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Boron and other elements in sporophores of ectomycorrhizal and saprotrophic fungi

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Abstract Fungi are usually thought not to have a boron (B) requirement. It is not known if mycorrhizas take up B from low concentrations that are common in forest soils, as fungi might also immobilise B. Here, we studied the B concentrations in sporophores of 49 ectomycorrhizal and 10 saprotrophic fungi to assess whether B is translocated in mycelium or not. Additionally, P and metal concentrations were measured for comparison. Variability both within species and between species was very large, as the lowest measured B concentration was 0.01 mg kg^{-1} in *Amanita muscaria*, and the highest was 280 mg kg^{-1} in *Paxillus involutus*. There was no clear difference between saprotrophic and mycorrhizal fungi. The majority of species did not accumulate B at more than 0.01–3 mg kg^{-1}, but there were some species that consistently had median concentration values higher than 5–6 mg kg^{-1} and much higher maximum values, particularly *Paxillus involutus, Lactarius necator* and several *Russula* species. Most species increased their B concentration in B fertilised plots, but there were exceptions, particularly *Rozites caperatus* and *Lactarius camphoratus.* Boron concentrations did not correlate with those of other elements. In conclusion, B is translocated in the mycelia of most of the studied species. The differences between species may be due to differences in their water use, or carbohydrates used in translocation. It remains to be studied, if B concentrations in mycorrhizas or mycelia in soil are in the same order of magnitude as the larger ones found here, and if this has any effects on the host plants.

Keywords Boron · Fungus · Mycorrhiza · Saprotroph · Sporophore

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Introduction

The mycorrhizal symbiosis is essential to the majority of plant species for acquisition of nutrients from soil. In return, mycorrhizal fungi obtain carbohydrates from the host, usually glucose and fructose, which are either transformed to other carbohydrates in the mycelium, or consumed rapidly (Smith and Read 2008; Nehls 2008). However, little is still known about the mycorrhizal function regarding many other nutrients than nitrogen and phosphorus.

Fungi are thought not to require boron (B) for their own metabolism, although B has long been known to be an essential micronutrient for plants (Marschner 1995). The first hypotheses concerning B in fungi indicated that B could be sequestered in unavailable forms by fungal carbohydrates; Lewis (1980) suggested that long-distance translocation of B is possible only in vascular plants, as they make use of sucrose for transport. Sucrose and glucose do not complex with B, but many other simple carbohydrates have high affinity for complex formation with B. Some common fungal carbohydrates, particularly mannitol, form complexes with B readily.

Now it has been shown that the mannitol-B complex is mobile in some plant species (Hu et al. 1997). There are plant species that use polyols, such as mannitol and sorbitol in long-distance carbohydrate translocation, and these species are more efficient in redistributing B within the

plant than other species (Brown and Shelp 1997), even though the relationship between polyols and B mobility in plants is not always direct (Lehto et al. 2004a). Mannitol may have a role in long-distance carbohydrate translocation also in mycorrhizal fungi, although direct evidence for this is still lacking (Nehls 2008). Therefore mannitol could be suggested to increase B mobility in mycelia rather than decrease it. However, B can be sequestered by different compounds which have two hydroxyl groups in *cis*-position, and these are not well explored in fungi. Therefore, boron sequestration in an unavailable form with carbohydrates in the fungal part of mycorrhizas is a possibility.

If mycorrhizal fungi do sequester B, there will be minimal B translocation in the mycelium. In this case, there should be minimal levels of boron in sporophores of both mycorrhizal and other fungi, as the nutrition of sporophores is dependent on translocation in mycelium. Conversely, B concentrations in sporophores similar to those of plants would indicate that translocation occurs to the fungus either from soil solution or from the source of carbohydrates, which is decomposing organic matter in the case of saprotrophs, and the host plant in the case of ectomycorrhizal fungi.

In plants, B is thought to be taken up as undissociated boric acid (Hu and Brown 1997). Boric acid has high membrane permeability, and it is taken up passively with water, unless the external concentration is very low (Dannel et al. 2002; Fitzpatrick and Reid 2009). Facilitation by channel-mediated uptake (Dordas and Brown 2001) and active B transporters have been shown in annual plants (Tanaka and Fujiwara 2008). Nevertheless, the long-distance translocation of B is linked to the transpiration stream, as it has often been found that foliar B levels are increased when water is available (Hu and Brown 1997; Möttönen et al. 2005; Sutinen et al. 2006).

It is often stated that the difference between deficiency and toxicity inducing amounts of B is narrow. However, this holds for external concentrations such as fertiliser applications more than internal B concentrations. When B availability is high, many plants, including boreal forest trees, may increase their foliar B concentrations to a great degree. For example, in Norway spruce fertilisation experiments, B concentrations were less than 1 mg kg^{-1} at the lowest in unfertilised trees, but they rose up to 50–60 mg kg^{-1} after fertilisation, without signs of toxicity (M. Möttönen, M. Räisänen and T. Lehto, unpublished).

We have shown previously that the external mycelium of *Paxillus involutus* mycorrhizas takes up isotopically marked boron which is translocated to the foliage of the host plant (Lehto et al. 2004a). However, in this study, the applied B concentration was very high, 50 mM, and questions about the quantitative importance of mycorrhizal B uptake from realistic external concentrations remained to be studied.

