

1 **Variation in fine root biomass along a 1000 km long latitudinal climatic gradient in**
2 **mixed boreal forests**

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12

13 **Abstract**

14

15 **Aim:** Geographical patterns in distribution of fine root biomass (FRB) and in fine root
16 contribution to below-ground nitrogen and carbon stocks remain poorly known. Here, we
17 examine FRB, together with the ecological factors governing it, along a 1000 km long
18 latitudinal gradient in unmanaged mixed boreal forests. Specifically, we investigate whether
19 among-site variation in FRB could be explained by climatic, plant community and soil
20 characteristics.

21 **Location:** Northern Europe.

22 **Major taxa studied:** Vascular plants.

23 **Methods:** We sampled fine roots (diameter ≤ 2 mm; trees and field layer vegetation
24 combined) twice per growing seasons in 2015 and 2016 from ten sites spaced one degree of
25 latitude between 60° N (near Saint Petersburg) and 69° N (near Murmansk, Russia). We dried

26 and weighed alive fine roots and measured carbon and nitrogen concentrations in their
27 tissues. The obtained values were correlated with climatic, plant community and soil
28 characteristics.

29 **Results:** Total FRB varied significantly among study sites, but this variation was not
30 explained by either latitude or by climatic, plant community or soil characteristics. However,
31 fine root density increased towards the North in the organic layer. The fine root carbon and
32 nitrogen concentrations did not show latitudinal variation, but carbon concentration increased
33 with soil moisture.

34 **Main conclusions:** Total FRB and fine root contribution to below-ground nitrogen and
35 carbon stocks in mixed boreal forests did not exhibit linear latitudinal pattern and could not
36 be accurately predicted from climatic, plant community and soil characteristics. The fine root
37 system became more superficial towards the North, suggesting the poleward increase in
38 vulnerability of boreal forests to abiotic risks (forest fires, drought). Our results suggests that
39 future climate may shift FRB and also carbon and nitrogen cycling from organic layer to
40 mineral soil and increase the volume of soil utilized by fine roots for water and nutrient
41 uptake.

42

43 **Keywords**

44 Below-ground ecology, fine root nitrogen and carbon concentrations, plant community
45 characteristics, podzol soils, thickness of soil organic layer, weather conditions

46

47 **Introduction**

48

49 Multiple studies exploring global patterns in terrestrial ecosystems are limited to above-
50 ground subsystems, because empirical data from the below-ground subsystems are scarce and

51 often insufficient for confident generalisation (but see Fierer, Strickland, Liptzin, Bradford &
52 Cleveland, 2009; Peng, Guo & Yang, 2017). Nevertheless, exploration of below-ground
53 processes driving in particular carbon (C) and nutrient dynamics is vital for understanding
54 ecosystem responses to environmental changes (Finér, Mannerkoski, Piirainen & Starr, 2003;
55 Helmisaari, Derome, Nöjd & Kukkola, 2007).

56 The importance of plant roots for functioning of terrestrial ecosystems is difficult to
57 overestimate. They not only exploit soil resources, particularly water and macronutrients such
58 as nitrogen (N) and phosphorus (P), and transport them to above-ground plant parts, but serve
59 the basis for multiple below-ground food webs, which are associated with alive and decaying
60 roots. Due to the poor dispersal capacity and long survival of many soil organisms, legacy
61 effect of plant roots on the soil has substantial effects on ecosystem structure and functions
62 (van der Putten *et al.*, 2009). This gives paramount importance to the understanding of
63 patterns in distribution of root biomass at different spatial and temporal scales and to the
64 identification of factors that drive these patterns.

65 On average, 22–32% of the annual net primary production in forest ecosystems is
66 allocated to fine roots (i.e., roots less than 2 mm in diameter) and transferred to the soil by
67 root turnover (Jackson *et al.*, 1997; Yan & Chen, 2010; McCormack *et al.*, 2015). Moreover,
68 fine root litter decomposes and releases C and N slower than the above-ground leaf litter
69 (Palviainen *et al.*, 2004; Eldhuset, Kjønaas & Lange, 2017), and up to 70% of the soil C
70 stock in boreal forests can be derived from roots and root associated micro-organisms
71 (Clemmensen *et al.*, 2013). Therefore, quantification of fine root biomass (FRB hereafter)
72 and identification of the climatic and other factors which are related to fine root C and N
73 content are critical for estimating the role of fine roots as sources of C and N and for
74 modelling C and nutrient cycling in current and future climates.

75 The effects of environmental changes on different organisms are often predicted from
76 comparisons between ecosystems that exist at different latitudes, and geographical gradients
77 have been recently promoted as natural laboratories for studying the potential impacts of
78 changing climate on terrestrial organisms (De Frenne *et al.*, 2013). In contrast to above-
79 ground ecology, this approach is still infrequent in below-ground ecology, and only a handful
80 of studies (e.g., Helmisaari *et al.*, 2007; Lehtonen *et al.*, 2016, Finér *et al.*, 2017) explored
81 latitudinal changes in FRB. Intriguingly, some of geographical patterns identified by the
82 analysis of data extracted from multiple publications (Yan & Chen, 2010), like the poleward
83 decrease in FRB, contradict the results of the case study conducted in boreal forests
84 (Helmisaari *et al.*, 2007). Even more importantly, shortage of empirical data hampers the
85 understanding of the relative importance of various drivers contributing to formation of
86 geographical patterns in FRB.

