Variation in the chemical quality of woody supplements for nursery growing media affects growth of tree seedlings

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Abstract

Tree seedlings are produced in tree nurseries. However, nursery-grown seedlings often exhibit poor performance after outplanting due to the lack of adaptation to harsh natural conditions. These nursery-grown seedlings do not necessarily possess well-developed ectomycorrhizal symbionts, which help to obtain nutrients and increase resilience in exchange for seedling photoassimilated carbon. To improve the quality of the seedlings in natural conditions, we sowed spruce seeds on growing media with the addition of wood chips, i.e. stemwood chips or polyphenol- and resin acid-rich knotwood chips. Wood chips were chosen because they are common forest side-streams, and their compounds have shown a potential to improve mycorrhization and seedling fitness. Wood chips initially decreased the growth of seedlings. However, this effect levelled off with time and depended on the quality of the wood. Wood chips had no effect on mycorrhization. Further testing of the wood material showed that wood chips seemed to decrease seedling growth via nitrogen (N) immobilisation rather than a direct toxic effect. The phenomenon of N immobilisation on wood chips could be explored further to develop a slow-release N source, aptly reflecting N availability in natural conditions. Slow-release N source based on wood chips could be beneficial both to increase survival in natural conditions and for environment protection.

Keywords Forest nursery · Growing media · Nitrogen uptake · Tree seedlings · Wood chemistry

Introduction

Norway spruce (*Picea abies* (L.) Karst) is one of the most economically and ecologically important tree species in Central and Northern Europe (Johnson and More 2004). Spruce wood is commonly used for timber constructions and pulpwood for paper and furniture. Due to high demand for its wood and relatively high productivity, as well as tolerance to various soil conditions and herbivores, spruce is widely used in forest stand regeneration (Savill 2013). Compared to bare-rooted seedlings the planting of

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containerised Norway spruce seedlings has been shown to be a more reliable regeneration method (Grossnickle 2000; Miina and Saksa 2013). Forest tree seedling production in Finland, as in the whole of Fennoscandia, therefore relies on a containerised production system (OSF: Natural Resources Institute Finland <https://statdb.luke.fi/>) with the use of peat products as growing media.

The prevalent nursery growing methods include fertilisation with soluble mineral nutrients, which reduces the relative size of seedling roots and decreases ectomycorrhizal fungi (EMF) colonisation and diversity (McAlister and Timmer 1998; Flykt et al. 2008). Ectomycorrhizal fungi are crucial for tree water and nutrient uptake. EMF deficiency in nursery growing seedlings therefore impedes tree fitness and increases vulnerability to pathogens after outplanting (Quoreshi and Timmer 2000; Klavina et al. 2016). In addition, Norway spruce seedlings after outplanting suffer from herbivorous damage (Huitu et al. 2009). The reason is that herbivores (voles, ungulates, insects) prefer planted nursery seedlings due to their high nitrogen (N) content and low content of defensive compounds, i.e. phenolics and alkaloids (Burney and Jacobs 2013; Virjamo et al. 2013; Karst et al. 2015; Muiruri et al. 2019). As environmental reasons impede the use of peat (Boldrin et al. 2010), there is an urgent need to search for peatless growing media that provide better chances of survival after outplanting.

Wood fractions and other forest side-streams have already shown some potential to enhance the growth rate of Norway spruce seedlings via an improvement in mycorrhization and root/shoot ratio (Vaario et al. 2009). In forest stands, the occurrence of natural seedlings is positively correlated with decaying wood substrate (Alaspää et al. 2016). Decaying wood logs are known to host diverse fungal communities including mycorrhizal species, especially in later decay phases (Rajala et al. 2010, 2012, 2015; Rinne-Garmston et al. 2019). Since wood N concentration increases with decay phase due to symbiotic nitrogen fixation and transfer of N from the soil (Rinne et al. 2017), advanced decay phase wood is probably a better N source for seedlings than fresh wood substrate. Moreover, wood compounds may increase seedling resilience via the activation of plant's defence metabolism (Mandal et al. 2010). For example, lignans (a group of polyphenolics) exert strong antioxidant activity (Harmatha and Dinan 2003; Korkina 2007; Balík et al. 2017). Lignans are thus potentially very effective inducers of plant defence. Lignans reside almost exclusively in knots, not in the stemwood (Willför et al. 2004, 2005; Piispanen et al. 2008; Wijayanto et al. 2015). Knotwood is a typical forestry side-stream that is abundantly accessible from forest cutting residuals. In addition, knotwood contains a high amount of terpenes, which also act as defensive compounds (Maninen et al. 2002; Adamczyk et al. 2011, 2013).