The approach taken in the present study is to measure the B concentrations in sporophores to assess whether B is mobile in fungi in field conditions. The hypothesis tested was: Boron is not taken up and translocated by macro fungi at ecologically relevant external concentrations. Therefore sporophores of both saprotrophic and mycorrhizal fungi should have considerably smaller B concentrations than plants or soil due to the sequestration of B in a non-mobile form with fungal carbohydrates.

The aim of the study was to determine the B accumulation in sporophores of mycorrhizal and saprotrophic fungal species in different kinds of boreal forest stands including B fertilisation experiments. Additionally, concentrations of metals and phosphorus were studied in part of the material for comparison.

Materials and methods

Field sites

Sporophores were collected between July and October in 1999–2003 in several locations in Eastern Finland. In these forests the dominant tree was either *Pinus sylvestris* or *Picea abies*, but each site had some individuals of the other conifer and/or other tree species, particularly *Betula pendula* and *B. pubescens*, *Alnus incana* and *Populus tremula*. The nomenclature of the fungal species follows Hansen and Knudsen (1992).

One of the field sites was an untreated forest site in Kukkola, Joensuu (*Picea abies* stand, fertile Oxalis-Myrtillus type site according to the classification of Cajander 1949) (62°36′N, 29°45′E), and another was a forest park in the city of Joensuu (*Pinus sylvestris* stand, medium-fertile Vaccinium site), where there was also a small stand of *Larix sibirica*.

Other sites were field fertilisation experiments in *Picea abies* dominated stands. The Pyhäselkä experiment (62°25′ N, 29°58′E) is a single-tree fertilisation experiment with the factorial combinations of B as borax (2 kg B ha^{-1}) and N as urea (180 kg N ha^{-1}), grass-herb type. In this experiment each fertilised plot was a circle around a spruce tree, radius 2.5 m, and there were 36 replicates for each treatment combination (control, B, N, B+N). The fertilisers were applied in June 2000 (Räisänen et al. 2009). The first samples were collected from this site five weeks after the fertilisation, but most were collected in August-September of 2002 and 2003, two or three years after fertilisation.

Samples were also collected from long-term fertilisation experiments of the Finnish Forest Research Institute in Heinävesi (62°26′ N, 28°38′ E) and Kannonkoski (62°59′ N, 25°16′ E). The site in Heinävesi was Myrtillus type, and Kannonkoski less fertile Vaccinium/Myrtillus type, both

with mature *Picea abies*. Plots (30 m×30 m plus 5 m border) with factorial combinations of the treatments boron (B), nitrogen (N) and phosphorus (P) were used (Lehto and Mälkönen 1994: Möttönen et al. 2003). The N and P treatment was first applied in 1959 and repeatedly afterwards, totalling 534 kg ha^{-1}N at Heinävesi and 1136 kg ha^{-1}N at Kannonkoski, and 104 kg ha^{-1} P at Heinävesi and 109 kg ha^{-1} P at Kannonkoski (Möttönen et al. 2003). Boron was applied once, 1.5 kg ha^{-1} B as borax in June 1989. Samples were collected in 1999, 2002 and 2003. Hence the time between B fertilisation and sampling was at least ten years.

The sporophores were not in a specific developmental stage, but we avoided collecting very young and clearly aging sporophores; in those cases where we visited the same site regularly (Pyhäselkä 2002–2003) the developmental stages are the most comparable. To test the aging effect on B concentrations, we collected sporophores of *Amanita muscaria*, *Suillus grevillei* and *Paxillus involutus* growing near each other (less than 10 m distance), but of different age and size. In these data, there was no indication at all for an aging effect on B concentrations (data not shown separately).

Nutrient analysis

If there were several sporophores of the same species in a plot at any one sampling occasion, they were combined for nutrient analysis. If there was visible soil on the surfaces, this was removed by wiping with clean tissue. However, the samples were not washed in order not to wash away any intercellular B. The sporophores were initially dried either at room temperature or at 30°C to prevent sudden outburst of the liquid contents, and then in a forced-ventilation oven at 40°C until there was no further weight loss. The results were calculated against subsamples further dried at 105°C. The samples were ground in a ball mill (Planetary Monomill "Pulverisette 6", Fritsch GmbH, Idar-Oberstein, Germany). The B concentration was determined using the azomethine method in1999–2002 (Halonen et al. 1983), and ICP-OES in 2003 (see below). In 2002, additionally calcium concentration was analysed from part of the specimens (HCl digest, atomic absorption spectrometry). In 2003, B, P, K, Ca, Fe, Mg, Mn, Cu, Zn and Al were determined from microwave wet digests with ICP-OES (Iris Intrepid, Thermo Elemental, Franklin, MA, USA) after microwave (MARS 5, CEM Corp., USA) wet digestion in nitric acid (suprapur) plus H_2O_2 in teflon containers (method based on SFS-EN ISO 11885:1998 and EPA 3051). Standard leaf samples were used to control the procedures. If the B concentration measured was below detection limit, this is marked as 0 without decimal point in the tables.

Data analysis

The sporadic occurrence of the sporophores because of weather conditions and also local peoples' collection activity caused unevenness in the data. Samples of altogether 99 fungal species were collected between 1999 and 2003. However, for many species there was only one sample from any one of the three sampling locations: non-fertilised sites in Joensuu, the new Pyhäselkä experiment, and the old Heinävesi and Kannonkoski experiment. These samples were not further analysed. The B data are shown as minimum, maximum and median in order to explore the variability in the data. Minima and maxima can be subject to sampling intensity bias.

