG had a lower ($P = 0.001$ – 0.040) abundance of this bryophyte than LARG and LAN in both these years (Fig. 4) suggesting an effect of N-form and dose.

Discussion

In this field study the plot-scale application of ARG and AN resulted in significantly increased basal area growth compared to the control. However, the stimulation of growth was similar for the two N sources. Hence our data do not support the hypothesis that ARG addition would result in a greater tree growth response than AN addition. We also predicted that unwanted side effects of N addition on ground vegetation and mycorrhizal fungi would be lesser from ARG than AN addition. We found that effects differed between the N forms mainly as the bryophyte *Pleurozium schreberi* decreased more from ARG, and as EcM sporocarps declined from AN, but not from ARG.

Our investigation of mycorrhizal colonization of pine root tips one year following the N addition, showed no effect on the overall root tip colonization from neither of the N forms. The responses of ectomycorrhizas to N addition are known to be variable. Some studies report no changes in coniferous forests (Fransson et al. 2000; Peter et al. 2001; Berch et al. 2009; Hay et al. 2015), while others report significant reductions in abundance and changes in species composition (Lilleskov et al. 2002; Frey et al. 2004; Berch et al. 2006; Jones et al.

Fig. 4 The abundance (hits per plot) of five species/species groups in the forest floor vegetation in 2007 (before N addition), in August 2008 (two months after N addition), and in August of 2009 and 2013 in plots of the five treatments. Treatments with rastered bars were significantly different from the control

($P < 0.05$) in the GLMM. Bars with different letters indicate significant pair-wise contrasts based on a GLM ($P < 0.05$). Observe that the following contrasts were included in the analyses: LAN-LARG, HAN-HARG, LAN-HAN and LARG-HARG

2012). The N addition effects on ectomycorrhizal sporocarp production were, however, more pronounced. The overall abundance of sporocarps on AN plots was less than half of that on ARG or control plots. Mainly it was sporocarps of *Cortinarius* spp. that declined from AN additions. There was a significant dose effect as the high AN dose caused a significantly larger decline of sporocarps than the low AN dose. Also the high ARG dose caused a significant decline of sporocarps in comparison to control plots, while the low ARG dose had no such effect. Negative effects of inorganic N addition on *Cortinarius* spp. sporocarp production have previously been reported from experiments with annually repeated N additions, and may last for as much as 50 years after the termination of N additions (Strengbom

et al. 2001; Hasselquist and Högborg 2014). Noticeable was also the strong increase of *Lactarius rufus* (Scop:Fr) Fr sporocarps on high ARG plots. The proportion of this species of the total species pool was more than doubled on high ARG plots compared to low ARG plots and control plots. Other studies have shown that inorganic N addition may promote both tree root colonization and sporocarp production of *Lactarius* spp. (Strengbom et al. 2001; Hasselquist and Högborg 2014; Suz et al. 2015) and our result corroborate *L. rufus*' capacity to proliferate under circumstances of elevated N supply, although only when N was supplied in an organic form.

Nitrogen addition effects on boreal forest floor vegetation have been extensively investigated mainly in experiments with repeated N additions (see for

example Nohrstedt 2001 and references therein; Nordin et al. 2009; Hedwall et al. 2013; Manninen and Tolvanen 2013). Here we show that the proliferation of *A. flexuosa* commonly seen as a response to continuous N additions, also occurs already a few months following one single N application, independent of the N form added. The strongest increase in *A. flexuosa* was, however, seen the year after the N additions when the abundance was three times higher on both high AN and high ARG plots than on control plots. An effect of N dose was apparent as the abundance was significantly higher in high N plots than in low N plots although it was not proportional, i.e. although the N dose was three times higher on high N than on low N plots the *A. flexuosa* abundance was only c 30% higher on the high N plots. Five years after the N additions the abundance of *A. flexuosa* had declined, but was still higher on N addition plots than on control plots with a remaining N dose effect only on ARG plots. The very similar responses of *A. flexuosa* to AN and ARG addition was surprising since previous N uptake studies have shown that while this species has a very high capacity to proliferate on inorganic N and in particular on nitrate, it does not readily take up ARG (Persson et al. 2003; Nordin et al. 2006). However, following ARG addition high levels of ammonium were retained on ion exchange capsules suggesting that ammonium could have been a major N source for *A. flexuosa*. A decreased abundance of *A. flexuosa* over time has been observed also in other N addition experiments (i.e. Nordin et al. 2009) and may be due to both reinstated N limitation and suppression from N enhanced growth of the tree canopy (Strengbom et al. 2001).

In contrast to the immediate response to N addition displayed by *A. flexuosa*, a negative effect from the N additions on the abundance of the dwarf shrub *V. vitis-idaea* was detected only after five years. Studies have shown that light and moisture conditions are more important than N supply in explaining the abundance of *V. vitis-idaea* (Mäkipää et al. 1999; Palmroth et al. 2014) and we suggest that the long-term negative response of this species to the N additions is mainly due to tree canopy suppression. For the bryophyte dominating the site, *P. schreberi*, an immediate negative effect of N addition was expected as bryophytes have a high capacity to take up N, particularly as ammonium and amino acids (Forsum et al. 2006; Wiedermann et al. 2009), and bryophyte decline in high N environments have been repeatedly reported (Hedwall et al. 2010; Verhoeven

et al. 2011; Gundale et al. 2013) and subjected either to direct toxic effects or to increased competition from vascular plants (Turetsky et al. 2012 and references therein). Due to the absent immediate effect on bryophyte abundance, it may be that the observed long-term decline of *P. schreberi* was more related to suppression from competing vegetation than to any immediate toxic effects from the N additions.

Understanding how N addition affects structures and functions directing northern ecosystems' long-term capacity for carbon sequestration is key to fully apprehend how management measures, like N additions, can be directed to interlink N and carbon dynamics for effective climate change mitigation (Mack et al. 2004; Janssens et al. 2010; Maaroufi et al. 2015). The less negative effect of ARG compared to AN on EcM fungi is interesting in this perspective as C derived from EcM fungi may remain more recalcitrant than C input via plant litter (Clemmensen et al. 2013, 2015). Our short-term result showing different EcM responses to ARG and AN additions merits further investigations revealing the responsible mechanisms incorporating ARG and AN in different types of forest ecosystems before making conclusions on the long-term large-scale ecosystem effects.

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