However, these wood compounds do not leave other non-defence-related metabolic and soil processes untouched. For example, polyphenolics and terpenes may affect enzymatic activities in soil and bind organic N compounds (Joanisse et al. 2007; Hagerman 2012; Adamczyk et al. 2015, 2017), potentially affecting seedling growth and fitness. Altogether, the improvement of nursery-grown seedling performance and opportunities for competitive success seem to rely on a build-up of a root system that can provide sufficient nutrients and water, and the development of an effective defence against pathogens and herbivores, both of which involve beneficial symbioses with mycorrhizal fungi (Read 1996; Grossnickle 2000; Karst et al. 2015).

Here we examined the effects of two chemically varying wood materials, standard stemwood chips and knotwood chips, on the early performance of nursery tree seedlings. We aimed to obtain a holistic understanding of the effects of woody substrate addition to

nursery growing media on plant growth, N uptake, and symbiotic associations in roots. To achieve this goal we conducted the following series of experiments:

- (a) First aim was to observe the effect of wood chemistry on Norway spruce seedling growth and the potential to form mycorrhizal symbiosis. We therefore established nursery experiments in which the seedlings grew on substrate with the addition of standard stemwood chips (SW), knotwood chips (KW), or stemwood in various stages of decomposition.
- (b) Second, we aimed to decipher the mechanisms behind seedling growth reduction after the addition of knotwood observed in the first experiment. We hypothesised that the reduction in growth was due to the immobilisation of N on the wood chips. We thereby established a second nursery experiment in which we grew the seedlings with wood chips soaked in a nutrient solution, as well as with wood chips with immobilised ^{15}N . Here we traced the effect of wood chips on N availability for spruce seedlings. We also performed a laboratory experiment that aimed to estimate N immobilisation potential on wood chips.
- (c) Third, we assessed spruce seed germination rates and the effect of lignan extract purified from knotwood on the seeds to rule out the toxicity of lignan-rich knotwood chips.

Materials and methods

Nursery experiment 1 Effect of knot- and stemwood chips on seedling performance.

In the first nursery experiment, spruce seeds (seed orchard R01-89-1001 Sv 110) were sown in Plantek-81F containers (volume 85 cm³, BCC Ab) supplemented with mixtures of wood chips, peat, and vermiculite (2–4 mm, SP-Minerals, Finland). The conventional growing media (control) was a mixture of Sphagnum peat (90% in volume, pH 4.3, NPK 16–4–17 kg/m³, Kekkilä-BVB Oy), and 10% (v/v) of vermiculite. The treatments were obtained by replacing part of the conventional growing media with woody material as follows: 10% (volume) and 30% of knotwood chips (KW) or stemwood chips (SW). Wood chips were obtained from freshly cut wood logs of Norway spruce harvested from southern Finland (Ruotsinkylä, 60.369090, 24.972714). The chips were stored in a +4 °C cold room before use. Spruce knotwood contains five times more extractives than SW. Moreover, KW contains far more lignans and resin acids than SW (Nisula 2018).

Nursery experiment 1 was arranged in a randomized design. Altogether, 486 seeds (two PL81 containers per treatment, which makes 2×81 seedlings per treatment) were sown in May 2016. Seedlings were grown outdoors at the Luke Haapastensyrjä experimental nursery in southern Finland (60° 36' 59.6" N 24° 25' 52.3" E) for three growing seasons and fertilised with Superex fertilisers (Kekkilä-BVB Oy, NPK 12-5-27) to keep the conductivity around 1 mS m⁻¹.

