

Early root growth and architecture of fast- and slow-growing Norway spruce (*Picea abies*) families differ – potential for functional adaptation

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Complete List of Authors:	Hamberg, Leena; Luonnonvarakeskus, Management and Production of Renewable Resources Velmala, Sannakajsa Sievänen, Risto; Luonnonvarakeskus Kalliokoski, Tuomo; University of Helsinki, Forest Science Pennanen, Taina; Luonnonvarakeskus, Management and Production of Renewable Resources
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3 **1 Early root growth and architecture of fast- and slow-growing**
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6 **2 Norway spruce (*Picea abies*) families differ – potential for**
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9 **3 functional adaptation**
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16 Running title: Root growth differs in spruce
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22 **7 Leena Hamberg ^{1*}, Sannakajsa M. Velmala ¹, Risto Sievänen ¹, Tuomo Kalliokoski ²,**
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24 **8 Taina Pennanen ¹**
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26

27 ¹ Natural Resources Institute Finland, Helsinki, Finland
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30 ² Department of Physics, University of Helsinki, Helsinki, Finland
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39 ***Correspondence:**

40
41 Leena Hamberg, Natural Resources Institute Finland, P.O. Box 2 (Latokartanonkaari 9), FI-
42
43 00790 Helsinki, Finland email: leena.hamberg@luke.fi, tel. +358 29 532 5400
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50 **Keywords:** seedlings, phenotypes, fine roots, WinRHIZO™, mixed models
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3 19 **Abstract**
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6 20 The relationship between the growth rate of aboveground parts of trees and fine root
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8 21 development is largely unknown. We investigated the early root development of fast- and
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10 22 slow-growing Norway spruce (*Picea abies* L.) H. Karst. families at a developmental stage
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12 23 when the difference in size is not yet observed. Seedling root architecture data, describing
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14 24 root branching, were collected with the WinRHIZO™ image analysis system, and mixed
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16 25 models were used to determine possible differences between the two growth phenotypes. A
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18 26 new approach was used to investigate the spatial extent of root properties along the whole
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20 27 sample root from the base of one-year-old seedlings to the most distal part of a root. The root
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22 28 architecture of seedlings representing fast-growing phenotypes showed ca. 30% higher
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24 29 numbers of root branches and tips, which resulted in larger root extensions and potentially a
25
26 30 better ability to acquire nutrients. Seedlings of fast-growing phenotypes oriented and
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28 31 allocated root tips and biomass further away from the base of the seedling than those growing
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30 32 slowly, a possible advantage in nutrient limited and heterogeneous boreal forest soils. We
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32 33 conclude that a higher long-term growth rate of the aboveground parts in Norway spruce may
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34 34 relate to greater allocation of resources to explorative roots that confers a competitive edge
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36 35 during early growth phases in forest ecosystems.
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37 **Keywords:** seedlings, phenotypes, fine roots, WinRHIZO™, mixed models

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39 Introduction

40 The relationship between tree phenotype, root proliferation and the spatial distribution of fine
41 roots is largely unknown. For example, in Norway spruce (*Picea abies* L.) H. Karst., some
42 genotypes grow faster and repeatedly outcompete others in long-term field experiments, but
43 whether this relates to root development has seldom been investigated (Korkama et al. 2006,
44 Velmala et al. 2014a). Resource allocation to roots may be a crucial part of a tree's strategy
45 in competition with conspecifics and other species within a forest.

46 Variation in early root structure, such as larger root systems, implies great
47 differences in the potential of growing roots to acquire nutrients and water in heterogeneous
48 forest soils that may lead to better growth in later stages of long-lived trees. Small roots, also
49 called fine roots, are traditionally defined as roots less than 2.0 mm in diameter, but in more
50 recent investigations only roots less than 1.0 or 0.5 mm in diameter are considered fine roots
51 (Pregitzer 2002, Pregitzer et al. 2002, Bagniewska-Zadworna et al. 2012, Beidler et al. 2015,
52 McCormack et al. 2015). Fine root tissue is nutritionally expensive to produce and maintain
53 due to its high concentration of nitrogen, metabolic activity and respiration rate (Pregitzer et
54 al. 1997, Pregitzer 2002) as well as its turnover (Leppälammı-Kujansuu et al. 2014). In young
55 tree seedlings, two functionally different fine root types can be found, i.e., pioneer roots and
56 fibrous roots (Zadworny and Eissenstat 2011, Bagniewska-Zadworna et al. 2012; Zadworny
57 et al. 2017). In developing root systems, the function of pioneer roots is soil exploration and
58 assurance of long-distance, rapid transport of water and nutrients as well as provision of a
59 framework for the whole root system. Pioneer roots are long and thick, not colonized by
60 mycorrhizal fungi, they grow and extend rapidly in the soil both horizontally and vertically,
61 and branch intensively since they typically quickly produce fibrous roots responsible for
62 absorption of water and nutrients (Zadworny and Eissenstat 2011; Bagniewska-Zadworna et
63 al. 2012). The production of short-roots, also called root tips, or exterior links by Fitter and

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3 64 Stickland (1991), and associations with mycorrhiza increase the surface-area of roots and
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5 65 thus the uptake of nutrients and water for a given amount of carbon (Ostonen et al. 2007,
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7 66 2013). Root tips have a specific, dynamic role in root systems especially in absorbing water
8
9 67 and acquiring nutrients, with high biomass and turnover (Wang et al. 2006).

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12 68 Norway spruce adapts its root growth in response to environmental conditions
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14 69 (Kalliokoski et al. 2008). Stand characteristics have significant impacts on the architecture of
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16 70 coarse roots and affect the amount of root tips in the organic layer (Helmisaari et al. 2009,
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18 71 Kalliokoski et al. 2008, 2010). Furthermore, carbon allocation and the longevity of structural
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20 72 compounds differ with thickness and the classification of roots, implying huge functional and
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22 73 physiological heterogeneity within the outer rhizosphere (Keel et al. 2012).

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25 74 Boukcim and Plassard (2003) showed that the number and total length of root
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27 75 tips were higher in a fast-growing spruce family compared to a slow-growing family.
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29 76 Accordingly, we have previously found some differences between seedling roots of six
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31 77 Norway spruce families showing contrasting growth performance in long-term field trials
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33 78 (Velmala et al. 2014a, b). The density of outermost ectomycorrhizal root tips (number of root
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35 79 tips per root length) was lower in fast-growing than slow-growing families when seedlings
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37 80 were still of the same size. Beyond these associations between root tips and phenotypic
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39 81 characteristics, a genetic component driving root tip formation and root growth in Norway
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41 82 spruce was found by comparing 55 clonal genotypes. The density of root tips among spruce
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43 83 clones varied almost twofold, indicating huge adaptive potential, with root tip density being
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45 84 moderately heritable ($H^2 = 0.41$) (Velmala et al. 2013).

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49 85 However, evidence on the relationship between root structuring and shoot
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51 86 growth rate is still incomplete as spruces were not included in the study where root traits of
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53 87 fast- and slow-growing plants were compared (Comas and Eissenstat 2004). Bouckim and
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55 88 Plassard (2003) compared only two Norway spruce families, while in other spruce root
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3 89 studies comparing fast- and slow-growing phenotypes (Korkkama et al. 2006, 2007, Velmala
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5 90 et al. 2013, 2014a, b), no architectural analyses of root systems were performed. Especially,
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7 91 studies including complete root segments extending from the base of a seedling to the most
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10 92 distal part of a root are missing.

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12 93 We compared the early root formation of six Norway spruce families showing
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14 94 contrasting growth performance in long-term field trials before any differences in
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16 95 aboveground growth were found. We were interested in determining whether there are
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18 96 differences in root biomass, spatial extension of root systems and ability to form root
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21 97 branches. Detailed architectural data on whole sample roots originating from the base of a
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23 98 seedling were used in these analyses. The data included information on all root pieces
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25 99 originating from each branching point. We hypothesized that seedlings of fast-growing
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27 100 families would display lower root tip density (i.e., number of root tips per root length, see
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29 101 Velmala et al. 2014a, b). Furthermore, we hypothesized that in seedlings of fast-growing
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31 102 families, the length of sample roots is higher and includes more side branches (see Fig. 1)
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33 103 further away from the base of a seedling than in seedlings of slow-growing families. To the
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35 104 best of our knowledge, no previous intraspecific studies have concentrated on spatial
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37 105 differences in tree roots, i.e., differences in root branching intensity along the length of
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39 106 sample roots.
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44 45 108 **Material and Methods**

46 47 48 109 *Seedlings*

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51 110 The study was performed using six Norway spruce seed orchard families that originated from
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53 111 southern Finland and were selected for breeding purposes (Table 1). Three families were
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55 112 classified as fast-growing (good and excellent growth; family codes 298, 612, 1162) and
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57 113 three as slow-growing families (stunted; family codes 394, 427, 1183) based on stem height
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3 114 measured in several long-term field trials. These contrasting groups of spruce families are
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5 115 hereafter referred to as the fast- and slow-growing phenotypes.
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7 116 Seeds were germinated under optimal growth conditions in nursery containers
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9 117 with unfertilised light *Sphagnum* peat (PP03, Kekkilä, Vantaa, Finland) in a glasshouse at the
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11 118 end of April 2011. Seedlings were transplanted after six weeks into Plantek-81F containers
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13 119 (vol. 85 cm³, BCC) filled with sieved forest humus, which was excavated from the uppermost
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15 120 layer of fine sandy till of a Norway spruce stand located in southern Finland. The humus
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17 121 acted as a natural source of forest microbiota, e.g. ectomycorrhizal fungi. In August, sieved
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19 122 forest humus (30 ml) was added again to the base of each seedling. Seedlings were grown
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21 123 outdoors in an open forest nursery field (62.625 N, 27.122 E, Supplementary Table 1)
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23 124 following common seedling production practises in Finland, i.e., irrigated regularly and
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25 125 fertilized once a week according to the Kekkilä Forest-Superex fertilisation program (NPK
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27 126 22-5-16) - each seedling was provided with approximately 10 mg N during the growing
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29 127 season. The seedlings overwintered outdoors under natural snow cover. In the following
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31 128 summer, seedlings were grown in the same open nursery field and fertilized once a week
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33 129 according to the Kekkilä Forest-Superex fertilisation program with a slightly higher N load:
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35 130 the conductivity of peat was 2 mS cm⁻¹. In early August 2012, at 16 months of age and after
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37 131 two growing seasons, nine replicate seedlings from each family were sampled (Fig. 1a).
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43 132 Seedlings were removed from containers, the shoot was detached from roots
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45 133 and shoot height was measured from the base to the top of a seedling (mm) in order to
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47 134 examine if the phenotypes differed in height. Roots were washed gently with tap water and
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49 135 stored in water at +4 °C.
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54 137 ***Description of root data***
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3 138 In most cases, there were altogether ca. five root branches per seedling. Three uppermost
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5 139 sample roots per seedling were selected for the architectural analysis by sampling down from
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7 140 the root collar (Fig. 1a). Thus, altogether 162 detached roots were investigated in detail. The
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9 141 whole sample root from the base of a seedling up to the most distant part of a root was
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11 142 included in the architectural analysis. Fresh sample roots were kept in water on large Petri-
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13 143 dishes when scanned with an Epson perfection V700 Photo scanner (SeikoEpson
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15 144 Corporation), and analysed using the WinRHIZO™ image analysis system professional
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17 145 software (2013) in order to obtain detailed link-based data for statistical analyses (see below).
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19 146 Here, a link is defined as a piece of root between two branches or a piece of root forming a
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21 147 root tip (Fig. 1b).