87 Positive correlations have been observed between tree FRB and basal area in boreal
88 single-tree species stands (Finér *et al.*, 2007; Helmisaari *et al.*, 2007; Lehtonen *et al.*, 2016),
89 although the allocation of tree biomass to fine roots was reported to increase towards higher
90 latitudes (Helmisaari *et al.*, 2007; Reich *et al.*, 2014; Lehtonen *et al.*, 2016). At the same
91 time, 1% to 43% of the total FRB in boreal forests belongs to field layer vegetation, and this
92 percentage increases with increasing latitude (Finér *et al.*, 2003; Helmisaari *et al.*, 2007).
93 Furthermore, plant species diversity may have positive effects of on below-ground
94 productivity (Ma & Chen, 2016). Thus, from the ecosystem perspective, it is important to
95 measure FRB simultaneously for both top-canopy and field layer plants, and consider both
96 biomass and diversity of above-ground vegetation as explanatory variables in the analysis of
97 FRB.

98 Most of fine roots in boreal forests, which are generally N-limited, grow in the organic
99 layer (Helmisaari *et al.*, 2007; Finér *et al.*, 2017), where the soil N pools are the highest

100 (Dawud *et al.*, 2017). Experimental studies showed that the addition of N significantly
101 decreased root production (Peng *et al.*, 2016), indicating the importance of soil N
102 concentrations in explaining variation in FRB.

103 Last but not least, both between- and within-year variation in FRB is highly related to
104 the variation in air temperature and soil moisture (Makkonen & Helmisaari, 1998; Chen *et*
105 *al.*, 2017). Similarly, C:N ratio of fine roots increased with decreasing mean annual
106 temperature in European forests (Zadworny *et al.*, 2017). Thus, not only climate
107 characteristics at study sites, but also actual weather conditions preceding root sampling
108 should be taken into account as possible drivers of FRB.

109 Here we (i) report spatial and temporal variation in the total (including trees and field
110 layer vegetation) biomass of alive fine roots and rhizomes (diameter ≤ 2 mm) and the
111 concentrations of C and N in these below-ground plant parts in pristine mixed boreal forests
112 located along a latitudinal gradient and (ii) explore factors that could explain this variation.
113 Our study included the following explanatory variables: latitude and climate (long-term
114 averages of air temperature and precipitation) of study sites; weather characteristics (air
115 temperature and precipitation) of the summer seasons 2015 and 2016; plant community
116 characteristics (stand basal area, cover of field layer vegetation, diversity of tree and field
117 layer vegetation); and soil structural and chemical properties (moisture, granulometric
118 composition, thickness of organic layer, N, P and potassium (K) concentrations, pH). We
119 hypothesized that 1) FRB increases with an increase in latitude; 2) the distribution of FRB in
120 soil becomes more superficial towards the higher latitudes; 3) fine root N concentrations
121 decrease towards the North; 4) FRB is tightly related to the plant community characteristics;
122 and 5) between- and within-year variations in FRB are related to variation in weather
123 conditions. We tested our hypothesis by measuring FRB and all explanatory variables in ten
124 unmanaged stands along a 1000 km long latitudinal gradient in Russia.

125

126 **Material and methods**

127

128 *Study sites*

129

130 The data were collected from ten mature pristine forests located from 60°N near St.
131 Petersburg to 69°N close to Murmansk in NW Russia (Fig. 1). These sites were selected in
132 2008 for studies of insect herbivory as being closest to the round degrees of latitude along the
133 road linking mentioned cities, and therefore they were not biased in respect of soil or forest
134 characteristics. All sites had similar forest type; the tree species pool (Table S1) consisted, in
135 the decreasing order of importance, of Scots pine (*Pinus sylvestris*), downy birch (*Betula*
136 *pubescens*), Norway spruce (*Picea abies*), European aspen (*Populus tremula*) and goat
137 willow (*Salix caprea*). The field layer vegetation was dominated by dwarf shrubs, except in
138 the northernmost site, where the herb *Cornus suecica* was dominant (Table S2).

139 At each site, all samples were collected within the plot of 100 × 100 m size. The basal
140 area of the tree stands was measured by relascope at five randomly selected points, and the
141 cover of field layer vegetation was evaluated visually in 10 randomly selected plots 1 × 1m
142 size. The species diversity was quantified by the Shannon index from the measurements of
143 tree stand basal area and field layer vegetation cover. The long-term mean annual air
144 temperature and precipitation (Table S3) were estimated using New_LocClim (FAO, 2006).
145 The average air temperature and the amount of precipitation in May-August of 2015 and of
146 2016 (when FRB was measured; Table S3) were obtained from the records of the
147 meteorological stations located at 5–29 km distances from our sites (www.rp5.ru).