Nursery experiment 2 Effect of nutrient-soaked knotwood and stemwood on seedling performance and EMF colonisation.

In the second nursery experiment established in 2017, spruce seeds (seed orchard R01-89-1001 Sv 110) were sown in Plantek-81F containers (volume 85 cm³, BCC Ab) supplemented with mixtures of wood chips, *Sphagnum* peat, and vermiculite, as in nursery experiment 1. The conventional growing media (control) was similar to that in nursery experiment 1. The treatments were obtained by replacing part of the conventional growing media with woody material, as presented in Table 1. Lignan 1

Table 1 Treatments in the Nursery experiment 2

Treatment	Abbreviation	Wood material	Nutrient addition	Fungal inocula
5% knotwood + N	5% KWN	5% knotwood	Soaked	–
5% stemwood + N	5% SWN	5% stemwood	Soaked	–
10% knotwood + N	10% KWN	10% knotwood	Soaked	–
10% stemwood + N	10% SWN	10% stemwood	Soaked	–
10% knotwood	10% KW	10% knotwood	–	–
10% stemwood	10% SW	10% stemwood	–	–
10% knotwood + N + EMF	10% KWN + EMF	10% knotwood	Soaked	Mycelia inoculum
10% stemwood + N + EMF	10% SWN + EMF	10% stemwood	Soaked	Mycelia inoculum
Lignan extract 1	LIGNAN1	300 mL lignan 1	–	–
Lignan extract 2	LIGNAN2	100 mL lignan 2	–	–
5% decaywood 1	5% DECAF1	5% decay1 wood	–	Decay wood
10% decaywood 1	10% DECAF1	10% decay1 wood	–	Decay wood
5% decaywood 4	5% DECAF4	5% decay4 wood	–	Decay wood
10% decaywood 4	10% DECAF4	10% decay 4 wood	–	Decay wood
10% decaywood	10% DECAF	10% decay 3–5 wood	–	Dry decay wood
Conventional peat	CONVENTIONAL	–	–	–

treatment was obtained by mixing 300 mL of lignan extract 1 (130 mg/L) and 100 mL of Lignan 2 (360 mg/L) to 4 L of growing media. Lignans were extracted and purified from fresh spruce inner branches (dried and ground to ca. 2 mm chips with Fritsch Pulverisette mill, Fritsch GmbH, model 15,903, Idar-Oberstein, Germany) with water in a 3-L flowthrough extractor (Konepaja Viitosmetalli, Paaso) at 100–120 °C. Extraction 100–120 °C yielded a pure sample, which did not contain measurable amounts of resin acids (confirmed with gas chromatography-mass spectrometry) and was soluble in water. During flowthrough extraction, the majority of lignans is eluted in small volumes and thus at high concentration. The other lignans were recovered by concentrating the clear extraction solution.

Decay wood substrate that represented decay phases 1 and 4 was obtained from an unmanaged Norway spruce stand in southern Finland (Lapinjärvi 60° 39' 09 N 26° 07' 04 E), and was sampled for an earlier study (Rinne-Garmston et al. 2019). Decay phase 1 represented trees felled only a few years ago with wood still hard and bark intact, whereas in decay phase 4, the wood was very decayed and soft with no hard core (Mäkipää et al. 2018). The decay phases were classified as in Mäkinen et al. (2006). In addition, decay wood substrate that was dried at 100 °C and had therefore lost the majority of fungal activity was used for comparison. For nursery experiment 2, 36 seeds per treatment were sown in containers in May, and the seedlings were grown outdoors at the Luke Haapastensyrjä experimental nursery for three growing seasons. Some of the seedlings were inoculated with ectomycorrhizal fungi: R-SP02 *Piloderma olivaceum*, R-SP01-HK3 *Tylospora asterophora*, and R-FC03 *Cenococcum geophilum*. The ECM fungal strains are deposited in the microbial culture collection of Natural Resources Institute Finland, Helsinki. Fungi were isolated from the ECM root tips of young Norway spruce seedlings growing in natural habitats (Vuorinen et al. 2015). Inoculum was obtained by growing mycelia plugs in ½ MMN nutrient media (100 rpm shaking) for two months. Five mL of inocula per seedling was