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25 148 Soil humus ensured that the roots were highly ectomycorrhizal (>81%, Velmala
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27 149 et al. 2014b). On average, seedlings were colonized with ca. five ectomycorrhizal species and
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29 150 the fast- and slow-growing families did not differ in terms of their ectomycorrhizal
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31 151 communities. The most abundant ectomycorrhizal colonizers belonged to the genera
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33 152 *Amphinema*, *Piloderma* and *Thelephora* which comprise more than two thirds of the
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35 153 occurrences. The ectomycorrhizal fungal communities of the families are described in detail
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37 154 in Velmala et al. (2014b).

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41 155 After scanning, roots and shoots were dried at 60 °C for 24 h. Total biomasses
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43 156 of roots and shoots (g) were determined for three seedlings per family. Dry biomass (g) was
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45 157 measured separately for detached sample roots.

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48 158 In our data analyses, we used the developmental order-based classification of
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50 159 WinRHIZO™ (2013) that counts root orders from the main branch of a sample root outwards
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52 160 instead of the functional stream-order classification beginning from the outermost roots
53
54 161 inwards. This approach was chosen as our root systems were relatively small and simple
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56 162 (mean diameter < 1 mm, Table 2, McCormack et al. 2015), and because the developmental

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3 163 order-based classification provides more information on root architecture (i.e., on
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5 164 architectural differences between the fast- and slow-growing phenotypes, such as the length
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7 165 of the main branch of a sample root and the number of side branches along it, see Fig. 1b)
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9 166 which was the main focus of the present study. In the developmental order-based
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11 167 classification, the main branch of a sample root originating from the base of a seedling was
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13 168 considered a zero order root, and at the branching point the thicker branch continued the same
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15 169 zero order while the thinner one belonged to the next order. Thus, a root branch originating
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17 170 from the zero order root was considered a first order root branch, and a root branch
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19 171 originating from the first order root was considered a second order root branch, etc. (Fig. 1b).
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21 172 The developmental order-based classification is especially useful for roots with a clear main
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23 173 root axis or branch, and in which clear branches of higher orders can be observed, such as in
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25 174 young spruce seedlings in our study (Fig. 2). Unlike the developmental order-based
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27 175 classification of roots used in the present study, the stream-order classification is more
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29 176 suitable for describing the function of roots based on the division between absorbing and
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31 177 transporting roots so that the first three root orders representing absorbing roots are
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33 178 considered anatomically and physiologically different from higher root orders (Pregitzer et al.
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35 179 2002, McCormack et al. 2015, Liu et al. 2016, Norby and Iversen 2017). It seems that the
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37 180 functional stream-order classification has widely been used in studies where roots have been
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39 181 cut to small fragments (e.g., Chen et al. 2016; Ostonen et al. 2017; Zadworny et al. 2017),
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41 182 whereas in the present study, a whole sample root was investigated in detail. Yet, root tips,
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43 183 also known as exterior links, and interior links (other links than root tips) were investigated
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45 184 separately (Fitter and Stickland 1991) to ensure comparability to the functional classification.
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53 185 In the WinRHIZOTM (2013) data, the number, length (mm) and surface area
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55 186 (mm²) of each link of each order were available. WinRHIZOTM also calculated root volume
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57 187 per sample root (mm³). Furthermore, angle, in degrees, between each pair of two links
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3 188 originating from the same branching point was provided. Utilizing this information, we
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5 189 calculated the total number, length and surface area of links per sample root, and separately
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7 190 for zero to the highest order links of root tips and interior links. In addition, root tip density,
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9 191 i.e., the number of root tips divided by the total length of links, and the mean angle for root
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11 192 tips and interior links were calculated per sample root. A side branch was defined as one
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13 193 branch of a root that originates from the zero order root link and contains all branching links
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15 194 from that point (see Fig. 1b). Side branches include more than one link, and are thus
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17 195 separated from exterior links (i.e., root tips). Utilizing the LIGNUM software (Sievänen et al.
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19 196 2010), we determined the occurrence of side branches (Fig. 1b), the number of all links and
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21 197 root tips and the length of all links within each branch (both side branches and root tips were
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23 198 considered as branches) along a zero order root. The LIGNUM software was also used to
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25 199 visualize the roots.
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31 200 Utilizing link-based data, we calculated specific root length (SRL, ratio of root
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33 201 length to root dry mass, mm g^{-1}), specific root area (SRA, ratio of surface area to root dry
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35 202 mass, $\text{m}^2 \text{kg}^{-1}$) and root tissue density (RTD, ratio of root dry mass to root volume, kg m^{-3})
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37 203 for all seedlings.
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42 43 205 *Statistical analyzes*

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46 206 Differences in shoot height (height of a seedling from the base to the top, mm), and total
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48 207 biomass of the three sample roots (g), specific root length (SRL, mm g^{-1}), specific root area
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50 208 (SRA, $\text{m}^2 \text{kg}^{-1}$) and root tissue density (RTD, kg m^{-3}) between the fast- and slow-growing
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52 209 phenotypes were analyzed using linear mixed models (LMMs) in R with the *lme* function in
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54 210 library *nlme* ($n = 54$, six spruce families with nine seedlings in each, see Ostonen et al. 2013,
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56 211 Maherali 2014, R Core Team 2015, Pinheiro et al. 2016, Weemstra et al. 2016). In the
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3 212 LMMs, response variables were either the height, SRL, SRA or RTD of a seedling or the
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5 213 total biomass of three sample roots per seedling. As an explanatory variable, we used the
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7 214 long-term growth rate of a family (a factor with two levels: fast or slow), and as random
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9 215 factor we used the family (seedlings belonging to the same origin were given the same family
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11 216 code). Similarly, differences in the total biomass of roots and shoots, and root:shoot ratio,
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13 217 between seedlings of fast- and slow-growing phenotypes were investigated as above ($n = 18$,
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15 218 six spruce families with three seedlings in each).

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18 219 Detailed link data for each sample root of a seedling ($n = 162$, i.e., 54 seedlings
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20 220 with three sample roots in each), produced by WinRHIZO™ (2013), were analyzed using
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22 221 linear (LMMs) or generalized linear mixed models (GLMMs) in R. These models were
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24 222 estimated separately for root volume, root tip density, and number, angle and surface area of
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26 223 links, measured separately for each sample root of an individual seedling. These response
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28 224 variables were mainly investigated for the whole sample root, and for zero, first, second and
29
30 225 the combined third to fifth order links separately (Fig. 1). Count response models were
31
32 226 estimated assuming a Poisson distribution with log link function using the *glmer* function in
33
34 227 the *lme4* library (Bates et al. 2015). For other responses, a normal distribution was assumed,
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36 228 and the *lme* function in library *nlme* was used to estimate these models (Pineiro et al. 2016).
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38 229 The long-term growth rate of a seedling (a factor with two levels: fast or slow) was included
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40 230 as an explanatory variable. Furthermore, two nested random factors were included: family
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42 231 and seedling identity (as three sample roots per seedling within a family were investigated as
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44 232 an individual observational unit).

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47 233 Generalized additive mixed models (GAMMs) were used to investigate
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49 234 differences in root properties along zero order sample roots. The probability of occurrence of
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51 235 side branches, the number of root tips and all links, and the total length of links in branches
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53 236 along zero order links of sample roots were analyzed using the function *gamm* in library
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3 237 *mgcv* in R (Wood 2004, 2006, 2011). Models for seedlings of fast- and slow-growing
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5 238 phenotypes were estimated separately. Models for the probability of occurrence of side
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7 239 branches were estimated assuming a quasi-binomial distribution with logit link function in R.
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9 240 The response in this model was a factor with two levels: 0 = a side branch does not exist but a
10
11 241 root tip was visible, 1 = a side branch with more than one link existed. GAMMs for count
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13 242 responses (number of root tips, and all links per branch, i.e., both side branches and root tip
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15 243 branches were included) were estimated assuming a quasi-Poisson distribution with log link
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17 244 function, and the model for the total length of branches (mm) as a response was estimated
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19 245 with a log-linear model. As an explanatory variable we used the total length of sample root
20
21 246 links of zero order in cm and in a separate model the total length was scaled from 0 to 100%.
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23 247 These variables were smoothed when the models were estimated. As random factors we used
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25 248 family, seedling identity (as three sample roots per seedling within a family were investigated
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27 249 as an individual observational unit), and identity of a sample root (as several side branches
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29 250 and root tips existed per sample root length). GAMMs are able to find curvilinear
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31 251 relationships between two variables, as in these models, tiny regression lines are combined
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33 252 together to form a smooth line representing variation in data along an investigated
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35 253 explanatory variable (Wood 2004, 2006, 2011). *P*-values for the curves are given, but no
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37 254 parametric estimate for the smoothed explanatory variable is provided as a result of this
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39 255 analysis. Statistically significant results can be visualized by drawing a response curve based
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41 256 on the GAMM.
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258 **Results**

259 *Seedling results*

260 There were no differences in shoot height, specific root length, specific root area, root tissue
261 density, the total biomass of shoots or roots, nor in root:shoot biomass ratio between

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3 262 seedlings of fast- and slow-growing Norway spruce phenotypes (Table 3). Only total biomass
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5 263 of the three sample roots per seedling was indicatively higher in fast- than in slow-growing
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7 264 seedlings.
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11 266 *Sample root results*

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14 267 The thickest links (zero order) comprised less than 20% of the root links while more than
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16 268 80% of all links were of the 1st to 5th order (Table 2). In our data, root diameter decreased
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18 269 with increasing order (note that we used the developmental order-based classification of
19
20 270 counting orders outwards from the main branch of a sample root). Mean diameter and the
21
22 271 length of links did not differ between the seedlings of fast- and slow-growing phenotypes
23
24 272 although fast-growing seedlings showed slightly higher values. Half of the links were root
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26 273 tips while the rest were interior links.
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30 274 Root volume of the sample roots was indicatively higher in fast- than in slow-
31
32 275 growing seedlings ($n = 162$, $p = 0.10$), with predicted values based on the model being 319.7
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34 276 ± 26.7 and 238.3 ± 26.7 mm³, respectively. Also the number of side branches (single root tips
35
36 277 excluded, GLMM, $p < 0.01$) and the total number of links were higher in fast- than in slow-
37
38 278 growing seedlings (Tables 4-5, Fig. 2-4). In particular, first order interior links and root tips
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40 279 attached to them were more abundant in fast- than slow-growing seedlings (Table 5, Fig. 4F
41
42 280 and K), yielding higher total length values for the first order links in fast-growing seedlings
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44 281 (LMM, $p = 0.04$). The surface area of root tips was higher in fast-growing seedlings (LMM,
45
46 282 Table 4, Fig. 3L, $p = 0.04$), but root tip density (LMM, $p > 0.10$) and total length of the main
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48 283 root branch (of zero order) was similar in all seedlings (LMM, $p = 0.20$).
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52 284 The angle of root tips, i.e., exterior links, was indicatively smaller in fast-
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54 285 (60.5°) than slow-growing seedlings (62.6°, LMM, $p = 0.07$), while the angle of other links,
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3 286 i.e., interior links, was indicatively larger among fast- (31.4°) than slow-growing seedlings
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5 287 (30.2°, $p = 0.06$).
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7 288 In the sample roots of fast-growing seedlings, the probability of occurrence of
8
9 289 side branches was higher right next to the base and 10-15 cm further away along zero order
10
11 290 root links compared to the branching profile of slow-growing seedlings (Figs. 5A and 6A).
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13 291 Moreover, the total number of root tips and links per branch, and the total length of all branch
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15 292 links along zero order roots peaked closer to the base in slow-growing seedlings (Fig. 5B-D),
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17 293 i.e., ca. 5 cm from the base, while in fast-growing seedlings these values remained relatively
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19 294 high up to 10-15 cm (Fig. 6B-D).
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296 Discussion

297 The data supported our original hypothesis that seedlings of the fast-growing Norway spruce
298 phenotypes have more branches further away along the sample roots than those with slow
299 growth, although no differences in height or biomass between the groups could yet be
300 observed. This indicates that fast-growing seedlings allocate more resources to root growth
301 further away from the base of a seedling although – contrary to our hypothesis – the total
302 length of zero root branches did not differ between the two growth phenotypes.