For those species that had three or more samples from each B fertiliser level in either of the fertilisation experiments Heinävesi-Kannonkoski (HK) or Pyhäselkä, the assumptions for ANOVA were checked. A logarithm transformation was used to improve homogeneity of variances. There were no zero values (below detection limit) in the ANOVA data. The data from each experiment were first subjected to ANOVA with species, year, and all the fertilisation treatments as factors, and their interactions.

As the data did not show differences between the N and P fertilisation treatments, partly because of low numbers of samples for each treatment combination and year, these were combined in all cases. There were no trends found between the years in the HK experiments, but in Pyhäselkä years 2002 and 2003 showed some differences. However, as the differences were not consistent between different species, and there were too few data to fully assess the trends, the data for these two years were combined for the ANOVA. Eventually, ANOVA was done in both experiments with species and B treatment as factors.

Pearson correlations between B and the other nutrients measured were computed (log transformed data), but the correlations were low, and these results are not shown.

Results

Boron concentrations

The B concentrations of 49 (probably) mycorrhizal and ten (probably) saprotrophic species are reported (Tables 1, 2 and 3). Pyhäselkä experiment was fertilised in the year 2000, and sporophores of only three species were sampled there that year, about five weeks after the treatment (Table 2). In B fertilised plots, *Amanita muscaria* had a much higher B concentration than in any other time or place, 41.7 mg kg^{-1}. Similarly, *Marasmius bulliardi* remained at a low B level in the long-term B-fertilisation experiments (Table 3), and the only very high concen-

Table 1 Boron concentrations (mg kg^{-1} dry mass) in sporophores collected at two forest sites in Joensuu with no fertiliser treatment. Combined data from 2000 and 2002–03. Number of samples, median, minimum, and maximum shown.

Species	n	Median	Min	Max	Likely host or food source
Coprinus comatus	1	0	0	0	Litter
Rozites caperatus	21	0.51	0	1.58	*Pinus*
Hypholoma capnoides	1	0.64	0.64	0.64	*Picea* litter
Lactarius rufus	16	0.79	0.12	4.58	*Pinus*
Cortinarius semisanguineus	10	0.88	0.32	1.63	*Pinus*
Amanita muscaria	22	1.10	0.15	15.00	*Betula/Picea*
Suillus bovinus	30	1.13	0.10	4.99	*Pinus*
Leccinum aurantiacum	4	1.24	0.73	1.93	*Populus*
Suillus luteus	17	1.27	0.47	6.9	*Pinus*
Paxillus atrotomentosus	5	1.28	0.65	7.05	*Pinus* stump
Leccinum versipelle	2	1.29	1.18	1.40	*Betula*
Hydnym sp.	2	1.37	1.34	1.40	Litter
Boletus pinophilus	4	1.41	0.38	3.02	*Pinus*
Suillus variegatus	17	1.45	0.77	3.73	*Pinus*
Boletus edulis	9	1.76	0.41	11.26	*Picea*
Lactarius torminosus	2	1.82	1.66	1.98	*Betula*
Cantharellus cibarius	2	2.12	1.73	2.50	*Picea*
Lactarius mammosus	3	2.54	0.99	4.38	*Pinus*
Leccinum scabrum	7	3.14	1.78	4.6	*Betula*
Leccinum vulpinum	4	3.27	1.60	5.48	*Pinus*
Russula paludosa	7	3.28	0.50	10.12	*Pinus/Betula*
Russula claroflava	5	3.30	2.60	7.48	*Betula*
Armillaria mellea	3	3.33	2.68	3.42	*Betula* stump
Suillus grevillei	13	3.77	1.24	18.43	*Larix*
Lactarius trivialis	2	4.23	1.81	6.65	*Betula/Picea/Pinus*
Russula decolorans	14	6.82	2.25	12.88	*Pinus/Betula*
Paxillus involutus	44	8.84	1.10	280.02	*Betula/Picea/Pinus*
Lactarius necator	19	12.38	1.07	79.65	*Betula/Picea*
Russula emetica	2	13.13	11.76	14.50	*Betula/Picea*
Russula aeruginea	6	15.41	5.17	16.80	*Betula*

trations, up to 133 mg kg^{-1}, were in 2000 in Pyhäselkä (Table 2). As these values differed from the rest of the data for these species, and the short time since fertilisation provided an explanation for this difference, the sampling in 2000 was not included in the ANOVA.

The B concentrations in different species were highly significantly different from each other in ANOVA in each case for those species that had at least three specimens (Tables 1, 2 and 3; for fertilisation treatment, see additionally below). However, the different species formed a continuum rather than clear categories. The data are shown in the tables in the order from the lowest median B concentration to the largest in unfertilised plots.

In sites and plots without B fertiliser, *Paxillus involutus* had the highest maximum values, followed by *Lactarius necator, Lactarius trivialis,* most *Russula* species and also *Amanita porphyria* and *Hygrophorus olivaceoalbus* (Tables 1, 2 and 3).Yet these species could in some cases contain as little as about 1 mg kg^{-1} B, even *Paxillus*, which could have tens or hundreds of times higher concentrations even in unfertilised sites. Concentrations between 0.5–16 mg kg^{-1} were found in *Russula* species in unfertilised sites.