pipetted to the roots of seedlings in June. All the seedlings were fertilised with Superex fertilisers (Kekkilä-BVB Oy, Finland NPK 12-5-27) to keep the conductivity around 1 mS m⁻¹.

Nursery experiment 2 was arranged in a randomized design. Altogether, we used 36 seedlings per treatment. Seedlings were grown outdoors at the Luke Haapastensyrjä experimental nursery in southern Finland (60° 36' 59.6" N 24° 25' 52.3" E).

Laboratory experiment Immobilisation of N on wood chips.

We compared the ability of stemwood vs knotwood to immobilise ammonium and nitrate ions. Wood chips (10, 25, 50 mg) were weighed into Eppendorf tubes. Then, 250 µL of water was added, and after 10 min, 1 mL of 5 mM NH₄Cl or KNO₃ (9.5 mM, equal amount of N to 5 mM NH₄Cl) was added and mixed for 30 min on a planar shaker (200 rpm). No wood chips were used as a first blank. As a second blank, 1 mL of water instead of NH₄Cl or KNO₃ was used. After centrifugation (5 min, 12,000 g), the residual amount of NH₄Cl or KNO₃ was measured from supernatant using spectrophotometric methods (Adamczyk et al. 2019).

In the following laboratory experiment, we estimated the stability of N-bound to knotwood and stemwood chips. Wood chips (50 mg) with bound ammonium-N were prepared as above. The precipitates were washed with 1 mL of water, mixed for 5 min, and after centrifugation (5 min, 5000 g), supernatants were used to study the residual amount of ammonium as above. Later, the washing was repeated. We added 1 mL of 1 M KCl (a common extractant used in soil studies) to the wood chips, mixed it for 30 min in a planar shaker (200 rpm), and after centrifugation (5000 g, 5 min), we measured the residual amount of ammonium using the spectrophotometric method (Adamczyk et al. 2019).

Lab experiment was arranged in a randomized design with four replicates per each treatment.

Nursery experiment 3 Uptake of ¹⁵N immobilised on wood chips by nursery seedlings.

The third nursery experiment was conducted in 2017 with 1-year-old spruce seedlings from nursery experiment 1 (control treatment), to which small mesh bags (1 × 1 cm, 50 µm mesh size) containing 50 mg of wood chips with 5 mg of ¹⁵N-ammonium bound to it were added. These mesh bags allow fungal but not root ingrowth. In this experiment, we compared the seedling uptake of ¹⁵N bound to knotwood vs stemwood chips. Wood chips with immobilised ¹⁵N-ammonium were prepared as above for the laboratory experiment and washed with water to remove unbound-N. We used 1.25 mM NH₄Cl for knotwood chips (KW) and 5 mM NH₄Cl for stemwood chips (SW) to obtain an equal amount of bound-N for both wood chip types. The amount of bound-N to wood chips was measured as above. Later, wet wood chips with immobilised ¹⁵N were placed in the mesh bags and transferred to the growing media of spruce seedlings. We used the following treatments: (1) mesh bags filled with KW with immobilised ¹⁵N; (2) mesh bags filled with KW with immobilised ¹⁴N; (3) mesh bags filled with SW with immobilised ¹⁵N; and (4) mesh bags filled with SW with immobilised ¹⁴N. The amount of N in every treatment was equal.