148

149 *Soil characteristics*

150

151 Soil was sampled 20–22 August 2014. In each study plot, we selected three representative
152 locations some 20–40 m apart, and in each location excavated a pit of 30 × 50 cm in area and
153 50–60 cm in depth. One side of this pit was aligned to measure minimum, maximum and
154 average depth of each of the three soil horizons (organic layer, eluvial E-horizon and illuvial
155 B-horizon) and to collect samples for measurements of soil characteristics. These samples
156 were collected from the middle of each soil horizon, one sample per pit, yielding a total of 90
157 samples (10 sites × 3 pits × 3 horizons).

158 Soil type was identified on-site according to FAO (2015). Soil moisture was calculated
159 as the relative difference between its wet weight and weight after drying to constant mass at
160 +105°C. Soil particle size distribution was measured using vibratory sieve shaker AS 200
161 Control (Retsch GmbH, Haan, Germany); in the analysis, we used the percentage of the
162 fraction with <2 mm grain size. Samples for other measurements were air-dried and sieved (2
163 mm). pH was measured in soil water extract (1:25 for organic layer; 1:2.5 for mineral layers)
164 using potentiometric pH-meter 150MH (Izmeritel'naya Tekhnika Ltd., Moscow, Russia). For
165 chemical analyses, soil samples were extracted by 1M ammonium acetate (1:25 for organic
166 layer; 1:10 for mineral layers; pH=4.65). The concentration of bioavailable P was measured
167 after Watanabe and Olsen (1965) using photometer KФK-3 (Zagorsk Optical and Mechanical
168 Plant, Zagorsk, Russia). The concentration of bioavailable K was measured by atomic
169 absorption spectrometry (AAS 360, Perkin Elmer, Waltham, USA). The total N was
170 measured according to the Kjeldahl method.

171

172 *Fine root sampling and chemical analyses*

173

174 The samples for measurement of FRB were collected four times, during the last weeks of
175 June and August of both 2015 and 2016, by using a cylindrical metal corer with an inner
176 diameter of 36 mm. We collected 10 samples per site in June of 2015, and 5 samples per site
177 in all other dates. We measured thickness of organic layer and mineral soil horizons and
178 divided each sample to the organic and 0-10 cm and 10-20 cm mineral soil layers; the deepest
179 sampled layer was often incomplete (i.e., its thickness was <10 cm). All alive fine roots
180 including mycorrhizas and rhizomes were separated by hand from the samples in laboratory
181 conditions and washed with water to remove the adhered soil. The separation of alive and
182 dead roots was based on root branching patterns, periderm colour, surface structure, root
183 elasticity and toughness.

184 The root samples collected in June 2015 were dried at +40°C until a constant weight for
185 element analyses, and a subsample was taken for dry mass assessment at +105 °C. The
186 samples collected in other dates were dried for 48 hours at +105 °C. Dry samples were
187 weighted to the nearest 0.1 mg. Subsequently, the fine roots sampled in June 2015 were
188 ground (IKA A10; IKA, Staufen, Germany), and C and N concentrations in these samples
189 were measured with LECO CHN-1000 Analyser (LECO Corporation, St. Joseph, MI).

190

191 *Intermediate calculations*

192

193 We analysed two characteristics of fine root distribution in the soil: FRB per unit of soil
194 volume (mg cm^{-3} ; density hereafter) and per unit of surface area (g m^{-2} ; biomass hereafter).
195 For calculation of density, we divided root weight by thickness of respective soil layer in a
196 sample (in cm) and then by 10.17 (i.e., the number of cm^3 in 1-cm layer of soil core of 3.6 cm
197 diameter). For calculation of biomass, we first disregarded the samples that did not include
198 the deepest (10-20 cm) mineral soil layer. In some of the remaining samples, this layer was

199 collected incompletely; in these cases, we divided root weight by the thickness of the
200 sampled part of this layer (in cm) and multiplied the obtained value by 10. The use of these
201 two variables, biomass and density, allowed us to uncover the changes in FRB, which
202 depended both on the thickness of soil organic layer (that varied from 1 to 16 cm in our
203 samples), and on root density in this layer.

204 In the calculation of site-specific values of FRB we took into account the stone content
205 of the mineral soil (Table S1). This variable was obtained by averaging a visually estimated
206 volume of stones (>50 mm in diameter) in soil samples of 25 × 25 × 30 (depth) cm, which
207 were collected for investigation soil invertebrates simultaneously with soil cores (25 samples
208 per study site in 2015–2016).

209

210 *Statistical analysis*

211

212 Our key research question concerned the latitudinal changes in FRB, and therefore the
213 latitude of study site was included as a covariate in the linear mixed model ANCOVAs,
214 which explored the sources of variation in soil characteristics, FRB, and fine root C and N
215 concentrations. In these models, latitude, layer (or soil horizon), year, and month nested
216 within the year (where appropriate) were treated as fixed factors, whereas study site and
217 individual sample (=core) were treated as random factors. The simultaneous involvement of
218 both site and latitude in our analysis is justified by the fact that our sites differ not only in
219 latitude, but also in a number of other characteristics (plant community structure, in
220 particular). Fine root density, FRB, and fine root C and N concentrations were square-root
221 transformed before analyses to normalize the data. All analyses were carried out with the
222 SAS GLIMMIX procedure. We facilitated the accurate *F*-tests of the fixed effects by
223 adjusting the standard errors and denominator degrees of freedom by the latest version of the

224 method described by Kenward and Roger (2009). The significance of the random factors in
225 these analyses was evaluated by calculating the likelihood ratio and testing it against chi-
226 squared distribution (as described in Littell, Milliken, Stroup, Wolfinger & Schabenberger,
227 2006). The relationships between soil characteristics (square root or log-transformed) and
228 latitude, as well as between fine root densities in different soil layers, were explored by
229 calculating Pearson correlation coefficients.

