

The effects of N-enriched rain and warmer soil on the ectomycorrhizae of black spruce remain inconclusive in the short term

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Abstract

- **Context** Warmer temperatures and anthropogenic N depositions are altering soil nutrient cycles and plant nutrition worldwide and are projected to rise dramatically in the future, particularly at the high latitudes. How much will such alterations affect symbiotic organisms such as ectomycorrhizae (ECM)?
- **Aims** The aim was to investigate the short-term effects of warmer soil temperatures and N-enriched precipitations on roots and ECM.
- **Methods** A field experiment was established during 2008–2010 in two black spruce (*Picea mariana*) stands of the boreal forest of Quebec, Canada. The ECM of 12 trees per site were counted and identified by morphotyping.
- **Results** After 3 years, soil heating markedly modified the proportions of vital root tips and ECM in the colder stand, while no effect of the N-enriched rain was observed on soil

chemistry and consequently on roots and ECM. Density and ramification of root tips were not affected by the treatments.

- **Conclusion** The hypothesis that the treatments would alter the ECM was confirmed only partially in the colder stand. The type of application and amount of N supplied were unable to substantially modify in the short-term growth conditions of roots and ECM, explaining the observed results.

Keywords Boreal forest · Nitrogen · Fungal diversity · Morphotyping · *Picea mariana* · Soil temperature

1 Introduction

In the boreal forest, the reduced evapotranspiration and low decomposition rates slow down the nutrient cycle and lead to a gradual accumulation of organic matter during forest stand successions, maintaining a huge part of the nitrogen in the ecosystem in a form unavailable for plants (Read et al. 2004; Rossi et al. 2009). The climatic models for Northeastern Canada estimate a warming of up to 3 °C over the next 50 years (Plummer et al. 2006), with effects already noticeable in the growth dynamics of the boreal species (Boulouf Lugo et al. 2012). The increase in surface air temperature and the changes in the dates of snowmelt will inevitably warm the soil, with expected increases during the growing season of 4 °C for 2050–2100 (Houle et al. 2012). Along with the increased anthropogenic N depositions, soil warming is expected to increase decomposition, thus favoring mineralization and enhancing N availability (Campbell et al. 2009). This may alter the slow nutrient cycles and, potentially, the dynamics of all living organisms in the soil. As the boreal forest represents 27 % of the world's forest cover and contains more than 30 % of all carbon present in the terrestrial biomes (Kasischke 2000), any change in this biome has obvious

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Contribution of the co-authors SR: experimental design, data analysis and paper writing.

AB: data collection and literature reviewing.

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ecological and economic relevance and should be predicted as soon as possible.

Investigations on increased nutrient availability have used large amounts of N, often spread in few applications directly on the forest floor, which can hardly be compared to a natural process (Gundale et al. 2011). On the contrary, most of the boreal region receives only 4 kg ha⁻¹ year⁻¹ or less of nitrogen, while past studies have added quantities of 1–3 orders of magnitude higher, representing a fertilization rather than a natural deposition (Gundale et al. 2011). In the past, warming of trees was regularly performed on seedlings in a growth chamber or nursery (Domisch et al. 2002), which precluded the possibility of extending the results to the whole stand. More recently, warming has been more suitably carried out in the field, with the challenging technical problems related to works in natural and remote locations (Clemmensen et al. 2006).

In 2008, a field experiment was established in two mature stands of the boreal forest of Quebec, Canada, simulating increases in soil temperature and nitrogen-enriched rainfall (Lupi et al. 2012a). After only 3 years of treatment, the effects on trees were slight but significant. In particular, the major findings concerned the phenology of roots, which showed earlier resumptions and longer durations of growth in warmed soils (Lupi et al. 2012a). The root system of trees is a resource for which a number of symbionts compete, and root colonisation is a necessary condition for survival and reproduction of mycorrhizal fungi (Bruns 1995). Besides, boreal forest conifers allocate high proportions of their photosynthates for the metabolism of belowground organs to feed and sustain as wide as possible a variety of ectomycorrhizae (ECM) (Read et al. 2004). In turn, ECM proliferate within the organic horizons where they intensely exploit the N stored within the litter and in the organic matter for improving plant nutrition. Thus, ECM fungi and communities are expected to be markedly affected by alterations of soil. Moreover, ECM are the more dynamic components of the partnership and respond directly and more rapidly to environmental disturbances and climate change than their long-living hosts (Staddon et al. 2002, Deslippe et al. 2011).

Because of their central role in several biogeochemical processes of the ecosystem, any modification of the ECM may generate feedbacks on other components of the system, such as the soil or the vegetal partner. In addition to nutrient uptake, ECM symbioses improve the protection from soil pathogens and the tolerance to stress of trees. Consequently, any environmental disturbance could affect plants directly, via modifications of the growing conditions, and indirectly, by modifying the nature and abundance of mycorrhizae. Fine roots growing in warmer soils or at higher N availability are expected to experience increased ECM colonisations (Rossi et al. 2012) because of the higher species richness and mycelial growth (Clemmensen et al. 2006; Kranabetter et al. 2009). However, Lilleskov et al. (2008) observed contrasting and

species-specific responses of ECM, with the frequency of certain morphotypes either increasing or decreasing with N availability. This paper investigated the ECM 3 years after the beginning of the experiment by Lupi et al. (2012b). We expected that the longer growing periods observed in certain treatments may correspond to modifications of the relationships of host–symbionts and, consequently, to changes in the presence of ECM. A hypothesis was proposed that soil heating and nitrogen addition by simulated rain would rapidly and substantially alter the ECM both in terms of presence and diversity of species. The hypothesis was tested by comparing ECM abundance and richness between the treatments. Root tip vitality was also analysed in order to quantify the eventual effects of the treatments on the absorbing parts of the root system.