Nursery experiment 3 was arranged in a randomized design with ten replicate mesh bags per treatment. Mesh bags were buried in the rhizosphere of 1-year-old nursery seedlings (seed orchard R01-89-1001 Sv 110) with one bag per seedling, and the seedlings were grown in the nursery for three months in Plantek-81F containers (volume 85 cm³, BCC Ab). In the autumn of 2017, the seedlings were sampled, and the ¹⁵N content in the plants (roots, needles) and mycorrhiza was analysed by isotope-ratio mass spectrometry coupled to an elemental analyser (Thermo Finnigan). An amount of three mg of ground material was weighed into tin cups and introduced to the EA-IRMS via an autosampler.

The analytical precision was better than 0.2 ‰ for the $\delta^{15}\text{N}$, based on replicates of the standards. The nitrogen isotope values were calculated as atom% ^{15}N excess (atom% ^{15}N sample—atom% ^{15}N natural abundance), and for the treatments with seedlings, given as ^{15}N content ($\mu\text{g }^{15}\text{N}$) in needles, mycorrhizal root tips and roots by taking into account the mass, nitrogen content, and atom% ^{15}N excess values of each plant pool.

EMF colonisation of the seedlings

The mycorrhizal status of the seedlings was determined by gross microscopy of the outer mantle. The root tips were counted per fine root length, and the colour and texture of the tips (morphotype) were examined under a stereomicroscope (Agerer 1997). The identification of the inoculated EMF morphotypes was confirmed with direct sequencing of the ITS1 region, as described in Velmala et al. (2013).

Statistical analyses

To determine significant differences between seedling growths on different media, a one-way ANOVA was used, followed by Tukey's test. The difference was reported as significant when $P < 0.05$. The assumption of normality was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests, and the homogeneity of variances using Levene's test. These statistical analyses were conducted using SPSS software (22.0 version; IBM Corporation, New York, USA).

The relative abundances of EMF morphotypes were subjected to Detrended Correspondence Analysis (DCA) using PC-ORD, version 5.0, using a correlation distance measure. The data matrix consisted of the seedlings' colonisation degrees, as in Table 2.

Results

Effect of knotwood and stemwood chips on seedling performance

The growth of seedlings during the first year was negatively affected by the addition of wood chips, especially knotwood chips (Fig. 1). Growth slowdown also depended on the quantity of wood chips (10% vs 30%). During the following years (2017 and 2018), the differences levelled off, and the seedlings growing on media with 10% stemwood (SW) no longer differed from these of the control treatment.

Effect of nutrient-soaked knotwood and stemwood chips on seedling performance and EMF colonisation

The growth of seedlings was significantly affected by the treatment, both after the first growing season (Table 2) and after the second growing season (Fig. 2). After the first growing season, the lowest seedling growth was observed for unsoaked wood chips (Table 2). Soaking of the SW with nutrients significantly decreased growth reduction (Table 2). All the seedlings amended with nutrient-soaked KW remained smaller than the respective SW ones. Inoculation with EMF had no effect on the growth of the SW amended seedlings, but a trend of increased growth was seen in the inoculated KW treatment. The

Table 2 Root characteristics of the seedlings in the nursery experiment 3 after one growing season