230 The variables used to explain among-site variation in FRB and C and N concentrations
231 in fine roots (15 in total; Table S4) often correlated to each other, i.e. they were not truly
232 independent. Therefore we used principal component analysis (PCA) to combine explanatory
233 variables within each of three groups (weather, plant community and soil) and then calculated
234 the Pearson correlation coefficients between the site-specific values of the response variables
235 and the first PCA axis from each of three PCA analyses. When these correlations appeared
236 significant, we explored correlations of our response variables with individual explanatory
237 variables to uncover potential drivers of the detected patterns.

238

239 **Results**

240

241 *Soil characteristics*

242

243 The soils in all our sites were gleyic albic podzols and folic albic podzols formed on the
244 sandy material. The thickness of the organic layer, measured from soil cores (Table S1), was
245 independent of the latitude of the study site ($r=0.01$, $n=10$, $P=0.72$), whereas the volumetric
246 stone content of the mineral soil increased towards the North ($r=0.74$, $n=10$, $P=0.01$). The
247 remaining soil characteristics (Table S5) generally did not change with latitude (Table S6).
248 However, in the B-horizon only, the percentage of soil particles less than 2 mm in size

249 increased with an increase in latitude ($r=0.69$, $n=10$, $P=0.03$), and plant-available soil P
250 concentration showed the opposite pattern ($r=-0.66$, $n=10$, $P=0.03$).

251

252 *Fine root density*

253

254 Fine root density varied among the soil layers, study sites and individual samples; but, on
255 average, it showed no latitudinal changes (Table 1). However, the latitudinal pattern was
256 discovered when soil layers were analyzed separately: density of fine roots increased towards
257 the North in the organic layer ($r=0.69$, $n=10$, $P=0.03$), but did not change with latitude in the
258 0-10 and 10-20 cm mineral soil layers (Fig. 2). Fine root density was also affected by the
259 study year and sampling season (Table 1), being in 2016 significantly higher than in 2015 in
260 all soil layers (Fig. 3a). Furthermore, in the organic layer fine root density in August was
261 higher than in June, but no seasonal differences were found in the mineral layers (Fig. 3b).

262 At the site-specific level, fine root density did not correlate between the organic layer
263 and mineral soil layers (0–10 cm: $r=0.33$, $n=10$, $P=0.36$; 10–20 cm: $r=0.27$, $n=10$, $P=0.45$),
264 whereas strongly correlated between these two mineral layers ($r=0.83$, $n=10$, $P=0.003$).

265

266 *Fine root biomass*

267

268 FRB (the sum of organic layer and the upper 20 cm of mineral soil) greatly varied between
269 study sites (Table 2), but was not related to any of the fixed-effect variables, including
270 latitude, year and month of data collection, or their interaction (Table 3). Among-site
271 variation in total FRB was not explained by plant community, soil or weather characteristics
272 (Table S7). In the organic layer, FRB was explained by the soil characteristics (Table S7),
273 because it increased with the thickness of this layer ($r=0.70$, $n=10$, $P=0.02$). We also detected

274 significant decrease in FRB in the organic layer with an increase in summer temperatures
275 along our climatic gradient ($r=-0.66$, $n=10$, $P=0.04$).

276

277 *Concentrations of carbon and nitrogen in fine roots*

278

279 The concentrations of N in fine roots varied among the study sites, but not those of C (Table
280 S8 and Table 4). Neither C nor N concentrations were related to the latitude. Variation among
281 individual samples within a site was significant for N but not for C (Table 4). Consistently,
282 root C concentrations did not correlate between the organic layer and the upper mineral layer
283 ($r=0.21$, $n=10$, $P=0.56$), whereas root N concentrations demonstrated strong positive
284 correlation between these layers ($r=0.73$, $n=10$, $P=0.02$).

285 Among-site variation in fine root C concentrations was strongly associated with soil
286 characteristics but not related to either plant community characteristics or weather conditions
287 (Table S7). Among soil characteristics, fine root C concentration was most strongly
288 associated with soil moisture ($r=0.74$, $n=10$, $P=0.02$). The variation in N concentrations was
289 independent from all groups of explanatory variables (Table S7).

290

291 *Carbon and nitrogen stocks in fine roots*

292

293 Fine root N stocks varied from 4.0 to 8.4 g m⁻² (mean 6.1 g m⁻²) and C stocks from 224 to
294 547 g m⁻² (mean 388 g m⁻²) among our sites (Table 2). Nitrogen stock was explained by soil
295 characteristics (Table S7), primarily due to its positive relationship with the thickness of the
296 organic layer ($r=0.64$, $n=10$, $P=0.04$). Carbon stock did not correlate with any group of the
297 exploratory variables (Table S7).