2 Material and methods

2.1 Study sites

The study was conducted in two mature, even-aged and monospecific black spruce [*Picea mariana* (Mill.) BSP] stands of the boreal forest of Quebec, Canada. The first site (abbreviated as BER) was located near lake Bernatchez, in the Monts-Valin (48°51' N, 70°20' W, 611 m a.s.l.). The second site (SIM) was at a lower altitude, in the réserve faunique des Laurentides, within the Simoncouche research station (48°13' N, 71°15' W, 350 m a.s.l.). The stands are growing on gentle slopes (8–17 %) and drained glacial tills. They originate from forest fires that occurred in 1870 and 1922 for BER and SIM, respectively. The climate is continental, with short summers and long, cold winters with mean annual temperatures of 0.2 and 1.9 °C for BER and SIM, respectively, and a total May–September rainfall of 413.6 mm (Lupi et al. 2010, Boulouf Lugo et al. 2012). Snow cover lasts from November to May, reaching a depth of up to 150 cm in both sites. Because of its higher altitude and latitude and shorter snow-free periods, trees in BER experience shorter growing seasons and grow in more humid and cold soils than those in SIM (Rossi et al. 2011b). According to Boulouf Lugo et al. (2012), during 2002–2010, the average growing season was 125 and 109 days in SIM and BER, respectively. The soils of both sites were podzol with a mor-type humus (Table 1). The LFH (organic) horizon was 10 and 18 cm in depth in SIM and BER, respectively. In SIM, the maximum rooting depth was frequently limited by a shallow bedrock.

2.2 Experimental design

In each site, a square plot of 60×60 m was delimited and divided in twenty-five 12×12-m squares, of which 12 acted as experimental units with the others being buffer zones (Fig. 1).

Table 1 Soil description of the two studied sites

	BER	SIM
Soil type	Podzol	Podzol
Humus type	Mor	Mor
	Of, 0–11 cm	Of, 0–2 cm
	Om, 11–16 cm	Om, 2–8 cm
	Oh, 16–18 cm	Oh, 8–10 cm
Profile description	Ae, 18–23 cm	Ae, 10–26 cm
	Bhf, 23–26 cm	Bf, 26–45 cm
	B, 26–46 cm	C, >45 cm
	C, >46 cm	

Of organic fibric, *Om* organic mesic, *Oh* organic humic, *Ae* surface eluvial horizon, *B* buried horizon, *Bhf* enriched with organic matter, *Fe* and *Al*, *Bf* enriched with *Fe* and *Al*, *C* cemented horizon

In each experimental unit, a tree was chosen with upright stem and healthy overall appearance. On average, trees were 16 and 17 m tall, with a diameter at breast height of 17 and 20 cm in BER and SIM, respectively. Different treatments were applied during 2008–2010, consisting of an increase in soil temperature (heated trees, abbreviated as H), a canopy application of artificial rain enriched with nitrogen (N-enriched trees, N) and a combination of heating and N enrichment (NH trees). The combination of the treatments resulted in three different levels (H, N, NH) and a control (C) that were organized in a random split plot design with three replications (Fig. 1).

For the H treatment, heating cables were installed during the autumn of 2007 between the organic and mineral soil layers, at about 20–30-cm depth, where the root system of black spruce is mainly localized (Ruess et al. 2003), following a spiral pattern at a distance of 90–200 cm from the stem collar. To account for potential root damage and soil disturbance during cable laying, non-heating cables were also installed around control trees. The power unit consisted of a diesel generator located 200 m from the site, and soil temperature was measured between the coils of the cables for three heated and three control trees. The treatment was carried out in

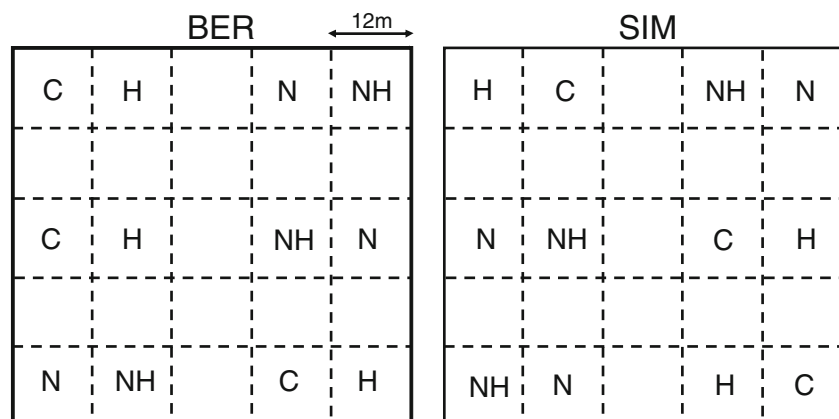
spring, during the period in which most cambial division takes place (Thibeault-Martel et al. 2008; Rossi et al. 2011a), and consisted of increasing the soil temperature by 4 °C in line with the forecasts for 2100 in the boreal forest of eastern Canada (Houle et al. 2012).