303 Longer branches and the larger number of side branches and root tips especially
304 further away from the base of seedlings (see Figs. 2 and 5) suggest that fast-growing
305 seedlings have higher potential to produce pioneer roots (Zadworny and Eissenstat 2011,
306 Bagniewska-Zadworna et al. 2012), enabling the growth of higher number of absorbing fine
307 roots. This may be a general characteristic that differentiates fast- from slow-growing
308 phenotypes. The formation of branches in roots is a vital process as it enables plants to extend
309 to a larger area and to increase their contact area with the soil (Pagès 2014, Beidler et al.
310 2015). When comparing various deciduous and evergreen tree species, Comas and Eissenstat

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3 311 (2004) found that roots of fast-growing tree species had a greater degree of branching, but
4
5 312 smaller diameters than slow-growing species. In our study, specific root length, SRL, did not
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7 313 differ between the seedlings of fast- and slow-growing phenotypes, although higher SRL
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9 314 values usually indicate thinner roots and greater root surface area (via longer roots with more
10
11 315 branches) for nutrient absorption, while lower values indicate thicker roots with lower surface
12
13 316 area (Craine et al. 2001, Comas and Eissenstat 2004, Maherali 2014, Weemstra et al. 2016).
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16 317 Interestingly, Ostonen et al. (2013) also did not find variation in SRL in Norway spruce along
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18 318 a latitudinal gradient. Most likely SRL is not an essential character regulating performance in
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20 319 spruce under different conditions. In Scots pine (*Pinus sylvestris* L.), however, SRL is
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22 320 dependent on latitude and, more specifically, on mean annual temperature (Ostonen et al.
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24 321 2017, Zadworny et al. 2017).

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27 322 In fast-growing seedlings, a larger branching angle of lower level interior links
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29 323 may relate to the ability to enlarge the soil contact area, while smaller angles of root tips may
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31 324 indicate a strategy of roots for more explorative growth further away from the base of a
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33 325 seedling in forest soils to better reach patches of nutrients (Beidler et al. 2015) and soil
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35 326 microbes (Pennanen et al. 1999) that may enable fast-growing seedlings to gather more
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37 327 resources for future growth. Especially, in combination with the observation that fast-
38
39 328 growing spruce seedlings bore more side branches further away from the base of the seedling,
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41 329 their ability to gain water, nutrients and symbiotic associations in highly heterogeneous forest
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43 330 soils may be significantly higher than that of slow-growing ones since, e.g., ectomycorrhizal
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45 331 fungi often occur in patches 3 - 17 m apart (Lilleskov et al. 2004).

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48 332 Ectomycorrhizal trees are more dependent on mycorrhizal hyphal foraging (i.e.,
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50 333 in absorbing water and acquiring nutrients) than arbuscular mycorrhizal trees which invest
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52 334 more carbon in constructing foraging roots (Eissenstat et al. 2015, Chen et al. 2016).
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54 335 Furthermore, fertilization may increase root proliferation and reduce mycorrhizal
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3 336 colonization (Eissenstat et al. 2015). Clear differences in fine root architecture between fast-
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5 337 and slow-growing Norway spruce origins have, thus far, only been found under mycorrhiza-
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7 338 free conditions in a study by Boukcim and Plassard (2003). They reported that the number
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9
10 339 and length of root tips were higher in a fast-growing spruce family, but the difference
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12 340 disappeared when roots were associated with an ectomycorrhizal fungus, i.e., it seemed that
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14 341 after mycorrhizal colonization, the fast-growing family did not anymore allocate resources to
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16 342 root growth. Our results were obtained under conventional growth conditions of spruce, and
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18 343 seedlings were cultivated in a substrate containing soil humus ensuring ectomycorrhizal
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21 344 colonization of the roots (> 81%, ca. five species per seedling with no differences between
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23 345 the seedlings of fast- and slow-growing phenotypes; Velmala et al. 2014b). Interestingly, in
24
25 346 our study, fast-growing seedlings invested differently to roots than slow-growing seedlings,
26
27 347 i.e., they had higher number of side branches, links and root tips, even after ectomycorrhizal
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29 348 colonization. As seedlings were cultivated under uniform conditions, the observed differences
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31 349 in root architecture most probably reflect variation in the genetic background of the spruce
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33 350 seedlings rather than environmental effects (see Fitter and Stickland 1991).

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36 351 In contrast to studies by Velmala et al. (2014a, b) and our hypothesis, we
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38 352 showed that root tip density does not differ between seedlings of fast- and slow-growing
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40 353 spruce phenotypes, although the number of root tips was higher in fast-growing seedlings.
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42 354 This may be due to the finding that also the total length of links was indicatively higher in
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44 355 fast-growing seedlings, thus resulting in similar densities between the phenotypes. Velmala et
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46 356 al. (2014a) reported that the root tip density of younger, 13-month-old Norway spruce
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48 357 seedlings grown in a homogenous growth substrate in a nursery was higher and that the roots
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50 358 were denser in seedlings of slow-growing phenotype, but also that the total number of root
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52 359 tips in a seedling did not differ. However, in Norway spruce at 12 years of age, roots of fast-
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54 360 growing clones supported higher ectomycorrhizal diversity and a greater number of root tips
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3 361 than the slow-growing ones (Korkama et al. 2006, 2007). Differences in these results
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5 362 estimating root tip densities may reflect either true differences in roots at different stages of
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7 363 seedling growth or different methods used to measure root tip densities. The WinRHIZO™
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9 364 analysis used here provided an estimate of large compartments of root systems compared to
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11 365 previous studies in which roots were sampled by a soil corer (Korkama et al. 2006) or cut into
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13 366 1-2 cm pieces (Velmala et al. 2013) as sampling was aimed only to detect ectomycorrhizal
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15
16 367 root tips. Still, both the mycorrhizal root tip-focused sampling and the present root
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18 368 architecture approach show that tree genotype affects root structure. This indicates that
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20 369 different strategies for root investigations are possible if limitations of the approach and
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22 370 comparability of the samples between treatments are considered.
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25 371 This is the first time the spatial distribution of root branch properties, i.e., side
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27 372 roots and root tips along main side branches, was investigated. We believe that this is an
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29 373 approach that could be utilized in future studies when complete root systems of small
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31 374 seedlings can be analysed. Furthermore, the use of developmental order-based classification
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33 375 allowed us to get detailed information on the location of root variables, such as side branches
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35 376 and root tips, within the root systems, e.g., along the main root branch of zero order.
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37 377 However, due to the limited number of observations, our results were often indicative, and
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39 378 therefore larger datasets are needed to provide a comprehensive view of the belowground
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41 379 development of tree seedlings.
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47 381 **Conclusions**

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50 382 The roots of 16-month-old seedlings of Norway spruce phenotypes with contrasting growth
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52 383 rates in long-term field experiments were investigated. After two growing seasons,
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54 384 aboveground parts of seedlings were still even-sized, but the differing growth phenotypes had
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56 385 different strategies for early root growth under uniform conditions. Fast-growing phenotypes
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3 386 showed higher numbers of root tips (i.e., exterior links) and interior links, and oriented and
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5 387 allocated root tips and biomass further away from the base of the seedling than slow-growing
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7 388 ones. The genetic background of spruce seedlings is likely to determine these differences
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9 389 since environmental conditions were similar for all seedlings. The ability of different spruce
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11 390 origins to control their root architecture may cause, in later life, differences in their ability to
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13 391 gain nutrients, water and mutualistic associations in heterogeneous forest soils, potentially
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15 392 explaining the systematic long-term growth differences and competitive relationships in
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17 393 forest ecosystems. We conclude that over time, small differences in these specific allocation
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19 394 patterns for root growth may create positive self-reinforcing mechanisms that underlie the
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21 395 higher growth rates and superior long-term growth performance of some trees in forests.
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27 397 **Conflict of Interest**

28
29 398 None declared.
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48
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52 53 54 409 55 56 410 **References** 57 58 59 60

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3 552 **Figure legends**
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11 554 **Figure 1.** Sampling design: (A) Altogether 54 seedlings from six spruce families (i.e., nine
12 555 seedlings per family), and their sample roots, three per seedling, were investigated. (B)
13 556 Schematic representation of a sample root with explanations for some root variables. A link is
14 557 a part of a root between two branches or the most distant part of a branch (i.e., root tip). All
15 558 side branches include more than one root link whereas a root tip includes one link only.
16 559 Angle is determined as degrees between two links starting from the same point. The order of
17 560 branches (and links within a branch) is indicated with a number as presented in the figure.
18 561 The zero order root links form the main root branch of a sample root, first order links form a
19 562 first order branch originating from the zero order root branch, second order links form a
20 563 second order branch originating from first order branch links etc. This developmental order-
21 564 based classification was utilized in this study.
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34 566 **Figure 2.** Schematic model of four sample root branches of Norway spruce (*Picea abies*)
35 567 seedlings representing: (A) a sample root with the highest number of links for fast-growing
36 568 seedlings, (B) a root containing a median number of root links for fast-growing seedlings, (C)
37 569 a root containing a median number of root links for slow-growing seedlings, and (D) a
38 570 sample root with the lowest number of links for slow-growing seedlings. The lengths, widths
39 571 and branching angles of the root links were analyzed with WinRHIZO™, and visual
40 572 rendering was done using the LIGNUM program (Sievänen et al. 2010).
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52 574 **Figure 3.** Number, total length and surface area of links among the sample roots of fast- and
53 575 slow-growing Norway spruce (*Picea abies*) seedlings based the predictions of linear or
54 576 generalized linear mixed models ($n = 162$). Values for the whole sample root, side branches,
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3 577 interior links, i.e., other links than root tips, and root tips, i.e., exterior links are presented.