Treatment	Germination %	2016 shoot, cm, SE	2017 shoot, cm, SE	Root tips, cm, SE	Uncolonized	Unknown infection	Young ECM	<i>Thelaphora terrestris</i>	<i>Rhizocyphus</i>	<i>Amphibema byssoides</i> sp.	<i>Wilcoxina</i> sp.	<i>Tylospora</i> sp.	<i>Cenococcium geophilum</i>	<i>Sebacinales</i>	<i>Cor-tinarius</i> sp.	<i>Ascomycota</i>
Conventional	96.3	8.3	± 0.780	± 0.202	9.03	± 0.01	19.0	49.9	8.8	11.1	5.9	0.0	0.0	0.0	0.1	5.2
5%SCN	91.4	7.7	± 0.729	67.14 ^a	± 2.687	9.41	± 0.02	16.1	74.4	5.5	3.8	0.0	0.0	0.0	0.0	0.0
5%KCN	86.1	5.7	± 0.614	54.42 ^b	± 2.930	9.08	± 0.01	20.5	70.4	8.1	1.0	0.0	0.0	0.0	0.0	0.0
10%SC	86.1	4.2	± 0.471	35.61 ^c	± 1.460	8.22	± 0.01	13.7	67.6	9.0	3.3	0.0	0.0	0.0	0.0	0.0
10%KC	97.2	3.3	± 0.471	34.86 ^c	± 1.581	8.09	± 0.02	7.0	72.2	13.9	0.0	5.9	0.0	0.0	0.1	0.9
10%SCN	91.7	5.4	± 0.437	63.89 ^b	± 2.286	9.23	± 0.02	9.4	84.6	4.3	0.0	0.0	0.0	0.0	0.0	0.0
10%KCN	91.7	4.8	± 0.345	43.19 ^{bc}	± 1.19	9.34	± 0.03	24.2	65.0	8.3	0.0	0.0	0.0	0.0	0.0	0.0
10%SCN + EMF	92.0	6.8	± 0.345	58.19 ^{ab}	± 2.140	7.99	± 0.01	16.5	58.9	8.3	13.0	0.0	0.0	0.0	0.0	0.0
10%KCN + EMF	94.4	6.2	± 0.570	51.83 ^b	± 1.818	9.16	± 0.01	19.0	69.5	8.5	0.9	0.0	0.0	0.0	0.0	0.0
5%DECAY1	83.3	6.1	± 0.651	63.47 ^b	± 2.413	9.62	± 0.03	19.8	73.5	5.6	0.0	0.3	0.6	0.0	0.0	0.5
10%DECAY1	77.8	6.0	± 0.425	59.14 ^b	± 2.653	9.58	± 0.02	11.4	72.2	11.4	4.1	0.0	0.0	0.0	0.0	0.9
5%DECAY4	72.2	7.5	± 0.663	69.72 ^{ab}	± 2.981	8.69	± 0.01	7.2	51.6	14.9	25.4	0.0	0.0	0.0	0.0	0.0
10%DECAY4	91.7	8.2	± 0.471	71.56 ^a	± 2.470	10.13	± 0.02	8.2	71.0	10.7	1.7	0.0	0.0	0.0	0.0	0.0
10%DECAY	93.8	7.7	± 0.747	73.90 ^{ab}	± 0.196	9.58	± 0.02	20.2	70.6	8.8	0.0	0.0	0.0	0.0	0.0	0.0
LIGNAN1	91.7	7.9	± 0.588	74.25 ^{ab}	± 1.896	8.50	± 0.00	13.9	60.0	11.2	14.9	0.0	0.0	0.0	0.0	0.0
LIGNAN2	100.0	7.2	± 0.593	71.67 ^{ab}	± 2.253	9.81	± 0.03	8.9	43.5	7.2	38.8	0.0	0.0	0.0	0.0	0.0

Treatments are SW stemwood chips, KW knotwood chips, N nutrient soaking of the wood chips, EMF EMF inoculation, DECAY 1, 2 decayed wood and decay stage, LIGNAN 1, 2 purified lignan extract in the peat, SE standard error

Fig. 1 Nursery experiment 1: Mean shoot heights of the seedlings during the three growing seasons. SW designates stemwood and KW knotwood chips; see other explanations in Table 1. Bars designate SE. Significant differences ($P < 0.05$) between treatments within the same year are indicated by different letters

Fig. 2 Nursery experiment 2: Mean shoot heights of the seedlings after two growing seasons. Treatments are SW, stemwood chips; KW, knotwood chips; N, nutrient soaking of the wood chips; EMF, ectomycorrhizal fungi inoculation; DECAY 1, 2 decayed wood and decay stage; LIGNAN 1, 2, purified lignan. % indicates the proportion of wood chips (v/v). More detailed information on the treatments in Table 1. Bars designate SE. Significant differences ($P < 0.05$) between treatments are indicated by different letters

decomposed wood of the 1st decay stage decreased seedling growth. However, the addition of more decomposed wood (decay stage 4 or mixture of decay stages 3–5) provided the same growth as the control. The growth of the seedlings in the controls was not negatively affected by the addition of purified lignan extracts. Root tip density was not significantly affected by any of the treatments (Table 2). The seedlings' EMF fungal communities were rather variable and did not respond to the inoculations or any other treatments (Table 2, Supplementary Fig. 1). The germination rate of the seeds showed variable trends, being highest in the Lignan2 treatment and smallest in decay wood treatments (Table 2).