The artificial rain was produced by sprinklers installed above the canopy of each tree. Each week, the equivalent of 2-mm rainfall was applied to the canopy, during the frost-safe period (June to September), for a number of weeks varying between 12 and 16. Rain was applied over a circular area of 3-m radius centred on the stem of each experimental tree, which allowed the canopy area to be covered. Control trees were irrigated with a water solution reproducing the chemical composition of natural rainfall over the studied sites (Duchesne and Houle 2008), while for N-enriched treatment, a threefold increase in ammonium nitrate (NH₄NO₃) concentration was used. Actually, the sites receive naturally 0.7–1 kg ha⁻¹ year⁻¹ of N, while N trees were supplied with an additional 0.35–0.5 kg ha⁻¹ year⁻¹ of N, representing approximately an increase of 50 % in N per year. The treatment with frequent N additions at low concentration provided directly to the canopy accurately imitated the natural N depositions expected within 50 years in Quebec.

2.3 Sampling and data collection

In October 2010, three soil cores (17 cm in length and 4.5 cm in diameter) per tree were collected at a distance of 150 cm from the root collar, sealed in plastic bags, immediately transported to the lab and stored at –10 °C in the dark. Cores were collected with a hand auger from the organic horizon, where the majority of black spruce fine root production occurs (Ruess et al. 2003), around the stem to achieve the greatest sampling efficiency for analysis of the ECM (Lilleskov et al. 2004). The analyses were performed within 8 months from sampling. Fifteen root fragments per sample were randomly chosen among those with undamaged and fully developed apical tips and were carefully cleaned in water under a

Fig. 1 Experimental design in BER and SIM with the location of control (C), heated (H) and N-enriched (N) trees. NH corresponds to trees subject to the combined treatment



stereomicroscope. Five root tips located at the distal portion of the fragment (i.e. situated away from the point of attachment) and representing the last formed apices of each rootlets were chosen and classified as vital or non-vital according to Montecchio et al. (2004). Preliminary analyses demonstrated that this sampling size was adequate to statistically represent the ECM of black spruce (Rossi et al. 2012). Moreover, the five distal tips allowed to observe the most recently produced tips and consequently those representing the response to the treatments. Non-vital tips showed a scurfy surface and an easily detachable cortex, with or without remnants of ECM mantle. Vital tips were well developed, turgid and inflated. The vital tips were classified as mycorrhized or non-mycorrhized according to whether the ECM mantle was present or lacking, respectively. The systems deriving from ramification of ECM were counted as one ECM, irrespective of the extent of ramification (Montecchio et al. 2004). The rootlet length was defined as the length of the root fragment where the five root tips were collected. The number of root tips and ramifications per unit of rootlet length was calculated for assessing their density along the rootlet. Damaged and not fully developed root tips were excluded from the observations.

All collected ECM were coded and classified into morphotypes by recording their morphological and anatomical characteristics under a stereomicroscope and a light microscope, at magnifications of $\times 40$ and $\times 400$, respectively, using the available literature (Agerer 1991; Goodman et al. 1996; Agerer and Rambold 2004–2011). ECM characteristics included the occurrence and type of ramification, colour and features of the mantle surface and type and features of the outer, middle and inner mantles. Also, when present, type and features of the emanating elements (hyphae, rhizomorphs, cystidia, laticifers) were observed and described in order to identify each morphotype as accurately as possible. The detection of the main microscopic (anatomical) features besides the macroscopic (morphological) characteristics allowed a more reliable and unambiguous identification of the ECM in the different morphotypes (Mehmann et al. 1995).

For assessing element concentration after treatments, soil cores were collected in October 2010, at the end of the 3 years of experiment. Soil was air dried at 65 °C for 48 h and sieved through a 2-mm mesh. The amount of total carbon (C_{tot}) and nitrogen (N_{tot}) extracted was determined using a combustion element analyser. Potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), aluminium (Al), iron (Fe), sodium (Na), sulphur (S) and pH were determined using an unbuffered ammonium chloride (NH_4Cl ; 1 mol L^{-1} , 12 h) solutions.

2.4 Statistics

Generalised linear mixed models [GLIMMIX procedure in SAS 9.2 (SAS Institute Inc., Cary, NC)] were used for

analysing the proportion of vital and mycorrhizal tips. Generalised linear mixed models (GLMMs) were modelled with logit-link functions, where the response variable was implemented as the ratio between the number of events (e.g. number of vital tips) and the number of trials (e.g. number of observed tips). Element concentration, number of morphotypes, densities of root tips and ramifications (i.e. tips and ramifications per unit of rootlet length) were compared with generalised linear models (GLMs). Data for ramifications were log transformed to respect the assumption of normality in data distribution. Overall tests for the significance of the fixed effects based on factorial comparisons were evaluated with type III hypotheses.