298

299 Discussion

300

301 *Changes in fine root biomass along the latitudinal gradient*

302

303 Our results did not support our first hypothesis of the increase in total FRB along the
304 latitudinal gradient, which was based primarily on the results by Helmisaari *et al.* (2007),
305 neither they agree with the conclusions by Yan & Chen (2010) on the poleward decline in
306 FRB at the global scale. The study by Helmisaari *et al.* (2007) covered the same range of
307 latitudes in Finland, relatively close to our gradient, but focussed on single-species stands,
308 whereas we collected our data from mixed stands. The combination of species with different
309 functional traits may utilize varying environmental conditions better than single species
310 stands (Loreau *et al.*, 2001) and result in increased fine root production (Ma & Chen, 2016).
311 In our study, FRB was not related to plant community characteristics; however, it remains in
312 question whether FRB in mixed and single-species stands responds differently to
313 environmental conditions.

314 Our gradient was relatively short, and the number of study sites (ten) was clearly
315 insufficient to detect weak correlations between FRB and latitude, or reject the hypothesis on
316 the presence of minor effects with statistical confidence. However, many other biotic
317 characteristics measured from the same sites showed significant latitudinal patterns. For
318 example, both losses of birch foliage to defoliating insects (Zverev, Zvereva & Kozlov 2017)
319 and abundance of arboreal spiders (Kozlov, Stańska, Hajdamowicz, Zverev & Zvereva, 2015)
320 decreased nearly fivefold with an increase in latitude from 60° to 69°N. Furthermore, from
321 the study by Helmisaari *et al.* (2007) we expected to find at least 1.5-fold differences in FRB
322 between our southern and northern sites; however, these expectations were not met.

323 We sampled mineral soil only to 20 cm depth, which means that we underestimated
324 total FRB. From the distribution of FRB by soil layers, we conclude that our sampling
325 covered at least 70% of the FRB. The underestimation in FRB might have been bigger for the
326 southern than for the northern sites because the rooting depth decreases with latitude (Jackson
327 *et al.* 1996). However, the effect of that underestimation was probably small, and careful
328 examination of our data (Fig. 2) suggests that correction for rooting depth is unlikely to
329 change our conclusion on the absence of linear changes in FRB with latitude of study site.

330 In spite of relatively small depth, from which we measured FRB, the mean FRB across
331 our sites (839 g m⁻²) appeared much higher than in the earlier studies conducted in boreal
332 forests (230 g m⁻²: Jackson *et al.*, 1997; 315 g m⁻²: Yuan & Chen, 2010; 419 g m⁻²: Finér *et*
333 *al.*, 2011). This difference may be partly due to methodological reasons, because Jackson *et*
334 *al.* (1997) excluded from their analyses the studies which combined FRB with biomass of
335 rhizomes. In contrast, we included rhizomes in our data (as explicitly stated in Materials and
336 Methods), because we had a dense cover of dwarf shrubs in our sites, and it was practically
337 impossible to separate rhizomes from roots, especially in the organic layer.

338 The basal area of the sampled stands decreased with latitude and was two times lower
339 in the northern than in the southern sites (Table 1). At the same time, the ratio between tree
340 FRB and stand basal area increased threefold within about the same range of latitudes
341 (Lehtonen *et al.*, 2016), which suggested that in our stands, based on changes in basal area,
342 tree FRB could be expected to increase with increasing latitude. As the total FRB did not
343 change, we conclude that the field layer FRB in our sites somewhat decreased towards the
344 North, opposite to the findings of Helmisaari *et al.* (2007). The basal areas of the stands
345 studied by Helmisaari *et al.* (2007) were higher (>25 m² ha⁻¹) in the southern sites than in this
346 study, which could explain the increase in the field layer FRB from the South to the North in
347 their study, as a result of a decrease in the shading of tree canopy. On the other hand,

348 Lehtonen *et al.* (2016) attributed these changes the ratio between tree FRB and stand basal
349 area to changes in soil nutritional quality, the characteristic that did not change with latitude
350 in our sites.

351 We found a clear support for our second hypothesis on more superficial fine root
352 system in the North than in the South, as the fine root density in the organic layer increased
353 with an increase in latitude. This finding corroborates the conclusions by Helmisaari *et al.*
354 (2007) who found both absolutely and relatively more fine roots of Norway spruce and Scots
355 pine in the organic layer in the northern than in southern Finland. The fine roots of the field
356 layer vegetation in boreal forests are generally located in the organic layer (Makkonen &
357 Helmisaari, 1998; Helmisaari *et al.*, 2007; Finér *et al.*, 2017); therefore we conclude that the
358 increase in fine root density in the organic layer in our northern sites resulted primarily from
359 the enhanced growth and/or redistribution of tree roots between soil layers. The higher soil
360 temperatures and soil N stocks (Dawud *et al.*, 2017) in the organic layer could be the reasons
361 to the higher fine root density in the organic layer in the harsh environmental conditions in
362 the North. These results suggest that the importance of fine roots as C pools increases
363 towards the North and C and nutrient cycles become more superficial with latitude. On the
364 other hand, the superficial root system increases vulnerability of boreal forests to abiotic
365 risks, such as forest fires, frost and drought, which occurrence may increase in future climate.