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5 578 Standard error values are not presented as these are not available for all model types.

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7 579 Statistically significant differences ($p < 0.05$) are indicated with an asterisk, and mean raw

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9 580 data values for spruce families are shown with a cross. See Table 4.

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14 582 **Figure 4.** Total number of links, the number of interior links (i.e., other than root tips), and

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16 583 root tips between the sample roots of fast- and slow-growing seedlings of Norway spruce

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18 584 (*Picea abies*) based on the predicted values of generalized linear mixed models (GLMMs, $n =$

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20 585 162). Values for different root orders are presented (note that the developmental order-based

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22 586 classification was used, see Fig. 1). Note that the numbers are extremely low in panels H and

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24 587 I. Standard error values are not presented as these are not available for this model type.

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26 588 Statistically significant differences ($p < 0.05$) between the fast- and slow-growing seedlings

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28 589 are indicated with an asterisk, and mean raw data values for spruce families are shown with a

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30 590 cross. See Table 5.

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36 592 **Figure 5** (A) The probability of occurrence of side branches, (B) the number of root tips per

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38 593 branch (composed of one or more links), (C) the number of all links per branch, and (D) the

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40 594 total length of branch links along the total length of sample root links of zero order expressed

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42 595 as a proportion (0-100%) for fast- and slow-growing Norway spruce seedlings. All responses

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44 596 are statistically significantly curvilinear (GAMMs, $p < 0.01$) and 95% confidence intervals

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46 597 are presented with dashed lines.

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52 599 **Figure 6** (A) The probability of occurrence of side branches, (B) the number of root tips per

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54 600 branch, (C) the number of all links per branch (composed of one or more links), and (D) the

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56 601 total length of branch links along the total length of sample root links of zero order expressed

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3 602 in cm for fast- and slow-growing Norway spruce seedlings. All responses are statistically
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5 603 significantly curvilinear (GAMMs, $p < 0.01$) and 95% confidence intervals are presented as
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7 604 dash line response curves.
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For Peer Review

2 **Tables**

4 **Table 1.** Origin, seed collection year and growth performance of the Norway spruce (*Picea*
 5 *abies* (L.) Karst.) families used in this study. The growth performance of two groups (fast and
 6 slow) has been assessed from 14 year old trees in seven to 10 growth experiments done
 7 earlier in the field. The table is modified from Velmala et al. (2014a).

Family code	Origin ^a		Collection year	Growth performance ^b	Succession rate ^c	Group
	Female	Male				
298	239	1515	2000	Good	89	Fast
612	5519	4309	1998	Excellent	102	Fast
1162	46	Open pollination	1989	Excellent	95	Fast
394	7133	Open pollination	1983	Poor	33	Slow
427	5514	Open pollination	1983	Poor	35	Slow
1183	217	Open pollination	1989	Poor	33	Slow

^a All trees are of southern Finnish origin, except male 4309 which originates from Germany.

^b Growth performance of the family was based on stem height (see Venäläinen 1993).

^c Proportional reference level is 50: values more or less than 50 indicate better or worse aboveground growth than average in Norway spruce, respectively.

Table 2. Mean diameter and length of root links, and the proportion of root tips and other links in fast- and slow-growing Norway spruce seedlings (*Picea abies*) based on raw data gathered from WinRHIZO ($n = 162$). Means with standard deviations (SD) are presented.

Variable	Fast ($n = 81$)	Slow ($n = 81$)
Mean diameter of links (mm)		
• All links	0.51 ± 0.08	0.50 ± 0.06
• Interior links ^a	0.65 ± 0.11	0.64 ± 0.08
• Root tips ^a	0.37 ± 0.05	0.35 ± 0.04
• 0 order links ^b	0.95 ± 0.21	0.90 ± 0.16
• 1 st order links	0.49 ± 0.08	0.47 ± 0.08
• 2 nd order links	0.37 ± 0.06	0.36 ± 0.06
• 3 rd to 5 th order links	0.35 ± 0.11	0.33 ± 0.12
Mean length of links (mm)		
• All links	2.95 ± 0.44	2.91 ± 0.52
• Interior links	2.93 ± 0.53	2.91 ± 0.64
• Root tips	2.98 ± 0.49	2.91 ± 0.54
• 0 order links	3.26 ± 0.73	3.32 ± 0.87
• 1 st order links	3.68 ± 0.64	3.52 ± 0.73
• 2 nd order links	2.15 ± 0.54	2.09 ± 0.46
• 3 rd to 5 th order links	1.02 ± 0.41	1.05 ± 0.43
Proportion of links (%) ^c		
• Interior links	51.40 ± 0.01	51.39 ± 0.01
• Root tips	48.60 ± 0.01	48.61 ± 0.01
• 0 order links	16.52 ± 5.80	17.77 ± 5.85
• 1 st order links	43.90 ± 3.84	44.48 ± 4.02
• 2 nd order links	33.04 ± 5.75	31.93 ± 5.59
• 3 rd to 5 th order links	6.55 ± 4.25	5.82 ± 4.42

^a Fitter and Stickland (1991) used the terms exterior links for root tips and interior links for other links than root tips

^b Note that the developmental order-based classification was used. Zero order links form the main branch of a sample root, first order links form branches originating from zero order links, second order links form branches originating from first order links etc. (see Fig. 1b). 3rd to 5th order links occurred in 100% and 94% of the roots of fast- and slow-growing seedlings, respectively

^c The proportion of interior or root tip links or a particular root order to the total number of links

25 **Table 3.** Differences in some variables describing growth between the fast- and slow-
 26 growing Norway spruce seedlings (*Picea abies*). Predicted values are based on linear mixed
 27 models (LMM) ($n = 54$). Means with standard errors (SE) are presented.

Variable	Fast ($n = 27$)	Slow ($n = 27$)	p
Shoot height (mm)	156.7 ± 12.6	145.6 ± 10.5	0.54
Specific root length (SRL, m g^{-1})	68.5 ± 3.5	70.8 ± 3.5	0.66
Specific root area, (SRA, $\text{m}^2 \text{kg}^{-1}$)	112.5 ± 5.7	111.4 ± 5.7	0.90
Root tissue density, (RTD, kg m^{-3})	61.0 ± 4.9	57.6 ± 4.9	0.66
Total biomass of shoots (g) ^a	0.903 ± 0.105	0.817 ± 0.105	0.59
Total biomass of roots (g) ^a	0.245 ± 0.025	0.229 ± 0.025	0.68
Root:shoot biomass ratio ^a	0.29 ± 0.02	0.28 ± 0.02	0.78
Total biomass of the three sample roots per seedling (g)	0.052 ± 0.004	0.040 ± 0.004	0.09

28 ^a Number of seedlings both in the fast- and slow-growing group was nine.

Table 4. Linear or generalized linear mixed models (LMMs and GLMMs, respectively) results relating to root characteristics of the sample roots of fast- and slow-growing Norway spruce (*Picea abies*) seedlings ($n = 162$). LMMs were used for the analysis of total length and the surface area of links, and GLMMs for the number of links. Statistically significant differences ($p < 0.05$) between fast- and slow-growing seedlings are in bold. Coefficients with standard errors (SE) are shown. See Fig. 3.

Variable	Intercept		Difference between the phenotypes (slow vs. fast)	
	Coeff. \pm SE	p	Coeff. \pm SE	p
Number of links				
• All links	5.652 \pm 0.075	< 0.01	0.251 \pm 0.106	0.02
• All branches ^a	5.469 \pm 0.082	< 0.01	0.273 \pm 0.116	0.02
• Interior links ^b	4.988 \pm 0.077	< 0.01	0.251 \pm 0.108	0.02
• Root tips ^b	4.931 \pm 0.074	< 0.01	0.251 \pm 0.105	0.02
Total length of links (mm)				
• All links	865.620 \pm 76.147	< 0.01	255.363 \pm 107.688	0.08
• All branches ^a	713.075 \pm 72.847	< 0.01	239.498 \pm 103.021	0.08
• Interior links ^b	444.905 \pm 42.182	< 0.01	126.845 \pm 59.655	0.10
• Root tips ^b	420.715 \pm 36.266	< 0.01	128.518 \pm 51.287	0.07
Surface area of links (mm ²)				
• All links	1409.306 \pm 126.738	< 0.01	444.250 \pm 179.235	0.07
• All branches ^a	990.104 \pm 101.524	< 0.01	369.317 \pm 143.577	0.06
• Interior links ^b	883.485 \pm 83.222	< 0.01	243.814 \pm 117.693	0.11
• Root tips ^b	525.821 \pm 47.255	< 0.01	200.436 \pm 66.829	0.04

^a Side branches and root tips (see Fig. 1b).

^b Fitter and Stickland (1991) used the terms exterior links for root tips and interior links for other links than root tips.

Table 5. Generalized linear mixed model (GLMMs) results relating to the number of links of the sample roots of fast- and slow-growing Norway spruce (*Picea abies*) seedlings in different root orders ($n = 162$). Statistically significant differences ($p < 0.05$) between fast- and slow-growing seedlings are in bold. Coefficients with standard errors (SE) are shown. See Fig. 4.

Variable	Intercept		Difference between the phenotypes (slow vs. fast)	
	Coeff. \pm SE	p	Coeff. \pm SE	p
Total number of links ^a				
• 0 order links	3.788 \pm 0.052	< 0.01	0.128 \pm 0.073	0.08
• 1 st order links	4.827 \pm 0.068	< 0.01	0.231 \pm 0.096	0.02
• 2 nd order links	4.538 \pm 0.091	< 0.01	0.301 \pm 0.128	0.02
• 3 rd to 5 th order links	2.790 \pm 0.191	< 0.01	0.394 \pm 0.269	0.14
Number of interior links ^b				
• 0 order links	3.764 \pm 0.053	< 0.01	0.132 \pm 0.075	0.08
• 1 st order links	4.383 \pm 0.084	< 0.01	0.265 \pm 0.119	0.03
• 2 nd order links	2.793 \pm 0.162	< 0.01	0.336 \pm 0.228	0.14
• 3 rd to 4 th order links	0.443 \pm 0.250	0.08	0.316 \pm 0.345	0.36
Number of root tips ^b				
• 0 order links	1		1	-
• 1 st order links	3.778 \pm 0.054	< 0.01	0.131 \pm 0.076	0.08
• 2 nd order links	4.328 \pm 0.082	< 0.01	0.285 \pm 0.115	0.01
• 3 rd to 5 th order links	2.691 \pm 0.186	< 0.01	0.397 \pm 0.263	0.13

^a Note that the developmental order-based classification was used. Zero order links form the main branch of a sample root, first order links form branches originating from zero order links, second order links form branches originating from first order links etc. (see Fig. 1b).

^b Fitter and Stickland (1991) used the terms exterior links for root tips and interior links for other links than root tips.