The morphotypes were used in a multivariate analysis to investigate the effect of the treatments on the most frequent ECM. Canonical discriminant analysis was applied separately for each site to find linear combinations of quantitative variables, represented by the frequencies of the morphotypes, which provide maximal separation between treatments, and successively test the hypothesis that the class means are equal in the population. The morphotypes were grouped by soil sample, with a resulting n of 36, and included in the analysis only if observed in at least three different samples. Wilks' λ was performed for overall comparison of the class means.

3 Results

3.1 Effects of treatments on soil conditions

Different absolute values but similar temporal patterns of soil temperature were observed at both sites. In winter, the temperature was close to 0 °C (Fig. 2). In spring, complete snowmelt was followed by an abrupt increase in soil temperature. The pattern of soil temperatures in summer and early autumn followed that of the air temperature, but with lower daily and seasonal variations (data not shown). Heating started in April, usually with a 2-week delay between SIM and BER to reflect the difference in air temperature and timing of snowmelt between the two sites. For the three studied years, the system successfully maintained the defined temperature differential of 4 °C until July, simulating years with earlier snow melts and consequently longer snow-free periods in early spring.

C_{tot} in the organic horizon varied between 375.0 and 483.0 g kg^{-1} , while N_{tot} ranged between 8 and 9.7 g kg^{-1} (Table 2). On average, the soil around N trees showed higher concentrations of N_{tot} in both sites. However, no significant effect of the treatments was observed on soil chemistry ($p > 0.05$), except for S in BER, where significantly lower concentrations were detected in the soil of H trees ($p < 0.05$). The two sites differed in terms of soil chemistry, with BER showing higher concentrations of C_{tot} , Ca, Mg and Fe. These

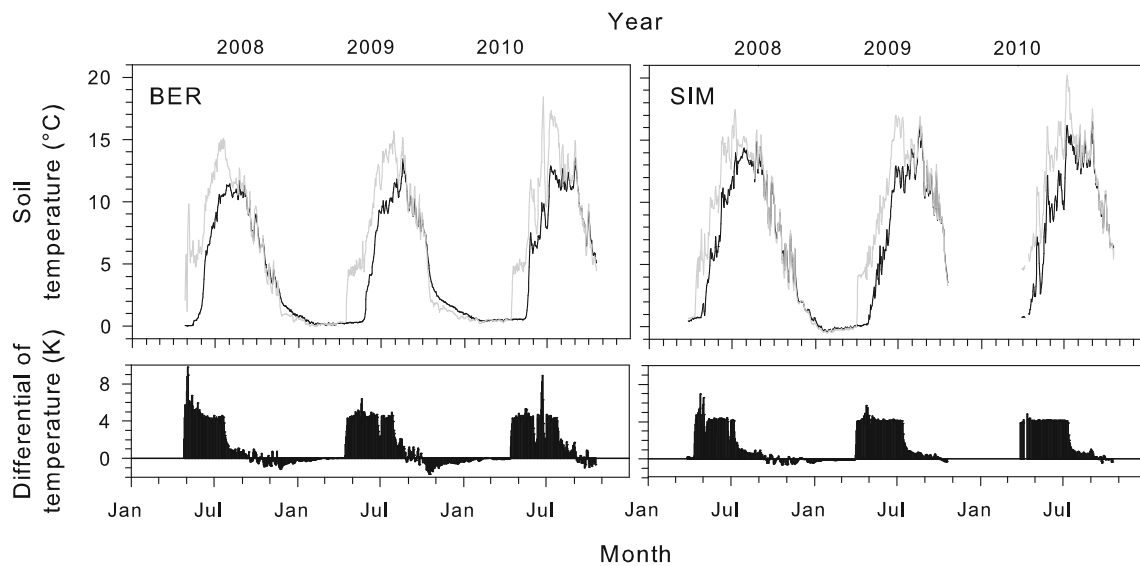


Fig. 2 Soil temperature in control (black line) and heated (gray line) trees and relative differential in BER and SIM during the 3 years of experiment

differences were confirmed both for all trees and C trees only. However, mean pH was higher in SIM, but this was not confirmed in C trees.

3.2 Root tips and ECM

In BER and SIM, vital root tips represented 94.0 and 90.5 % of the roots analysed, respectively (Fig. 3). A significant effect of the treatments was observed in BER, where greater proportions of vital root tips were detected for H trees ($p < 0.05$, Table 3), but no differences were found for N trees. Differences among treatments were not significant in SIM ($p > 0.05$, Table 3). Overall, 89.1 % of the root tips showed ECM, but wide differences were observed among treatments, mainly in H trees (Fig. 3). H treatment significantly reduced the

proportion of ECM in BER, with 83.1 and 91.3 % of ECM detected in heated and non-heated trees, respectively ($p < 0.0001$, Table 3). Although a small difference was observed in SIM with 90.0 vs. 92.0 % of ECM in heated and non-heated trees, respectively, no significant difference was detected ($p > 0.05$, Table 3). No effect was observed for the combined treatment $N \times H$ ($p > 0.05$). Compared to SIM, C trees in BER had greater proportions of vital root tips (91.1 vs. 92.8 %), but similar proportions of ECM (92.3 vs. 92.1 %).