366

367 *Variation in fine root carbon and nitrogen concentrations*

368

369 We did not find support to the hypothesis that fine root N concentrations decrease towards
370 the North. This result is easy to explain, because the fine root N concentrations indicate N
371 availability from soil (Helmisaari *et al.*, 2007; Lehtonen *et al.*, 2016), and soil N
372 concentrations did not change along our gradient. Furthermore, fine root N concentrations

373 were independent from climatic, plant community and soil characteristics. At the same time,
374 we found significant within-site variation in fine root N concentrations across all soil layers,
375 which may have masked a weak latitudinal trend, had it exist. The variation in fine root C
376 concentrations was small, from 49% to 51%, but this variation was clearly associated with the
377 soil moisture at the site-specific level.

378

379 *Factors explaining variation in fine root biomass among study sites*

380

381 Our study is likely the first one which applied latitudinal approach to investigation of soil
382 characteristics in natural ecosystems. The soils in all our sites are typical for the study region
383 (as described by Pereverzev, 2009; Goryachkin, 2010), but – keeping in mind that soil
384 characteristics were not accounted for in selection of study sites – it is indeed surprising that
385 these characteristics generally did not change along a 1000 km long latitudinal gradient. One
386 of the few statistically significant latitudinal changes, namely the increase in the volumetric
387 stone content towards the North, is consistent with the increase in the content of boulders in
388 unsorted moraine sand towards the centre of the Valdai glaciation (Lavrova, 1960).

389 The overall uniformity of soil characteristics across our sites allowed us to expect that the
390 larger part of variation in FNB should be explained by the characteristics of plant
391 communities and climate. However, these expectations were not met.

392

393 The earlier studies reported positive relationship between FRB and the stand basal area (Finér
394 *et al.*, 2007; Helmisaari *et al.*, 2007; Finér *et al.*, 2011; Lehtonen *et al.*, 2016). However, FRB
395 measured from our sites was not associated with plant community characteristics (including
396 stand basal area): the first PCA axis, combining the measures of plant abundance and
397 diversity, explained only 5% of among-site variation in FRB (Table S7). The plausible

398 explanation is that across the range of environments, plants adjust biomass partitioning to
399 above- and below-ground parts to achieve the maximum overall performance, and the rules of
400 this partitioning are not clear yet. Reich *et al.* (2014) found that the proportion of total forest
401 biomass in roots is greater in increasingly cold climates; however, mean annual temperature
402 explained only 1-11% of the total variation. Across our sites, we found indirect statistical
403 support for the conclusions by Reich *et al.* (2014) only when we analysed FRB in organic
404 layer. The largest part of the variation in the total FRB (19%) was explained by soil
405 characteristics, although the correlation between these variables did not even approach the
406 conventional level of statistical significance ($P=0.21$).

407 FRB in the organic layer was positively correlated with the tree species diversity,
408 indicating complementarily effects resulting from the more effective use of soil resources by
409 the higher filling of soil by roots of different plant species (Brassard, Chen & Bergeron,
410 2011; Ma & Chen, 2016). An increase in tree species diversity may also enhance the
411 probability of including species with high overall biomass production (Tobner *et al.*, 2016).
412 These species identity effects have increased FRB even more than tree species diversity
413 effects (Jacob *et al.*, 2013; Domisch, Finér, Dawud, Vesterdal & Raulund-Rasmussen, 2015;
414 Finér *et al.*, 2017). The species identity effect might have been important at our sites, which
415 are conifer dominated, and the increase in tree species diversity means increase in the
416 abundance of birch and other deciduous tree species, which have higher FRB than conifers
417 (Finér *et al.*, 2011; Lehtonen *et al.*, 2016).

418 As we hypothesized, the weather conditions affected FRB, but they could explain only
419 some of within-site patterns. In particular, the growing season of 2016 was on average 2 °C
420 warmer than of 2015, and the amount of precipitation was 170 mm larger; this may explain
421 why FRB in 2016 was significantly larger than in 2015 across our sites. Fine root growth and
422 mortality occur simultaneously and the seasonal fluctuations in FRB are controlled by

423 temperature and moisture conditions (McCormack & Guo, 2014; Chen *et al.*, 2017). The
424 between year differences in FRB was more clear in the mineral soil layers, where roots were
425 exposed to lower temperatures than in the organic layer during the growing season. Long
426 periods of air temperatures <13°C in 2015 could have particularly limited root growth in the
427 mineral soil (Vapaavuori, Rikala & Ryyppö, 1992; Domisch *et al.*, 2002).

428 We found that in the organic layer FRB in August was higher than in June. This result
429 is somewhat surprising, because the previous studies conducted in boreal forests did not
430 record seasonal patterns in FRB during the growing season (Persson, 1978; Makkonen &
431 Helmisaari, 1998; Børja, De Wit, Steffenrem & Majdi, 2008). The within-year variation in
432 FRB is controlled not only by the weather conditions but also by the competition between
433 shoots and roots of C and other resources (Radville, McCormack, Post & Eissenstat, 2016),
434 so the detected pattern is hard to explain.