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Figures

Figure 1

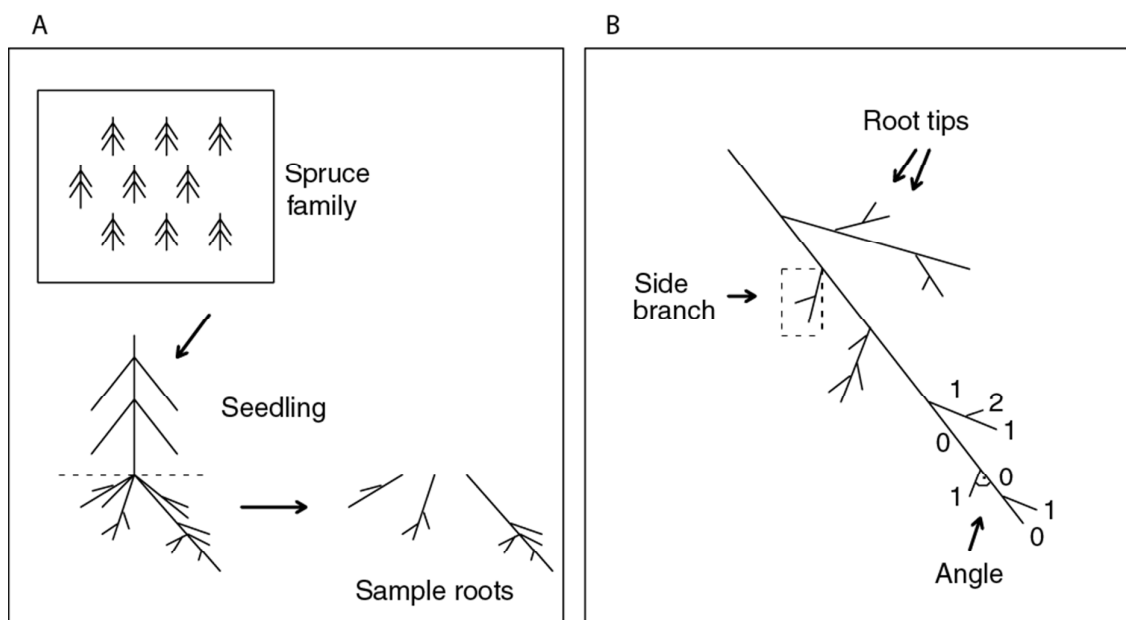


Figure 1. Sampling design: (A) Altogether 54 seedlings from six spruce families (i.e., nine seedlings per family), and their sample roots, three per seedling, were investigated. (B) Schematic representation of a sample root with explanations for some root variables. A link is a part of a root between two branches or the most distant part of a branch (i.e., root tip). All side branches include more than one root link whereas a root tip includes one link only. Angle is determined as degrees between two links starting from the same point. The order of branches (and links within a branch) is indicated with a number as presented in the figure. The zero order root links form the main root branch of a sample root, first order links form a first order branch originating from the zero order root branch, second order links form a second order branch originating from first order branch links etc. This developmental order-based classification was utilized in this study.

Figure 2

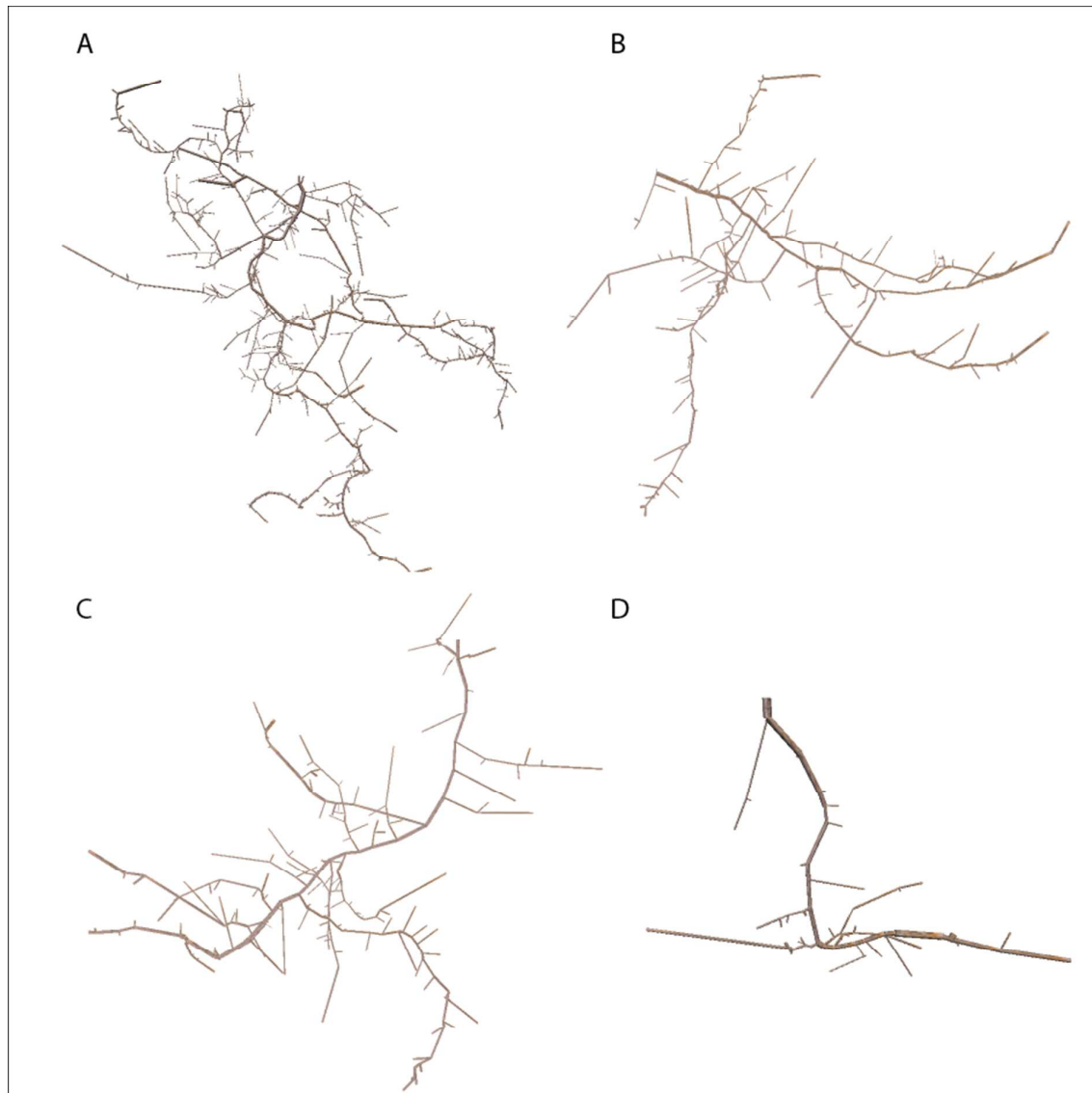
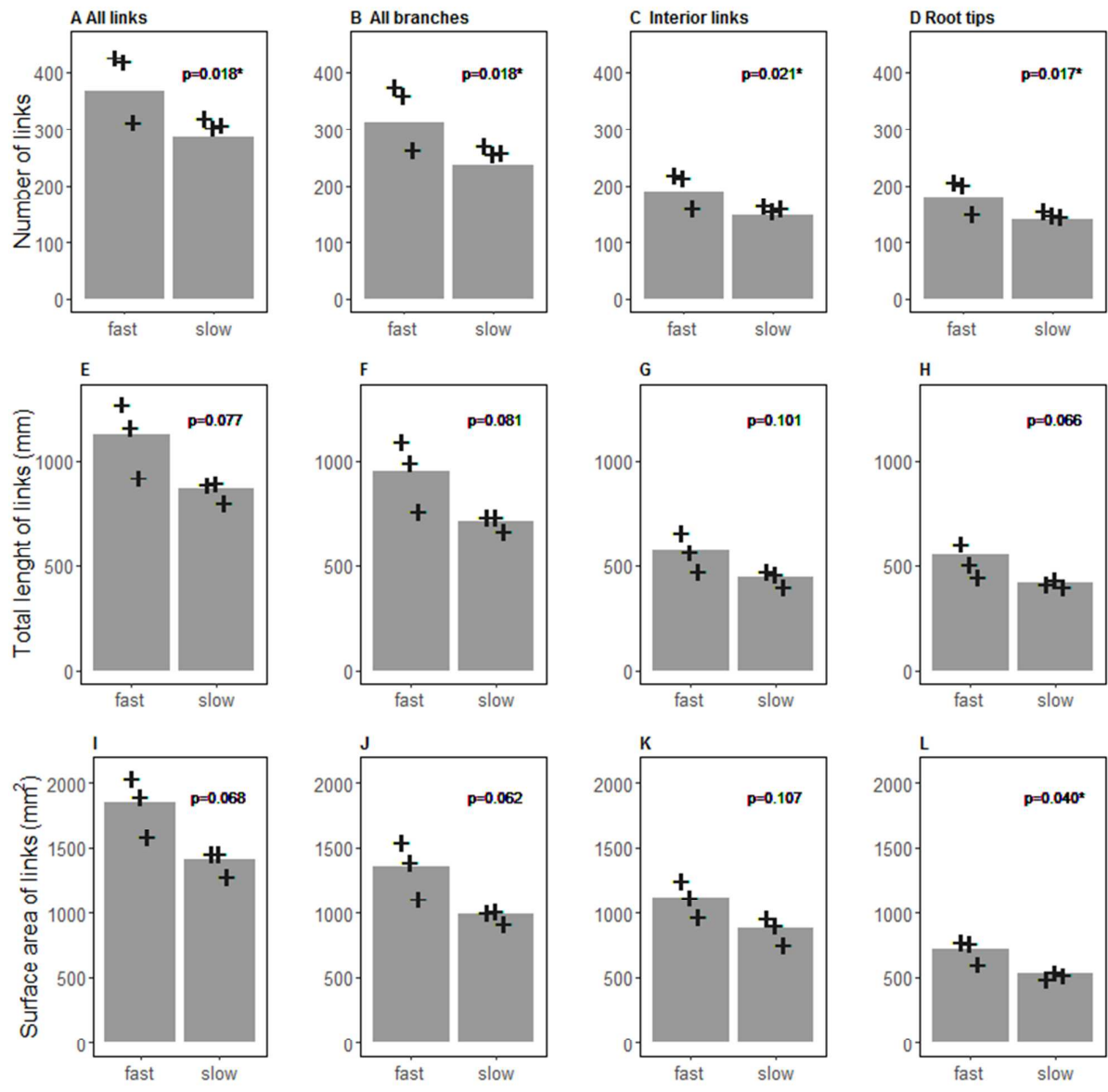