In one extreme, there were species with consistently very low B concentrations, less than 1 or 2 mg kg^{-1}: the saprotrophic species *Coprinus comatus* and the mycorrhizal species *Rozites caperatus, Lactarius rufus* and *L. camphoratus*. Also *Chalciporus piperatus, Tylopilus felleus, Laccaria laccata, Cortinarius traganus* and *C. gentilis* had consistently low concentrations, but there were not many samples of these species.

Several species associated with Scots pine accumulated consistently little B: *Cortinarius semisanguineus, Rozites caperatus, Lactarius rufus, Boletus pinophilus*. The *Suillus* species associated with *Pinus* remained mostly at a relatively low range of B concentrations (less than 1 to 5–7 mg kg^{-1}), unlike *Suillus grevillei* associated with *Larix* (Table 1). *Russula* spp. were very variable, and many of

Table 2 Minimum, maximum and median B concentrations (mg kg^{-1} dry mass) in sporophores collected at Pyhäselkä fertilisation experiment in 2000, and in 2002–03. Nitrogen fertilisation treatments pooled. 0, no B fertilisation, B, boron fertilisation in 2000. ANOVA results for B fertilisation effect are shown when n≥3. *P* values <0.05 in bold, between 0.05 and 0.10 in italics. m: mycorrhizal, s: saprotrophic

Year	Species	0				B				*P*	m/s
		n	Median	Min	Max	n	Median	Min	Max		
2000	*Amanita muscaria*					1	41.7	41.7	41.7		m
2000	*Marasmius bulliardii*	7	2.84	1.73	6.74	10	11.02	1.69	133.29		s
2000	*Paxillus involutus*	1	3.77	3.77	3.77	2	129.25	128.1	130.4		m
2003	*Boletus edulis*	3	0.27	0.19	0.53						m
2002	*Lactarius rufus*	2	0.48	0.44	0.53						m
2003	*Leccinum scabrum*	1	0.51	0.51	0.51	2	1.76	1.01	2.51		m
2003	*Cortinarius armillatus*	1	0.52	0.52	0.52	1	13.9	13.9	13.9		m
2003	*Inocybe sp.*	2	0.54	0.44	0.63	2	0.78	0.67	0.89		m
2002–03	*Lactarius deterrimus*	7	0.60	0.31	0.82	5	5.07	2.82	39.99	**<0.001**	m
2002–03	*Amanita muscaria*	8	0.80	0.33	1.39	10	1.43	0.01	9.14	0.292	m
2002–03	*Lactarius theiogalus*	17	0.08	1.87	0.66	25	0.51	8.24	1.23	**0.001**	m
2003	*Lactarius torminosus*	7	1.11	0.39	1.49	5	2.85	1.99	10.49	**0.003**	m
2003	*Collybia butyracea*	1	1.66	1.66	1.66	1	3.85	3.85	3.85		s
2002–03	*Russula aeruginea*	11	1.70	0.91	3.31	4	4.00	1.13	6.85	*0.065*	m
2003	*Tricholoma album*	2	1.82	1.32	2.32						m
2003	*Lactarius trivialis*	12	1.82	0.94	9.62	7	5.49	1.36	23.88	**0.045**	m
2003	*Stropharia hornemanni*	5	2.22	1.67	3.54	3	5.27	1.54	84.36	0.166	s
2002–03	*Lactarius necator*	14	5.19	1.2	13.13	14	48.80	18.56	92.98	**<0.001**	m
2002–03	*Paxillus involutus*	28	7.05	2.54	20.6	33	30.17	3.82	179.19	**<0.001**	m
2003	*Chalciporus piperatus*					2	1.41	1.28	1.53		m
2002	*Megacollybia platyphylla*					2	1.71	1.49	1.92		s
2002	*Boletus subtomentosus*					4	2.69	0.71	12.46		m
2002–03	*Laccaria laccata*					1	2.83	3	1.35		m
2003	*Lactarius helvus*					2	11.35	9.25	13.45		m

them are probably compatible with more than one tree species.

Effect of B fertilisation on B concentrations

In Pyhäselkä, there were nine species in the ANOVA, with at least 3 specimens from both B fertiliser levels (Table 2). In HK, there were 16 species in the ANOVA (Table 3). Species, B fertilisation and their interaction were all highly significant ($P<0.001$) in Pyhäselkä. In Heinävesi-Kannonkoski, species and B main effects were highly significant ($P<0.001$), but interaction was not significant ($P=0.227$). Each species was then subjected to one-way ANOVA to test the B effect in Pyhäselkä. In this experiment, the other species accumulated significantly more B in the fertilised plots, and only *Amanita muscaria* and *Stropharia hornemanni* were not significantly affected by B fertilisation, due to very large differences between individual samples (Table 2).

Among the species that had high B concentrations also in non-fertilised plots, most had significantly higher B concentrations in the B fertilised plots in Pyhäselkä (Table 2). Although *Rozites* and *L. camphoratus* increased in B concentration in the B fertilised plots in HK, the extent of the increase was only about 0.5 mg kg^{-1} for both species (Table 3). However, some species had low B concentrations (less than 2–3 mg kg^{-1}) in non-fertilised sites, but the concentration could increase considerably in fertilised plots (Tables 2 and 3). *Boletus subtomentosus* (n.s.), *Lactarius deterrimus, L. torminosus, Cantharellus cibarius* and *Hygrophorus olivaceoalbus* represented this pattern. *Cortinarius triumphans* and *Tricholoma album* also showed this pattern but there were few samples. Even *Lactarius theiogalus*, which was otherwise extremely low also in the B fertilised plots in the old HK experiment, had up to 8 mg kg^{-1} in Pyhäselkä. *Stropharia hornemanni* is the only saprotrophic species which had higher concentrations in B fertilised sites in Pyhäselkä (not significant), apart from the very high values in *Marasmius bulliardii* the first year.