435

436 **Conclusions**

437

438 Our results indicate that total FRB and fine root contribution to below-ground N and C stocks
439 in mixed boreal forests did not exhibit linear latitudinal pattern and could not be reliably
440 predicted from climatic, plant community and soil characteristics. The fine root system
441 becomes more superficial towards the North, suggesting that both C and N cycling is more
442 concentrated in the organic layer in the high latitudes and indicating the poleward increase in
443 vulnerability of boreal forests to abiotic risks (forest fires, drought). Future climate may shift
444 FRB and also C and N cycling from organic layer to mineral soil and increase the volume of
445 soil used for water and nutrient uptake.

446

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448

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452

453

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- 585
- 586

587 **Table 1** Sources of variation in density of fine roots (mg dry weight cm⁻³; mixed model
 588 ANOVA, type III sums of squares).

589

Effect	Explanatory variable	Degrees of freedom		Test statistics	F/χ^2 value	P
Fixed	Latitude	1	8.16	F	2.73	0.14
	Year	1	229.4	F	1.19	0.28
	Month	1	227.3	F	2.43	0.12
	Layer	2	447.9	F	4.97	0.0073
	Latitude \times Layer	2	448.3	F	9.39	0.0001
	Latitude \times Year	1	230	F	2.15	0.14
	Latitude \times Month	1	228	F	2.10	0.15
	Year \times Layer	2	451.1	F	7.59	0.0006
	Month \times Layer	2	451.6	F	8.28	0.0003
	Year \times Month \times Layer	3	478.2	F	2.86	0.04
Random	Site	1	-	χ^2	4.93	0.01
	Core	1	-	χ^2	4.93	0.01

590

591

592 **Table 2** Fine root biomass (FRB), nitrogen (N) and carbon (C) stocks in fine roots (g m^{-2}) by
 593 soil layers and study sites.

Site Layer	FRB	N stock	C stock	Site Layer	FRB	N stock	C stock
R60 Organic	185	1.5	93	R65 Organic	543	3.4	279
0-10 cm	215	1.7	80	0-10 cm	205	1.4	93
10-20 cm	131	0.8	51	10-20 cm	104	0.7	51
Total	531	4.0	224	Total	852	5.5	423
R61 Organic	390	3.2	197	R66 Organic	402	2.8	202
0-10 cm	313	2.3	138	0-10 cm	520	3.5	232
10-20 cm	237	1.5	89	10-20 cm	237	1.4	103
Total	940	7.0	424	Total	1160	7.7	537
R62 Organic	284	1.9	138	R67 Organic	544	3.7	275
0-10 cm	263	1.7	111	0-10 cm	209	1.3	97
10-20 cm	170	1.3	76	10-20 cm	183	1.7	72
Total	716	5.0	327	Total	936	6.7	444
R63 Organic	200	1.3	99	R68 Organic	400	3.0	202
0-10 cm	266	1.5	112	0-10 cm	292	1.9	130
10-20 cm	198	1.1	81	10-20 cm	1761	1.7	69
Total	665	4.0	293	Total	868	6.5	401

R64 Organic	505	4.5	257	R69 Organic	426	4.3	217
0-10 cm	288	2.1	123	0-10 cm	143	1.1	58
10-20 cm	281	1.9	120	10-20 cm	79	0.8	31
Total	1074	8.4	500	Total	648	6.1	306

595 **Table 3** Sources of variation in fine root biomass (mg dry weight m⁻²) in organic layer plus
 596 0–20 cm mineral soil layer (mixed model ANCOVA, type III sums of squares).

Effect	Explanatory variable	Degrees of freedom		Test statistics	F/χ^2 value	P
Fixed	Latitude	1	8.05	F	2.13	0.18
	Year	1	163.3	F	0.46	0.50
	Month	1	163.3	F	0.98	0.32
	Latitude × Year	1	163.3	F	1.05	0.31
	Latitude × Month	1	163.3	F	0.88	0.35
	Latitude × Year × Month	1	163.3	F	0.99	0.32
Random	Site	1	-	χ^2	22.4	<0.0001

597

598

599 **Table 4** Sources of variation in concentrations of C and N concentrations in fine roots (mixed
 600 model ANOVA, type III sums of squares, DF= degrees of freedom).

Response variable	Effect	Explanatory variable	Degrees of freedom		Test statistics	F/χ^2 value	P
Carbon	Fixed	Latitude	1	12.1	F	3.95	0.07
		Layer	2	148.3	F	1.86	0.16
		Latitude \times Layer	2	147.6	F	1.06	0.35
	Random	Site	1	-	χ^2	0.43	0.26
		Core	1	-	χ^2	0.43	0.26
Nitrogen	Fixed	Latitude	1	8.97	F	0.13	0.73
		Layer	1	125.5	F	0.71	0.50
		Latitude \times Layer	1	124.7	F	0.76	0.47
	Random	Site	1	-	χ^2	2.15	0.07
		Core	1	-	χ^2	2.15	0.07

601

602

603 **Figure captions**

604

605 Fig. 1. Location of the study sites.

606

607 Fig. 2. Fine root density (mean \pm S.E. based on the four sampling times) by study sites and
608 soil layers.

609 Fig. 3. The differences in fine root biomass (estimated marginal means + S.E.) between study
610 years (a) and between study months (b) in different soil layers. An asterisk indicates
611 significant ($P < 0.05$) difference between years (a) or months (b) within the layer.