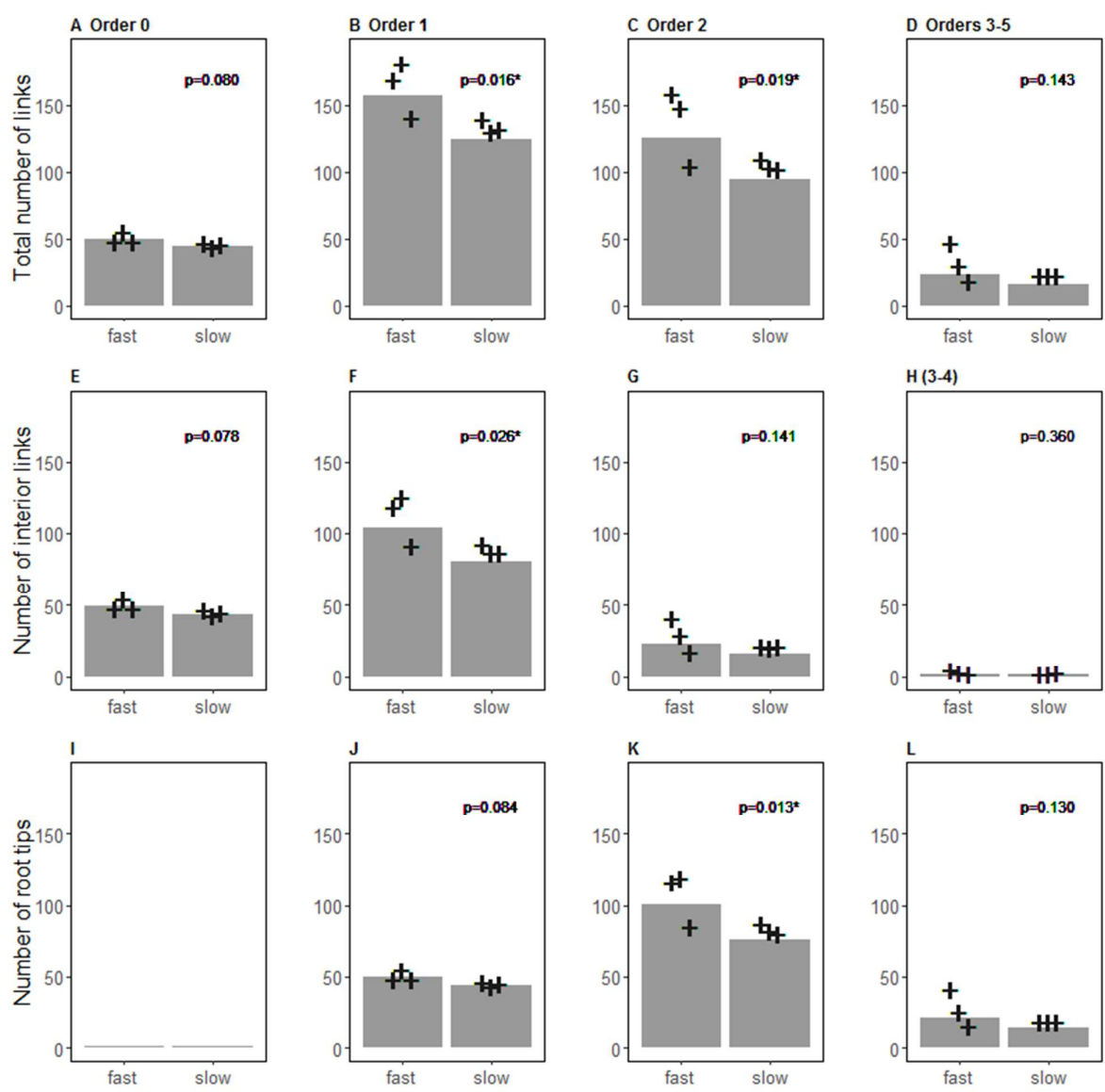
Figure 2. Schematic model of four sample root branches of Norway spruce (*Picea abies*) seedlings representing: (A) a sample root with the highest number of links for fast-growing seedlings, (B) a root containing a median number of root links for fast-growing seedlings, (C) a root containing a median number of root links for slow-growing seedlings, and (D) a sample root with the lowest number of links for slow-growing seedlings. The lengths, widths and branching angles of the root links were analyzed with WinRHIZO™, and visual rendering was done using the LIGNUM program (Sievänen et al. 2010).

Figure 3



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3 **Figure 3.** Number, total length and surface area of links among the sample roots of fast- and slow-growing Norway spruce (*Picea abies*)
4 seedlings based the predictions of linear or generalized linear mixed models ($n = 162$). Values for the whole sample root, side branches, interior
5 links, i.e., other links than root tips, and root tips, i.e., exterior links are presented. Standard error values are not presented as these are not
6 available for all model types. Statistically significant differences ($p < 0.05$) are indicated with an asterisk, and mean raw data values for spruce
7 families are shown with a cross. See Table 4.
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Figure 4



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3 **Figure 4.** Total number of links, the number of interior links (i.e., other than root tips), and root tips between the sample roots of fast- and slow-
4 growing seedlings of Norway spruce (*Picea abies*) based on the predicted values of generalized linear mixed models (GLMMs, $n = 162$). Values
5 for different root orders are presented (note that the developmental order-based classification was used, see Fig. 1). Note that the numbers are
6 extremely low in panels H and I. Standard error values are not presented as these are not available for this model type. Statistically significant
7 differences ($p < 0.05$) between the fast- and slow-growing seedlings are indicated with an asterisk, and mean raw data values for spruce families
8 are shown with a cross. See Table 5.
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Figure 5

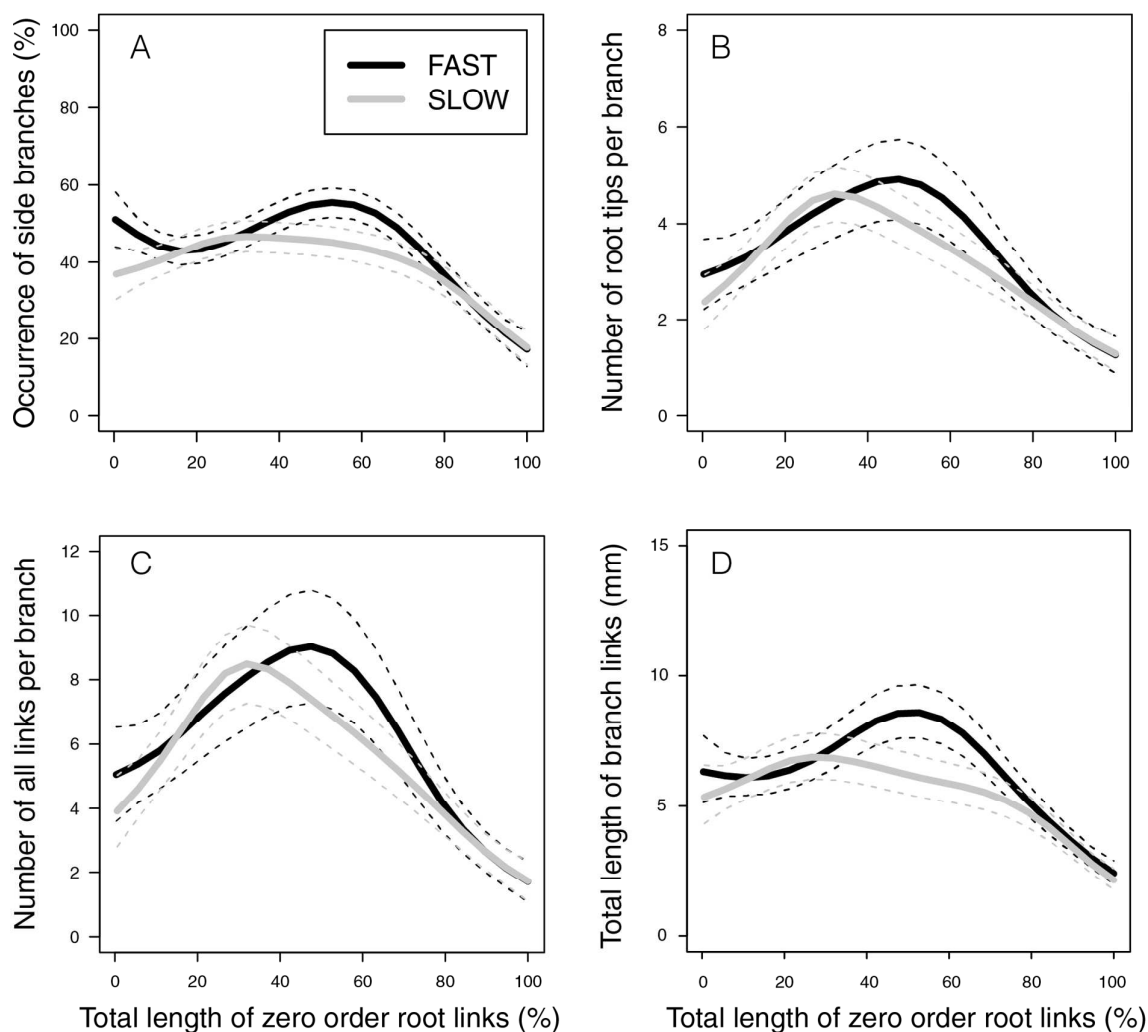


Figure 5 (A) The probability of occurrence of side branches, (B) the number of root tips per branch (composed of one or more links), (C) the number of all links per branch, and (D) the total length of branch links along the total length of sample root links of zero order expressed as a proportion (0-100%) for fast- and slow-growing Norway spruce seedlings. All responses are statistically significantly curvilinear (GAMMs, $p < 0.01$) and 95% confidence intervals are presented with dashed lines.