Some of the pine-associated species with low B levels, particularly *Suillus* species and *Cortinarius semisanguineus*,

Table 3 Boron concentrations (mg kg^{-1} dry mass) in sporophores collected at Heinävesi and Kannonkoski experiments. Nitrogen and phosphorus fertilisation treatments pooled. 0, no B fertilisation, B, boron fertilisation in 1989. Samplings in 1999–2000 and 2002–03 combined. ANOVA done when when n≥3. *P* for species main effect <0.001, B main effect <0.001, interaction not significant. m: mycorrhizal, s: saprotrophic

Species	0				B				m/s
	n	Median	Min	Max	n	Median	Min	Max	
Chalciporus piperatus	1	0.12	0.12	0.12	1	1.43	1.43	1.43	m
Boletus edulis	1	0.30	0.30	0.30	2	1.86	0.96	2.72	m
Tylopilus felleus	2	0.41	0	0.82	2	2.19	0.49	3.90	m
Rozites caperatus	5	0.44	0.15	0.64	4	0.79	0.51	1.22	m
Tricholoma album	2	0.63	0.35	0.91	1	9.47	9.47	9.47	m
Amanita muscaria	5	0.65	0.13	4.47	1	1.55	1.55	1.55	m
Lactarius camphoratus	8	0.71	0.53	1.10	5	1.01	0.71	1.34	m
Lactarius theiogalus	2	0.83	0.59	1.07	2	0.98	0.46	1.50	m
Cantharellus cibarius	3	0.89	0.58	1.38	5	9.68	2.58	10.04	m
Laccaria laccata	2	1.01	0.95	1.08	1	0.89	0.89	0.89	m
Amanita vaginata	1	1.08	1.08	1.08	1	4.41	4.41	4.41	m
Amanita virosa	1	1.26	1.26	1.26	1	3.07	3.07	3.07	m
Cortinarius gentilis	1	1.27	1.27	1.27	1	1.05	1.05	1.05	m
Boletus subtomentosus	7	1.31	0.67	1.85	3	1.44	0.95	10.73	m
Hypholoma capnoides	10	1.38	0.44	4.67	3	1.90	1.52	4.63	s
Marasmius bulliardii	4	1.57	1.35	1.78	5	1.53	1.18	2.24	s
Amanita porphyria	11	1.61	1.09	6.68	11	3.19	1.73	16.41	m
Clitocybe clavipes	10	1.74	0.78	13.58	4	1.45	1.24	2.32	s
Cortinarius traganus	2	1.88	1.88	1.88	2	1.73	1.52	1.94	m
Stropharia hornemanni	9	1.93	0.63	14.00	3	4.07	1.19	9.67	s
Lactarius rufus	1	2.57	2.57	2.57	4	1.81	1.23	2.07	m
Russula vinosa	4	2.67	3.40	5.86	5	12.61	4.28	15.87	m
Russula nitida	5	3.20	2.92	12.11	7	15.05	8.77	96.63	m
Russula xerampelina	2	3.22	2.36	3.07	3	4.47	2.70	7.19	m
Russula aeruginea	2	3.26	1.31	5.28	2	30.03	15.96	44.10	m
Russula emetica	3	3.60	2.60	4.31	6	9.27	2.45	84.01	m
Cortinarius triumphans	2	3.70	3.10	4.30	1	16.00	16.00	16.00	m
Russula decolorans	5	5.37	3.73	16.51	5	8.85	1.58	22.46	m
Lactarius trivialis	8	5.81	1.86	43.89	3	12.31	3.44	49.10	m
Hygrophorus olivaceoalbus	4	6.67	4.30	6.93	3	8.81	6.35	24.14	m
Lactarius necator	4	6.75	1.33	14.46	2	71.28	58.18	84.38	m
Russula fragilis	2	7.50	4.57	10.33	4	9.28	3.17	12.04	m
Russula vesca	1	8.57	8.57	8.57	3	6.08	4.01	9.12	m
Paxillus involutus	21	15.27	3.78	64.54	20	35.74	6.83	110.0	m

were not found in B fertilised plots in this study, and the effects of a high external supply remain unknown. The only cases where true comparison is possible are the pine-associated fungi that were found in some of the spruce-dominated stands, *Lactarius rufus* and *Rozites caperatus* (Table 3). Both species had low B concentrations also in B fertilized plots.

The B accumulation in sporophores in our data can be compared to the needle B concentrations in the fertilisation experiments, which varied between 0.7–8.1 and 11.2–52.0 mg kg^{-1} in the trees without and with B fertiliser in Pyhäselkä in November 2002 (Räisänen et al. unpublished; treatment means shown by Räisänen et al. 2009). In the old fertilisation experiments, the B concentrations in C needles in March 1999 varied between 1.7–26.4 mg kg^{-1} (no B fertiliser) and 11.2–63.8 mg kg^{-1} in individual trees in B fertilised plots (Möttönen et al. unpublished; plot means shown by Möttönen et al. 2003). In the same old experiments, *Paxillus involutus* and *L. trivialis* could have concentrations 44 and 64 mg kg^{-1}, and several other species over 10 mg kg^{-1} in the non-B fertilised plots. The total-B concentrations in the top 1 cm of the organic layer in the plots without any fertiliser and with only B fertiliser were 3.7 and 5.6 mg kg^{-1} two years after the fertilisation (Lehto and Mälkönen 1994; Lehto 1995).