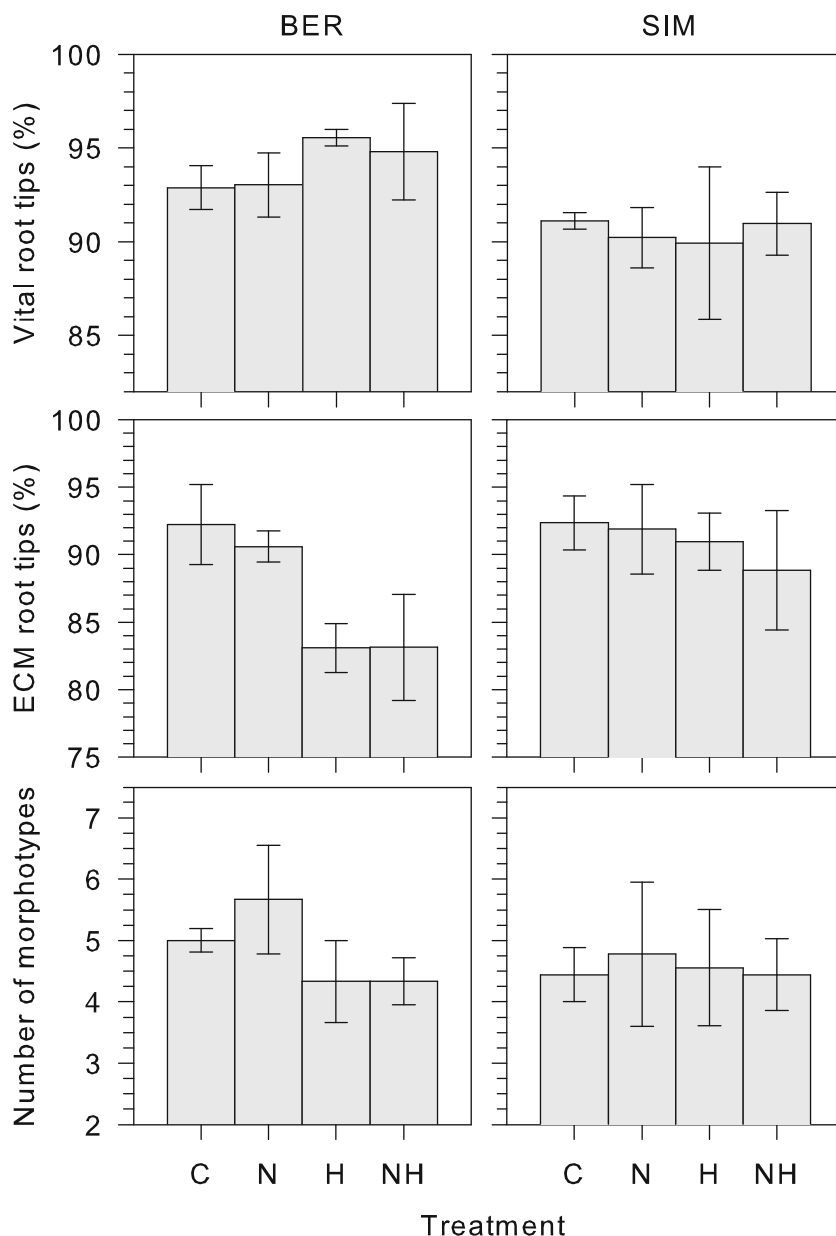
The analysed fragments had a length ranging between 0.2 and 2.3 cm. On average, 9.0 tips cm^{-1} were present, with root tips being slightly more abundant in BER. In BER, H trees showed fewer tips per centimetre of root than non-heated trees, but differences were not significant ($p > 0.05$, Table 3). Similarly, no effect of the treatments

Table 2 C and N content and exchangeable cations of the organic horizon measured after 3 years of treatment

	BER				SIM			
	C	N	H	NH	C	N	H	NH
C_{tot} (g kg^{-1})	428.3	483.0	454.7	462.0	375.0	426.0	426.0	387.7
N_{tot} (g kg^{-1})	8.5	8.9	8.0	8.7	9.7	10.9	11.1	10.3
K (mg kg^{-1})	471.0	401.7	504.3	505.7	552.0	410.3	523.7	529.7
Ca (mg kg^{-1})	2,636.7	2,920.0	2,260.0	2,030.0	1,893.3	1,503.3	1,830.0	1,346.7
Mg (mg kg^{-1})	444.0	356.7	366.3	402.3	332.0	276.7	282.3	328.0
Mn (mg kg^{-1})	78.1	43.0	102.4	63.9	142.8	124.3	131.0	129.7
Al (mg kg^{-1})	245.7	297.3	398.7	393.0	254.0	727.7	504.3	508.0
Fe (mg kg^{-1})	450.3	306.3	735.3	370.7	287.0	409.7	395.3	608.0
Na (mg kg^{-1})	24.7	25.3	24.0	26.7	29.7	31.3	33.0	30.3
S (mg kg^{-1})	91.3	83.3	70.7	72.7	103.3	106.7	123.7	103.3
pH	2.9	2.6	2.7	2.6	2.8	3.1	3.1	3.1

C control, H heated, N N enriched, NH combined treatment

Fig. 3 Proportion of vital and ECM root tips and number of morphotypes per soil core recorded on black spruce in BER and SIM after 3 years of treatment reported as mean ± standard error. C control, H heated, N N enriched, NH combined treatment



was detected in the number of ramifications per centimeter of root ($p > 0.05$, Table 3). On average, 0.5 ramifications cm^{-1} were calculated, with C trees in SIM exhibiting the highest density of ramifications.