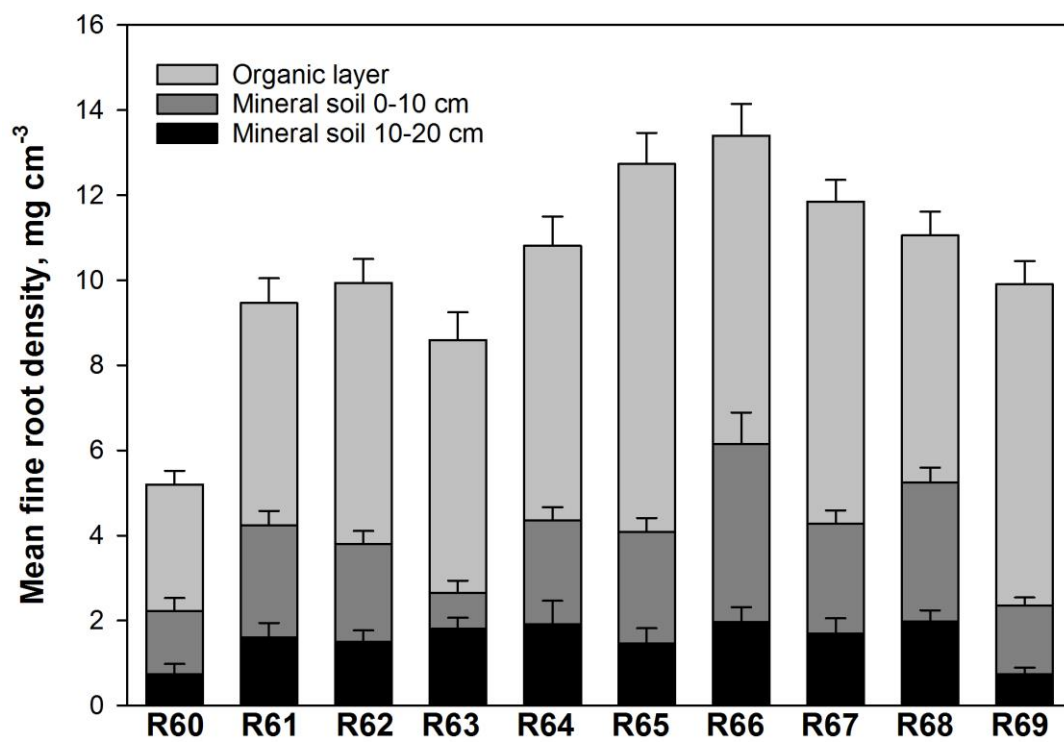
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613 **Figures**

614

615 **Figure 1**

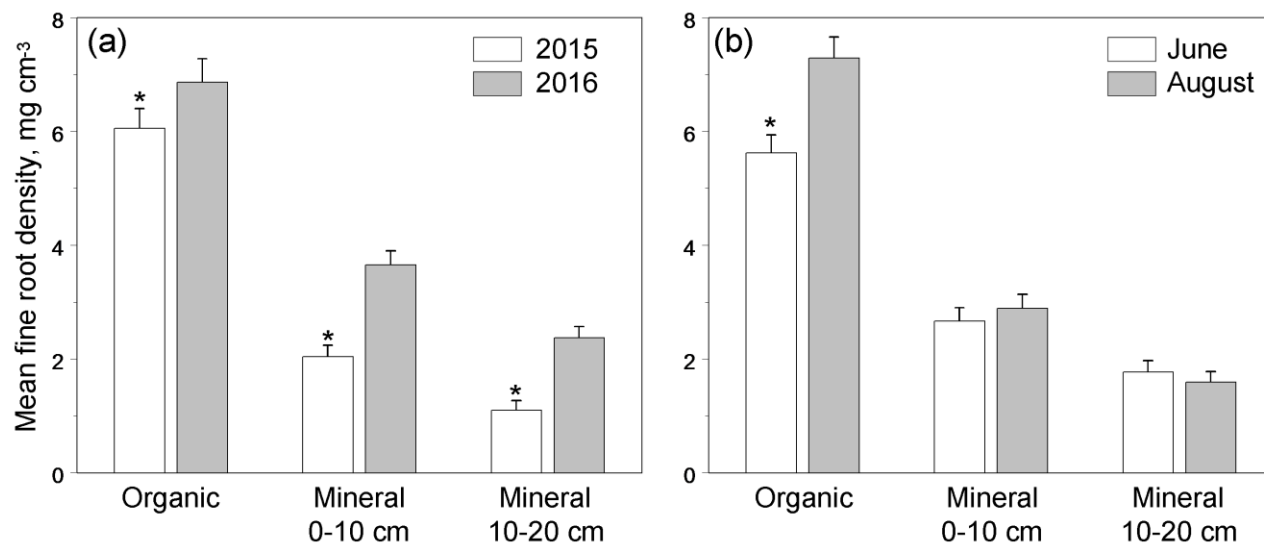
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618 Figure 2

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Figure 3