Other elements

The other elements were mostly not as variable between and within species as B, however, Al and Fe concentrations spanned over two orders of magnitude. The boxplots on element concentrations show several outliers with particularly high values, in the cases of Al, Fe (Fig. 1) and Ca, Mn and Zn (Fig. 2). However, considering the large variability in the fungi, these outliers may well be realistic. Among the species, the element concentrations of *Paxillus involutus* in general were in the higher end, and also more variable than other species in this data set.

Discussion

The lowest measured B concentration in these data was 0.01 mg kg^{-1} in *Amanita muscaria* in a B fertilised plot in Pyhäselkä, and a few values remained below detection limit. The largest concentration was measured in *Paxillus involutus,* 280 mg kg^{-1}, being four orders of magnitude larger than the smallest. It is not usual for element concentrations to vary so much in live organisms, but B is different from most other elements in this respect (Tyler 2005). It is still to be explored whether this variability is of any consequence to fungi.

Both saprotrophic and ectomycorrhizal species could have either very low or very high B concentrations. This indicates that B was translocated to some extent in almost all species, and the direction of B translocation is not directly dependent on the nutrient source of the fungus. As both mycorrhizal and saprotrophic groups included species that were very different from each other, the differences between species in terms of B accumulation remain the main result of this study.

Some fungi were capable of concentrating B in great excess of the concentrations found around the vegetative mycelium in the organic layer, while others excluded B.

Macro nutrients and metal concentrations have been measured in sporophores in some studies (Tyler 1980, 2005; Ohtonen 1982; Chudzynski and Falandysz 2008), but B has not often been reported. Tyler (2005) measured also B in sporophores in a beech forest, but the data were inadvertently left out on publication: *Clitocybe odora, Amanita citrina, Collybia butyracea* and *C. peronata* had B concentrations 1–6 mg kg^{-1}, and *Lactarius blennius* 123–125 mg kg^{-1} (Germund Tyler, pers. comm.). Additionally, *Rozites caperatus* in the same site had concen-

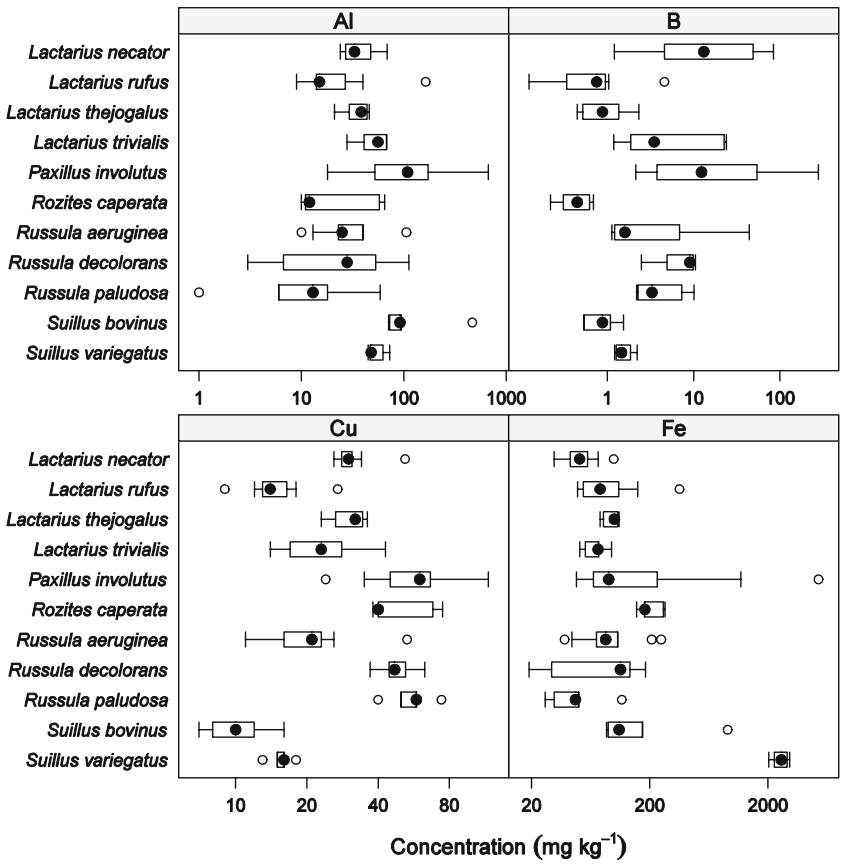

Fig. 1 Boxplots on element concentrations in a subset of sporophore samples collected in 2003 in unfertilised sites Notice the different, logarithmic x axes. *N*=6–11