3.3 Morphotypes

A total of 41 morphotypes were detected in the plots, consisting of a few dominant fungal species and many that

Table 3 *F* values resulting from the type III tests of fixed effects for GLMM and GLM models, where H and N correspond to the heating and N enrichment treatments

	BER			SIM		
	N	H	N × H	N	H	N × H
Vital root tips	0.18	5.98*	0.31	0.00	0.04	0.73
ECM root tips	0.56	37.37**	0.73	1.26	3.02	0.33
Density of root tips	0.79	1.72	0.31	0.55	0.32	0.16
Density of ramifications	0.05	3.18	1.59	0.08	1.20	0.44
Number of morphotypes	0.31	2.82	0.31	0.03	0.03	0.11

* $p < 0.05$, ** $p < 0.0001$, statistically significant differences

Table 4 Relative frequency (percent) of the 41 morphotypes detected on black spruce in BER and SIM after 3 years of treatment

Ectomycorrhizal morphotype	BER				SIM					
	C	N	H	NH	C	N	H	NH		
<i>Cenococcum geophilum</i>	18.7	23.7	20.7	23.8	– ^a	31.3	29.7	17.2	26.2	– ^a
<i>Hebeloma</i> sp.	21.1	27.4	8.8	15.9	– ^a	7.2	3.2	8.7	1.3	– ^a
Unknown fungus I	1.6	0.9	0.9	0	– ^a	14.1	0.5	9.2	5.5	– ^a
<i>Piceirhiza bicolorata</i>	1.2	0	0.4	3.4	– ^a	0.4	3	0.5	0.9	– ^a
Unknown fungus II	0.3	4.4	9.9	2.3	– ^a	3.5	7.3	0	22	– ^a
Unknown fungus III	3.8	6.5	2.2	5.1	– ^a	3.2	0	2.9	3.1	– ^a
Cortinariaceae I	0	1.8	1.1	0.2	– ^a	4.6	3.2	4.2	0.9	– ^a
<i>Lactarius</i> sp. I	0	6.5	8.8	21.6	– ^a	8.6	5.5	9.4	11.7	– ^a
Tricholomataceae	6.2	0.9	0.6	0	– ^a	0	6.6	12.3	1.5	– ^a
<i>Mycelium radicans atrovirens</i>	1.6	2.5	0	3.4	– ^a	0.7	0	0.2	0.7	– ^a
<i>Piloderma fallax</i>	0	1.2	0.4	0	– ^a	5.5	5.5	7.1	13.2	– ^a
Cortinariaceae II	2.2	3.5	14.7	8.6	– ^a	0	0.2	0	0.4	– ^a
Unknown fungus IV	5.4	1.1	0	0.4	– ^a	0.9	7.7	2	0	– ^a
<i>Inocybe</i> sp.	5.9	0.9	0	0	– ^a	4	0.7	1.1	0	– ^a
Unknown fungus V	2.8	1.6	0	0	– ^a	3.7	0	5.8	1.8	– ^a
<i>Cortinarius</i> sp.	0	0	3.9	2.3	– ^a	0	5.2	1.8	9	– ^a
<i>Russula</i> sp. I	0	0	0	6.8	– ^a	1.2	4.1	0.5	0	– ^a
Unknown fungus VI	1.6	3.5	0	2.4	– ^a	0.7	0	0	0.4	– ^a
<i>Amphinema byssoides</i>	0.5	1.9	0	1.9	– ^a	0	0	4.2	0	– ^a
<i>Neocudoniella radicea</i>	0	0	0.7	0	– ^a	0.2	0.9	0	0	– ^a
Unknown fungus VII	0	1.8	6.3	0	– ^a	0	0	0	0.2	– ^a
<i>Piceirhiza gelatinosa</i>	8.3	0.4	0	0.4	– ^a	0	0	0	0	
Unknown fungus VIII	0	0	0	0		0.5	0.9	6.1	0	– ^a
Unknown fungus IX	0.7	1.4	0	0	– ^a	0	0	0.9	0	– ^a
Unknown fungus X	0	0	0	0		9.5	3.2	0	0.7	– ^a
Unknown fungus XI	11.2	1.8	1.3	0	– ^a	0	0	0	0	
Unknown fungus XII	3.5	0.2	0	0	– ^a	0	0	0	0	
<i>Paxillus involutus</i>	0	0	0	0		0	0.4	0.9	0	
Sebacinaceae I	0.2	0.7	0	0		0	0	0	0	
Sebacinaceae II	0	0	0	0		0	0	0.2	0.5	
Unknown fungus XIII	0	0	0	0		0.2	0	2.2	0	– ^a
<i>Russula</i> sp. II	0	0	9.5	1.7		0	0	0	0	
Russulaceae	0	0	0	0		0	11.8	0	0	
Unknown fungus XIV	0	5.6	0	0		0	0	0	0	
Unknown fungus XV	0.7	0	0	0		0	0	0	0	
<i>Hydnellum</i> sp.	0.9	0	0	0		0	0	0	0	
Unknown fungus XVI	0	0	7.6	0		0	0	0	0	
<i>Tomentella</i> sp.	0	0	0	0		0	0.2	0	0	
Unknown fungus XVII	0	0	0	0		0	0	2.7	0	
<i>Russula</i> sp. III	1.7	0	0	0		0	0	0	0	
<i>Lactarius</i> sp. II	0	0	2.1	0		0	0	0	0	