Immobilisation of N on stemwood and knotwood chips in laboratory incubation experiment

Wood chips did not immobilise nitrate (data not shown). Ammonium was immobilised dose-dependently, with the highest immobilisation observed for the largest quantity of wood chips. Knotwood chips immobilised significantly more ammonium than stemwood chips (Fig. 3a). The immobilisation of ammonium on different wood chips also differed in terms of stability. Knotwood chips provided more stable immobilisation than stemwood chips after both

Fig. 3 Nitrogen immobilisation experiment: **a** Immobilisation of ammonium on stemwood (SW) and knotwood (KW) chips; **b** stability of immobilised N in the wood chips. Bars designate SE. Differences between treatments and amounts were always significant ($P < 0.05$)

extraction with water and with the common extractant, 1 M KCl (Fig. 3b). In summary, knotwood chips immobilised with high stability four times more N than stemwood chips.

Uptake of ^{15}N immobilised on wood chips by nursery seedlings

Spruce seedlings growing on conventional nursery substrate amended with wood chips with immobilised nitrogen took up ^{15}N from both stemwood and knotwood chips (Fig. 4). Labelled ^{15}N was detected in the needles, roots, and mycorrhizal root tips of the seedlings. However, the seedlings took up significantly more ^{15}N immobilised on stemwood than on knotwood. Knotwood and stemwood treatments showed different allocation patterns of ^{15}N . In knotwood treatment, we found slightly more ^{15}N in mycorrhizas than in root or needle pools. In stemwood treatment, the ^{15}N content in the mycorrhiza was slightly lower than in the roots.

Fig. 4 Uptake of ^{15}N immobilised on wood chips (SW—stemwood, KW—knotwood) by the spruce seedlings in the nursery. Difference between SW and KW were always significant. Bars designate SE

Discussion

We studied the effect of knotwood vs stemwood amendment on the growth of spruce seedlings, their N uptake, mycorrhization, and reactions between the wood chips and nutrients. Compared to conventionally used peat substrate (control) the addition of wood chips decreased the growth of seedlings dose-dependently. However, this effect levelled off with time. The decrease of growth was especially visible for knotwood chips. We hypothesised that the reason for the decreased growth of seedlings on wood chips was the result of wood chemistry, the quality and quantity of compounds affecting the immobilisation of N or the toxicity of the substrate, or both. The main composites of wood, i.e. cellulose, hemicellulose, and lignin (Rowell 2005) may not be responsible for changes in growth. However terpenes, phenolics and polyphenolics due to their chemical abilities can play an important role here (Joanisse et al. 2007; Adamczyk et al. 2015, 2017). For example, polyphenolics and terpenes present in both stemwood and knotwood chips may negatively affect enzymatic activities (Adamczyk et al. 2017) and some microbial communities (Kraus et al. 2003; Smolander et al. 2012). Moreover, polyphenolics and terpenes interact with organic N compounds decreasing their availability (Hagerman 2012; Adamczyk et al. 2018, 2019). Stemwood and knotwood chips differ in chemistry, particularly in their terpene and lignan content (Manninen et al. 2002; Piispanen et al. 2008). Lignans are a group of phenols that are characterised by coupling the two phenylpropanoid units by a bond between the positions in the phenyl propane side chains and their concentration in knots even reach up to 30% (w/w) (Willför et al. 2003, 2004, 2006; Huttula et al. 2018). Thus, these chemical differences in wood components have the potential to explain various seedling growths.