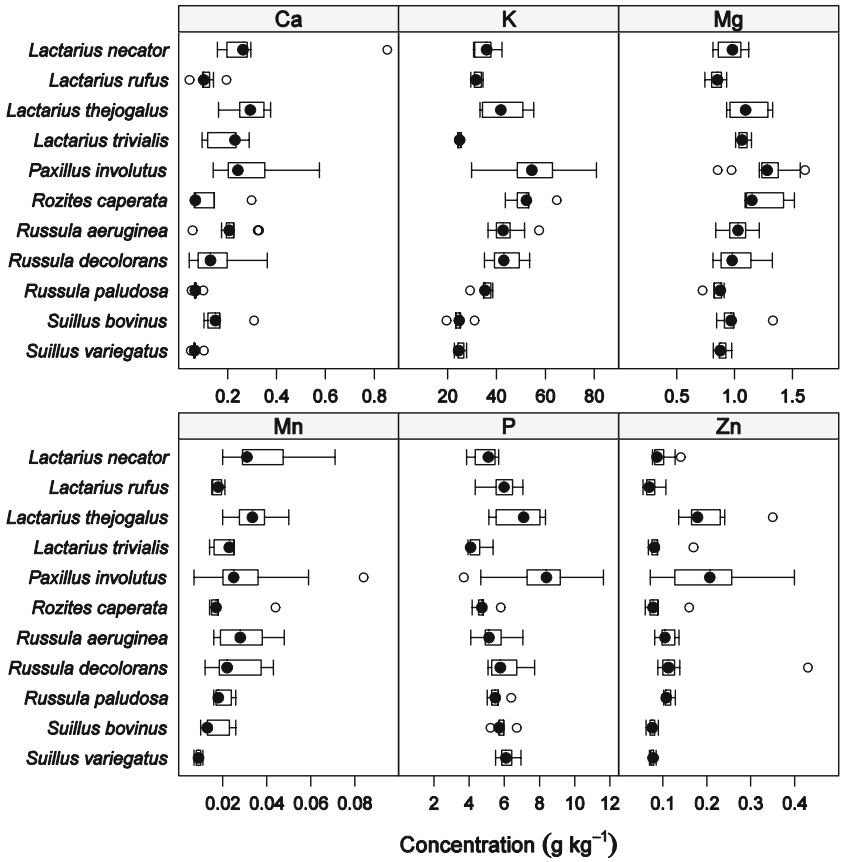

Fig. 2 Boxplots on element concentrations in a subset of sporophore samples collected in 2003 in unfertilised sites. Notice the different x axes. *N*=6–11

trations 0.1–0.3 mg kg^{-1} (G. Tyler, unpublished data). This agrees with our findings of very low B concentrations in *Rozites*. Also the values for *Amanita, Clitocybe* and *Collybia* are in the same range as our data on the same or related species, and *Lactarius blennius* resembles the high values found in *L. necator* and *L. trivialis*. The similarity of the results of Tyler and our results from different sites and different external B availability confirms that there are species that restrict B uptake and/or transport to the sporophore, and there are species that accumulate B in a different order of magnitude compared to tree foliage.

Copper has been found to accumulate in ectomycorrhizas (Berthelsen et al. 1995) and in sporophores (Tyler 2005). Berthelsen et al. (1995) found that almost all Cu in a Norwegian forest soil could be in mycorrhizas. Here the median Cu levels in sporophores varied from 10 to 60 mg kg^{-1}, while the needle concentrations at Heinävesi and Kannonkoski experiments were about 3 mg kg^{-1} (Möttönen et al. 2003). The same applies to Zn which was between 80 and 200 mg kg^{-1} in sporophores, and about 32 mg kg^{-1} in needles. Chudzynski and Falandysz (2008) found relative accumulation of Cu, Mn, Zn and other metals in *Suillus grevillei* sporophores; by contrast, in our data Mn levels were relatively low. Ohtonen (1982) found relatively little accumulation of Cu, Zn and Mn in *Lactarius rufus* and *Suillus variegatus*, which agrees with our results on lower concentrations of these metals compared to other species. Aluminium and Fe varied very much between the species, and the strong Fe accumulation in *S. variegatus* agrees with earlier results (Ohtonen 1982). Other elements did not show as large between-species variability. *Paxillus involutus* accumulated also most other elements more than other species, and showed particularly large variability in the concentrations of B as well as other elements. *Paxillus involutus* is not a genetically uniform species (Hedh et al. 2009), which may be one explanation for its large variability in element accumulation.

The Heinävesi and Kannonkoski experiments were in mature spruce stands, and over ten years had passed after the B fertilisation. Pyhäselkä experiment was in a younger spruce stand, and the first samples were taken directly after fertilisation. The immediate fertiliser effect appears to be stronger than in subsequent years, as the few samples collected in the fertilisation year, 2000, all had high B concentrations compared to the same species at another time or place. Year 2002 was exceptionally dry, and the number of sporophores was much smaller than in the other years (data not shown separately for the years). The

difference in the rainfall between the two years obscured any effect of the time passed after the fertilisation in the new Pyhäselkä experiment. In a comparison between the old experiments fertilised more than ten years ago and the new experiment, there are no obvious differences. Hence, more than ten years after the B fertilisation, there were still significantly higher B concentrations in the sporophores in B fertilised plots.

There was some consistency in the B accumulation patterns within genera, but also many exceptions. Particularly *Russula*, the genus with the largest number of species found here, tended to accumulate B. However, *Lactarius*, a genus close to *Russula*, included species with opposite tendencies: some of the least B accumulating and some of the most B accumulating species were in this genus. The host species may also have a role in determining the B accumulation in the fungus.