The characteristics of each morphotype are reported in the Supplementary material
 C control, H heated, N N enriched, NH combined treatment
^a Morphotypes included in the canonical discriminant analysis

were observed occasionally (Table 4 and Supplementary material). Although the selected morphological and anatomical characteristics allowed most morphotypes to be associated to a species, genus, or family, 17 morphotypes remained unidentified. *Cenococcum geophilum* was the most frequent

morphotype, with relative frequency ranging between 17.2 and 31.3 %. Most of the more frequent morphotypes, which belonged to the families of Cortinariaceae, Tricholomataceae and Atheliaceae, occurred in both sites, but only two (*C. geophilum* and *Hebeloma* sp.) were detected in all treatments

(Table 4). Nine morphotypes were observed in only one group belonging to the control or treatment.

A wide variation in the number of morphotypes was observed among samples in all treatments and at the two sites, which ranged between one and nine morphotypes per soil core (Fig. 3). On average, 4.7 morphotypes per soil core were identified in C plots. Overall, more morphotypes were detected in N trees (5.2 morphotypes per soil core). Moreover, fewer morphotypes seemed to be observed in H and NH trees of BER. However, no significant effect of the treatments was detected on the richness of morphotypes in either site ($p > 0.05$, Table 3).

Twenty-five morphotypes from each site were used in the canonical discriminant analysis (Table 4). Overall, canonical axis 1 represented between 0.45 and 0.60 of the variation accounted for, while a lower variation of 0.27–0.29 was explained by canonical axis 2 (Fig. 4). No discrimination was observed along canonical axis 3 (data not shown). In BER, three centroids were generated by the discriminant analysis, with control and H treatment being visibly separated by N and NH according to both canonical axes. A separation among centroids was also observed in SIM, with axis 2 discriminating between N trees and the other treatments. However, the wide variation among samples in both sites produced low values of Wilks' λ , which corresponded with non-significant F statistics ($p > 0.05$), meaning that no difference was detected between the control and the three treatments. Consequently, no effect of soil heating and nitrogen addition by simulated rain was observed on the overall occurrence of the most frequent morphotypes.

4 Discussion

In this study, trees of mature black spruce stands were subjected to the warmer soil temperatures and increased N depositions expected in the next future in the boreal forest of eastern Canada in order to investigate the short-term effects on ECM. The treatment of increased N deposition was unable to modify the concentration of N in the soil and, consequently, no significant effect was observed on root vitality and ECM presence. Soil warming markedly modified the proportions of vital root tips and ECM in one site, but no effect was observed for the combined treatment N \times H. Thus, these results only partially confirm the initial hypothesis that the treatments would rapidly alter root vitality and mycorrhization.

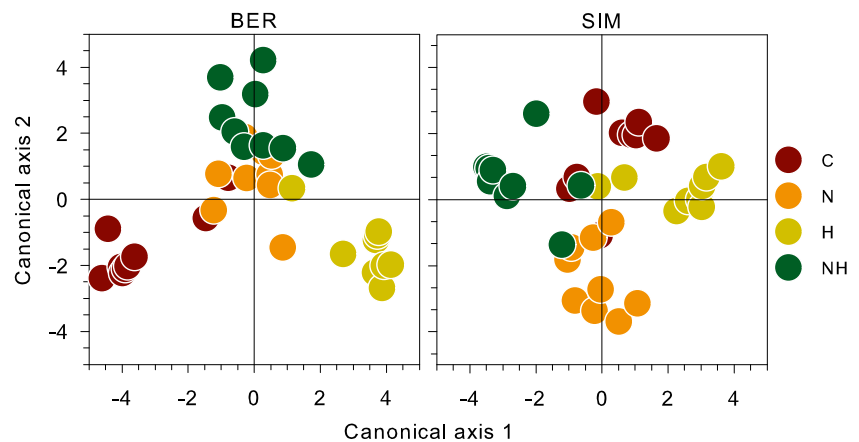
The significant reduction in ECM root tips exhibited in the heated treatment of BER contrasted with the results observed by Clemmensen et al. (2006), which reported modified allocations of resources towards aboveground biomass at the expense of fine root biomass and increased ECM colonisation of fine roots. It was suggested that warming may enhance the growth of ECM fungi by altering the physiology of their host

trees with an increase of autotrophic tissues and an enhancement of photosynthesis, which should allow more C to be allocated to symbionts (Deslippe et al. 2011). However, the heating treatments used by Clemmensen et al. (2006) consisted of small greenhouses constructed above the tundra vegetation for warming both surface soil and air temperature in the immediate vicinity of plants. On the contrary, the treatment applied in this study disentangled the air temperature, which remained unmodified, from that of soil, thus affecting only the belowground processes and the phenology of roots. No effect was either expected on the photosynthetic processes in the canopy of tall trees or observed on stem growth (Lupi et al. 2012a, 2012b). Moreover, the organisms monitored by Clemmensen et al. (2006) consisted of herbaceous plants and little shrubs, which could be assumed to show different metabolic rates and faster responses to environmental changes than larger trees. The presented experiment simulated the effects of a future climate change, although increases in soil temperature that are not complemented with air warming can only partially represent a natural phenomenon. This problem will persist until the technical challenges related to warming adult trees in remote fields will not be definitely resolved.