Figure 6

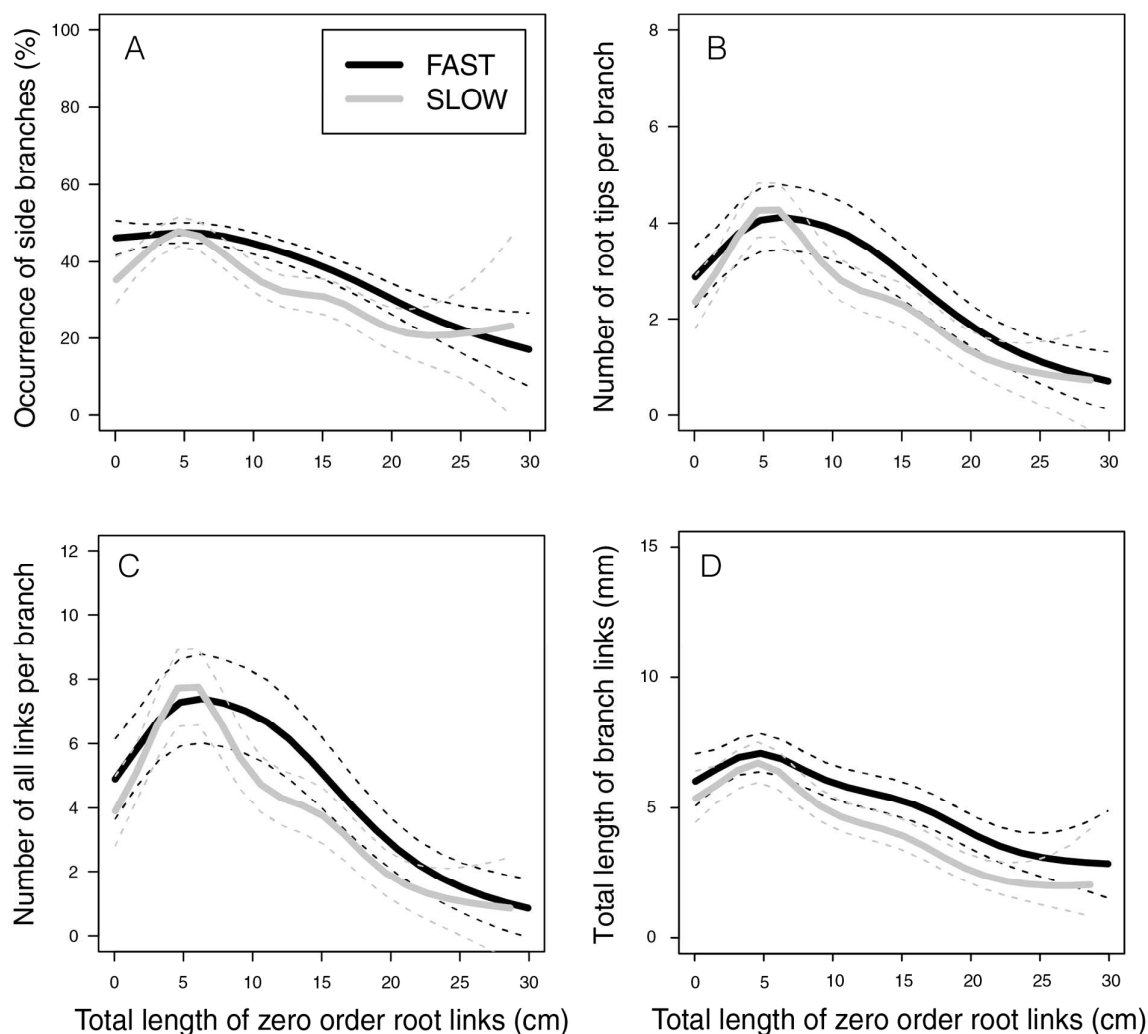


Figure 6 (A) The probability of occurrence of side branches, (B) the number of root tips per branch, (C) the number of all links per branch (composed of one or more links), and (D) the total length of branch links along the total length of sample root links of zero order expressed in cm for fast- and slow-growing Norway spruce seedlings. All responses are statistically significantly curvilinear (GAMMs, $p < 0.01$) and 95% confidence intervals are presented as dash line response curves.

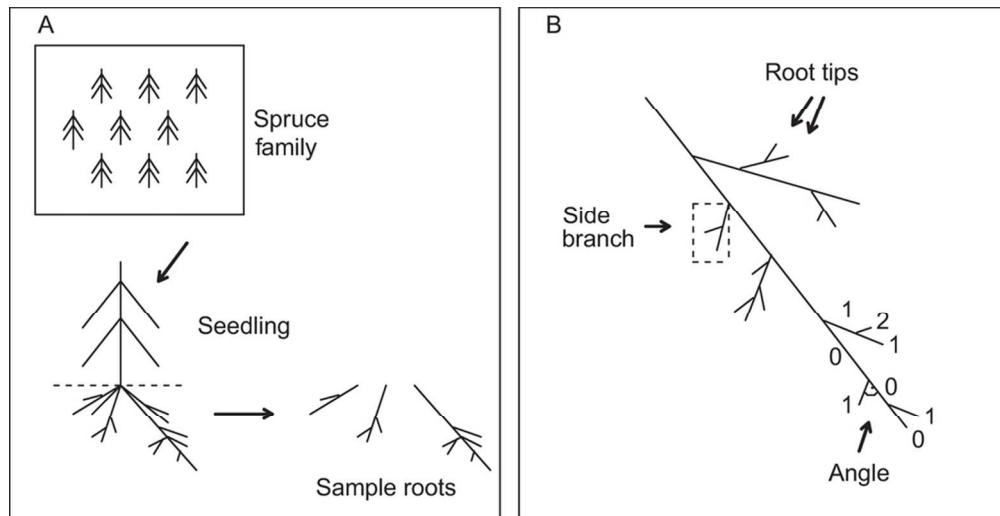


Figure 1. Sampling design: (A) Altogether 54 seedlings from six spruce families (i.e., nine seedlings per family), and their sample roots, three per seedling, were investigated. (B) Schematic representation of a sample root with explanations for some root variables. A link is a part of a root between two branches or the most distant part of a branch (i.e., root tip). All side branches include more than one root link whereas a root tip includes one link only. Angle is determined as degrees between two links starting from the same point. The order of branches (and links within a branch) is indicated with a number as presented in the figure. The zero order root links form the main root branch of a sample root, first order links form a first order branch originating from the zero order root branch, second order links form a second order branch originating from first order branch links etc. This developmental order-based classification was utilized in this study.

88x45mm (300 x 300 DPI)

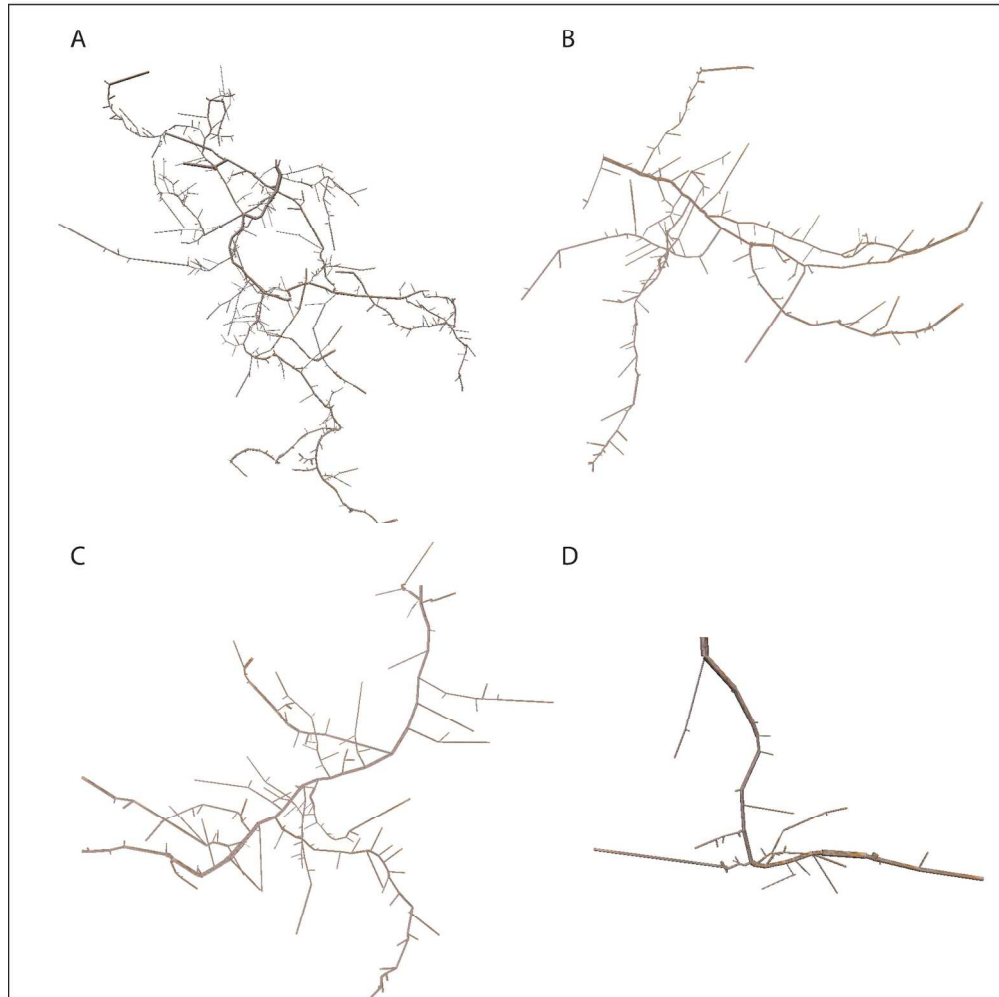


Figure 2. Schematic model of four sample root branches of Norway spruce (*Picea abies*) seedlings representing: (A) a sample root with the highest number of links for fast-growing seedlings, (B) a root containing a median number of root links for fast-growing seedlings, (C) a root containing a median number of root links for slow-growing seedlings, and (D) a sample root with the lowest number of links for slow-growing seedlings. The lengths, widths and branching angles of the root links were analyzed with WinRHIZO™, and visual rendering was done using the LIGNUM program (Sievänen et al. 2010).

172x186mm (300 x 300 DPI)

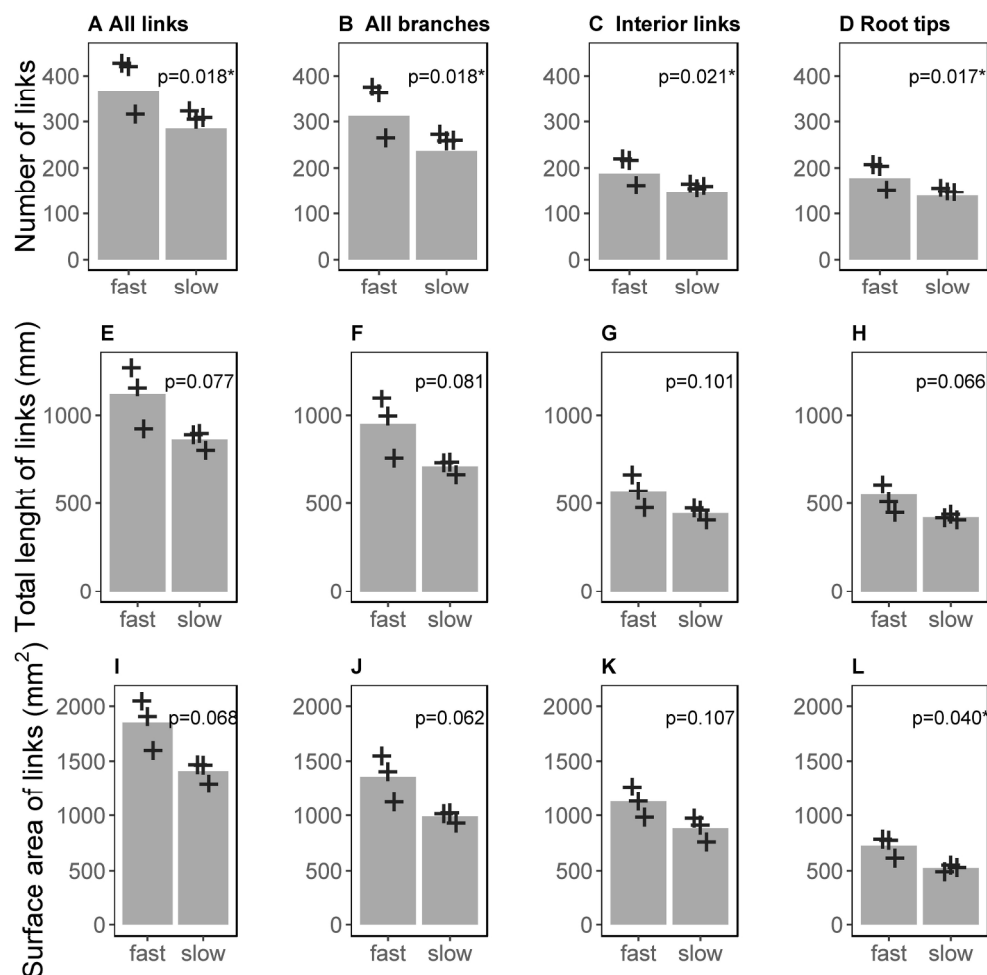


Figure 3. Number, total length and surface area of links among the sample roots of fast- and slow-growing Norway spruce (*Picea abies*) seedlings based the predictions of linear or generalized linear mixed models ($n = 162$). Values for the whole sample root, side branches, interior links, i.e., other links than root tips, and root tips, i.e., exterior links are presented. Standard error values are not presented as these are not available for all model types. Statistically significant differences ($p < 0.05$) are indicated with an asterisk, and mean raw data values for spruce families are shown with a cross. See Table 4.