Knotwood chips could decrease seedling growth via a toxic effect. However, there was no decrease of EMF diversity between treatments (Supplementary Fig. 1). In addition, purified lignan fractions seemed not to pose any negative effects for the seedlings' growth or root tip density. Moreover, the effect of pure lignans on seedling germination was positive rather than negative. Thus, like knotwood chips, pure lignan had no toxic effect on seedlings. We therefore estimated the effect of different wood chemistry on plant N nutrition, as this could have been the main mechanism of growth deceleration. The laboratory

study confirmed the differences between the immobilisation of ammonium on stemwood vs knotwood chips. The latter not only immobilised four times more N, but also formed more stable complexes with ammonium, suggesting that stronger N immobilisation may have been the reason for the enhanced reduction of seedling growth. To verify the role of N immobilisation on seedling growth, we immobilised ^{15}N on wood chips and added this source of N to the seedlings. In line with expectations, the uptake of ^{15}N from knotwood was about four times lower than from stemwood chips, confirming the role of N immobilisation in seedling growth reduction.

In nursery experiment 2, we soaked wood chips in nutrients to estimate the extent and persistence of nutrient immobilisation on woody fractions, and how this affected the growth of seedlings. Again, wood chips reduced seedling growth, especially knotwood, but soaking in nutrients levelled this effect off especially when stemwood chips were added. This means that most of the reactive sites of the wood chips able to react with N were saturated with N from soaking making it impossible to bind more N during seedling growth. This was supported by the dead wood amendments. Wood in the 1st stage of decay, chemically the closest to the fresh wood chips, decreased seedling growth similarly to the stem- and knotwood chip amendments. However, wood in the 4th stage of decay did not significantly decrease seedling growth. This may suggest that compounds responsible for decreasing seedling growth are quite decomposed in the early stages of decomposition and/or underlie leaching with precipitation. Indeed, wood decomposition by fungi is a complex process conducted via a multiple set of fungal species and their enzymes (Rajala et al. 2015). The decomposition of plant cell wall lignocellulose is initiated via a breakdown of covalent bonds and the release of dissolved sugars and lignin-derived aromatic compounds (Lundell et al. 2010).

The wood in the 4th stage of decay may also act as an EMF inoculum, as such wood amendments contain several EMF species (Rajala et al. 2011, 2015). However, this inoculum seemed unviable. The growing substrates were also inoculated with viable EMF mycelia to estimate whether it helped seedlings acquire more nutrients from the wood amendments. Ectomycorrhizal fungi are known for their ability to obtain N from recalcitrant sources, including N compounds in complexes with polyphenolics (Wurzburger and Hendrick 2009; Rineau et al. 2012). There were slight indications that EMF-inoculated seedlings were able to rebalance seedling growth more from knotwood treatment than from the respective stemwood. The inoculated strain *Tylospora* sp. is known to produce N-releasing enzymes (Velmalä 2014), but the colonisation degree by *Tylopora asterophora* remained marginal (2%), and no great impact of inoculation could therefore be expected. Thus, contrary to the previous study (Vaario et al. 2009) we observed no significant effect of wood chips on the mycorrhization of the seedlings, implying that stemwood and knotwood amendments did not show any toxicity for the EMF communities. Thereby, the nutrient availability of these amendments seemed to be the main factor affecting the seedlings' performance.

Conclusions

The set of experiments provided by this study suggests that the addition of wood chips to growing media has strong potential to affect seedling growth. However, the effect depends on the spatial location of the wood within logs and thereby on the wood chemistry. The addition of wood chips initially reduced the growth of the seedlings, but the effect levelled

off with time. We suggest that wood chips affected the growth of seedlings mainly via N immobilisation, rather than via a direct toxic effect. The immobilisation of N on wood chips, especially knotwood chips, has potential for developing a slow-release N source. As such, the use of slow-release N would be beneficial for plant growth, survival in natural conditions, and environmental protection. All in all, wood chips are an interesting amendment for growing media, but more work is needed to uncover the potential effects of wood amendments on nursery seedlings.

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Declarations

Conflict of interest We declare no conflict of interest.

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