The higher proportion of vital non-colonised root tips of H trees could be the result of a disproportionate growth between plant and fungi, with the root system being more affected than ECM mycelium by the warmer spring temperatures. Besides, the warmer conditions favourable for root growth could have reduced the mortality of non-mycorrhizal root tips, thus increasing their lifespan and proportion relative to the total amount of root tips. BER is located at higher altitude and latitude, experiencing shorter snow-free periods and growing seasons, and with wetter and colder soils than SIM (Rossi et al. 2011b). Consequently, temperature in BER is a more critical factor for the growth of plants and fungi, which might be more sensitive to warming. This could explain why significant results were detected only in heated trees in BER, although similar trends of root tip vitality and ECM colonisation were also observed in SIM. Water is not a limiting factor in the region, where precipitations are generally frequent and homogeneously distributed during the growing season (Rossi et al. 2011a; Belien et al. 2012). Also, part of the water lost by the warmer temperatures in the soil was compensated by the water supplied by the artificial rain. Thus, the effects of heating on soil moisture for fungal growth could be considered minor or negligible.

In this experiment, N addition was realized by simulating a natural rainfall, with small amounts of N added frequently over the growing season by sprinklers installed above each experimental tree. Just like natural precipitation on N-limited stands, a significant part of the N added was probably intercepted by canopy, stem and epiphytes such as lichens and mosses (Gundale et al. 2011), and only a residual

Fig. 4 Canonical discriminant analysis of the most frequent morphotypes identified in root tips of black spruce in BER and SIM after 3 years of treatment. *C* control, *H* heated, *N* N enriched, *NH* combined treatment



part reached the soil. Indeed, a ^{15}N tracer experiment ($^{15}\text{NH}_4\text{--}^{15}\text{NO}_3$, at a 98 % purity) realised in similar experimental conditions in a balsam fir stand in the boreal forest of Quebec has shown that aboveground vegetation is retaining 64 to 71 % of the recovered tracer as compared to 29–35 % for the soil (Gélinas-Pouliot 2013). The amount of N that reached the soils was probably readily immobilized within the large organic N pool with limited or inexistent effects on soil N concentration and N availability in the short term (David et al. 1998; D'Orangeville et al. 2013). Based on these results, the low amount of added N from our experiment was most probably rapidly immobilized in the canopy and in the forest floor organic N pool. Although this may have potentially affected the symbionts through an impact on the host tree, the impact on the ECM has been observed to be inexistent or undetectable.

Black spruce shows a rich mosaic of ECM associations, allowing a shift in its fungal partners according to the local conditions and to accommodate a range of climatic situations, which also explains the tolerance to edaphic extremes and the wide distribution of this species across the continent (Robertson et al. 2006). In this experiment, neither of the two treatments affected the richness of morphotypes and the proportion of the most frequent ECM, and non-significant differences were observed after 3 years of treatment on the overall occurrence of the most frequent morphotypes. In total, 30 and 33 morphotypes were characterized in the two studied sites, some of them being largely predominant among the ECM, and the majority being very infrequent or occasional. A similar species amount was reported previously in other black spruce stands (Robertson et al. 2006, Rossi et al. 2012), while a wider variability in the number of species was observed by Bruns (1995), who identified between 13 and 35 species from fruit bodies on very young stands or plantations. It is well known that DNA sequencing is the best way for taxa identification, although this technique still shows some levels of inefficiency (Tóth and Barta 2010). The less expensive morphotyping permits a significantly large amount of root tips to be sampled and observed, but can occasionally generate

lumping or splitting of taxa, and data produced by this method should be used with caution (Robertson et al. 2006). This study aimed to compare the effects of different treatments with data collected with the same procedure from control plots. Thus, the eventual systematic error allowed the results to be suitably interpreted in a relative point of view (e.g. by comparing treatments), even if not necessarily according to their absolute value.

5 Conclusions

This study tested the hypothesis that warmer soil temperatures and increased N depositions can modify root vitality and mycorrhization in the short term. No effect of N enrichment was observed on the soil chemistry and on the overall occurrence of morphotypes at both sites. Soil warming significantly modified the proportions of vital root tips and ECM in the colder stand, where temperature was more limiting. The initial hypothesis was thus only partially validated for soil warming. N concentration in the soil organic layer was insensitive to short-term increases in N concentration of rain. Boreal soils contain large amount of N sequestered in recalcitrant form of organic matter, which ECM may have access to, and thus, the relatively low amount of inorganic N added in the short term was unable to substantially modify the growth conditions of roots and ECM. The effects on root vitality and mycorrhization from the experiment lasting 3 years and investigating the short-term responses to warmer soil temperatures and increased N depositions remained inconclusive.

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