203x203mm (300 x 300 DPI)

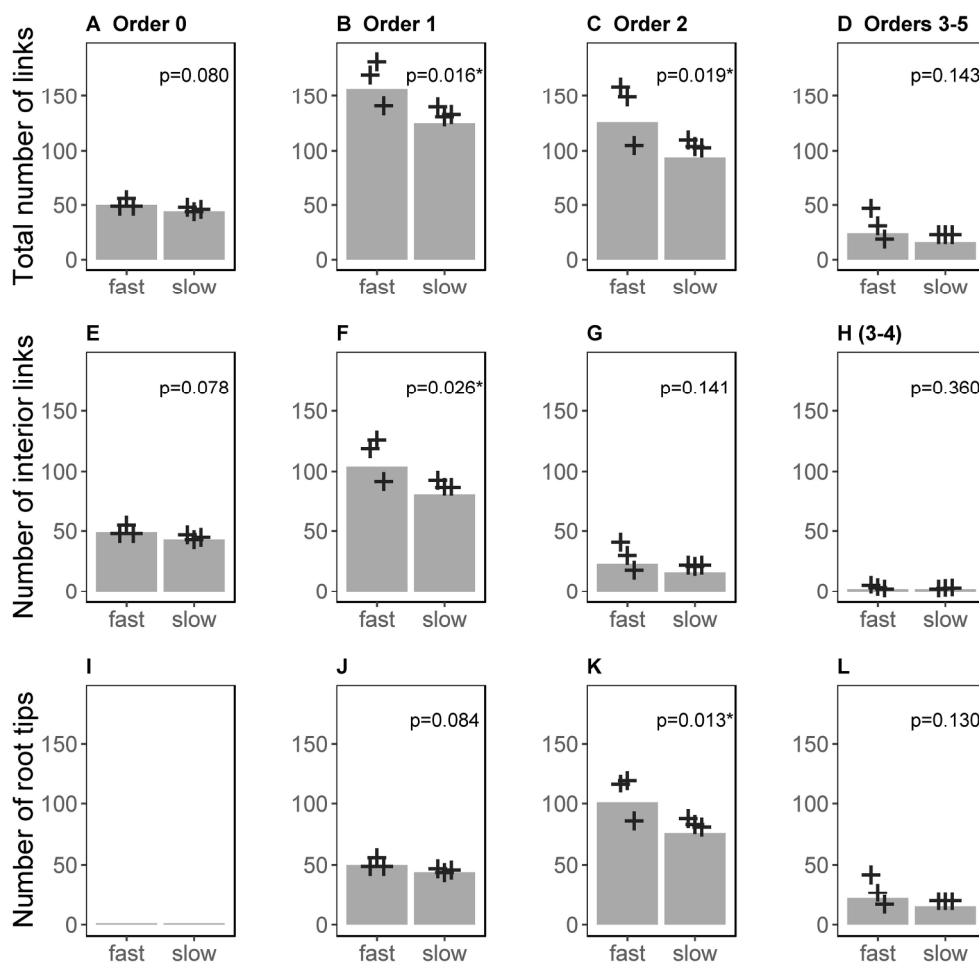


Figure 4. Total number of links, the number of interior links (i.e., other than root tips), and root tips between the sample roots of fast- and slow-growing seedlings of Norway spruce (*Picea abies*) based on the predicted values of generalized linear mixed models (GLMMs, $n = 162$). Values for different root orders are presented (note that the developmental order-based classification was used, see Fig. 1). Note that the numbers are extremely low in panels H and I. Standard error values are not presented as these are not available for this model type. Statistically significant differences ($p < 0.05$) between the fast- and slow-growing seedlings are indicated with an asterisk, and mean raw data values for spruce families are shown with a cross. See Table 5.

203x203mm (300 x 300 DPI)

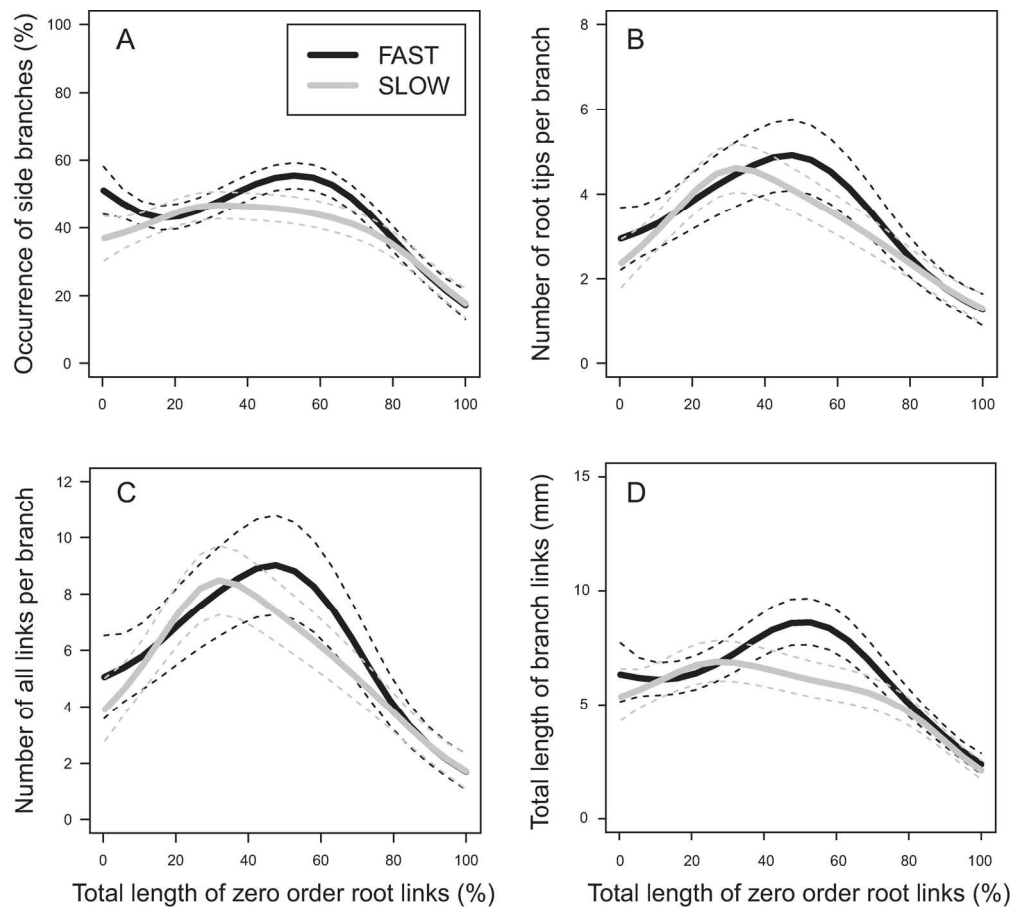


Figure 5 (A) The probability of occurrence of side branches, (B) the number of root tips per branch (composed of one or more links), (C) the number of all links per branch, and (D) the total length of branch links along the total length of sample root links of zero order expressed as a proportion (0-100%) for fast- and slow-growing Norway spruce seedlings. All responses are statistically significantly curvilinear (GAMMs, $p < 0.01$) and 95% confidence intervals are presented with dashed lines.

157x141mm (300 x 300 DPI)

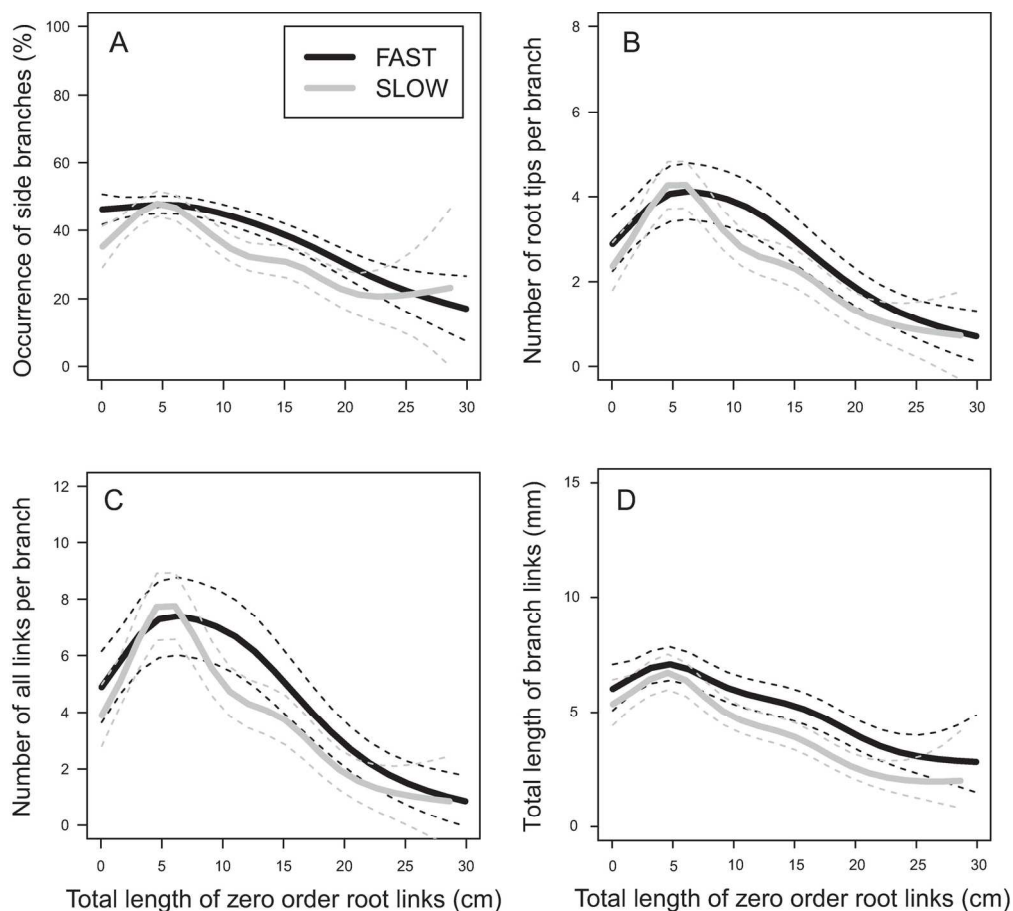


Figure 6 (A) The probability of occurrence of side branches, (B) the number of root tips per branch, (C) the number of all links per branch (composed of one or more links), and (D) the total length of branch links along the total length of sample root links of zero order expressed in cm for fast- and slow-growing Norway spruce seedlings. All responses are statistically significantly curvilinear (GAMMs, $p < 0.01$) and 95% confidence intervals are presented as dash line response curves.

157x142mm (300 x 300 DPI)