In these data, the species that were (most probably) associated with Scots pine accumulated consistently little B. Two of these, *Lactarius rufus* and *Rozites caperatus*, were found also in B fertilized plots, where they did not increase their B concentrations by contrast to most other fungal species. However, Scots pine is able to grow on drier sites than the other common tree species, and here the host species cannot be separated from the site effect. The fungal species associated with Scots pine are likely to be adapted to lower rates of evaporation from the sporophore surface than species on mesic sites. Lower amounts of water evaporated might lead to less B accumulation in their sporophores, if B is mobile with the transported water. In plants, xylem B transport is driven by transpiration, and more B accumulates in foliage when soil water is available (Hu and Brown 1997; Möttönen et al. 2005; Sutinen et al. 2006). Calcium concentrations in plants tend to be related to transpiration, because Ca is poorly remobilised in the plant (Michael and Marschner 1962). Here, the Ca levels in the pine-associated species *Lactarius rufus, Rozites caperatus* and *Suillus variegatus* were somewhat lower than those of other species, which is consistent with the suggestion of less water flow. However, the data on Ca does not allow strong conclusions.

The fungi with the high B concentrations in low-B soil may have access to soil B pools in the mineral layer that are not available to plants, possibly by an efficient uptake mechanism. The uptake of B by fungi from low concentrations has not been shown so far. Hence the mechanisms of differential B transport in different species still remain to be studied. It also remains open, if the species that accumulate B have a physiological or structural role for B, different from species that almost exclude B from the sporophores. There is considerable evidence that B is an essential element for animals (e.g. Goldbach and Wimmer 2007), and it is possible fungi may have a very low B requirement. However, so far the only B transporters identified in fungi (*Saccharomyces cerevisiae*) are B exporters that are assumed to protect yeast from excess B (Kaya et al. 2009).

The very high B concentrations in the sporophores of some species suggest more substantial transport than simply with the mass flow of water. A possible mechanism for concentrating B from soil is through (passive) uptake of boric acid, followed by rapid complex formation with compounds such as mannitol. This would allow continuous inflow of boric acid and thereby accumulation against a concentration gradient of boron (Hu and Brown 1997). This mechanism has been suggested for plant B uptake, and it could explain the high B concentrations also in fungi.

In plants, there are major differences between species in their ability to transport B in the phloem. Plant species with polyols such as mannitol and sorbitol transport B in the phloem in larger quantities than many other species (Brown and Shelp 1997; Lehto et al. 2004b, c). Trehalose and polyols –mannitol, arabitol and erythritol– are the most commonly found small-molecule carbohydrates in mycorrhizal fungi. Trehalose does not have the *cis*-diol structure that allows strong binding of B. It could be suggested that, in analogue to plants, fungal species with mannitol (and possibly arabitol) as a major carbohydrate would readily translocate B from one part of the system to another. This agrees with the findings that the most B-accumulating species in our study, *Paxillus involutus,* did have somewhat higher mannitol than trehalose concentrations, and one of the less B-accumulating species, *Amanita muscaria* had much higher trehalose than mannitol concentrations as reviewed by Nehls (2008). However, *Suillus bovinus* and *Tylopilus felleus* also had more mannitol than trehalose (Nehls 2008), although both species had consistently low B concentrations here. Trehalose has been found to occur in larger concentration than mannitol in many species of mycorrhizal Basidiomycetes, whilst some Ascomycetes may contain more mannitol; however, of the 28 species summarised by Nehls (2008), mannitol was detected in all but one. In conclusion, the carbohydrates do not provide a straightforward explanation to the great differences between species. Furthermore, the concentrations of these carbohydrates vary with environmental conditions, such as water availability (Shi et al. 2002) and temperature (Tibbett et al. 2002).

It is also conceivable that B may be translocated from the host plant as complexes with the simple carbohydrates that move from the host plant to mycorrhizal fungi. Mannitol and fructose complexes (Hu et al. 1997) are candidates for B translocation from plant to mycorrhizal fungus. Some species, including *Amanita muscaria*, are able to take up glucose and fructose simultaneously, while *Laccaria bicolor* takes up glucose preferentially (Martin and Nehls 2009). As *Amanita muscaria* was not one of the

B-accumulating fungi, fructose uptake from the plant may not be an explanation to the differences between species. Movement of mannitol from host to mycorrhizal fungus has not been shown, but Scots pine and Norway spruce do contain small amounts of mannitol (Lehto et al. 2004b). Such 'reverse' translocation of B could be harmful for plants in regions with low B. It is of interest to study whether this phenomenon occurs, as also water flow from host plant roots towards mycorrhizas occurs in some circumstances (Lilleskov et al. 2009).

The B accumulation in sporophores does not affect the B cycle much, as sporophores are an ephemeral part of the system. However, mycorrhizas and their external mycelium are much more persistent in soil. As an example, assuming that the biomass of mycelium in mycorrhizas plus external mycelium can vary between 180–900 kg ha^{-1} (Högberg and Högberg 2002; Wallander et al. 2001) and assuming a mean B concentration of the mycelia between 1–12 mg kg^{-1}; then the amount of B in the mycelia would vary between about 0.18 g ha^{-1}–10.8 g ha^{-1}. The annual uptake and transport to aboveground parts of mature Norway spruce has been estimated to be 5–10 g ha^{-1} (Aphalo et al. 2002). Hence the upper estimate of the B pool in mycelium is larger than the annual uptake by the trees. It is of consequence to the B economy of the site whether B is accumulated in mycelium, and if it is, whether this pool is mobile and whether it is potentially transported to the host plants.

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