



An integrated method for high-resolution definition of new diameter-based fine root sub-classes of *Fagus sylvatica* L.

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Abstract

- **Key message** Compared to the traditional approach, applying micrometric image analysis to fine root samples of *Fagus sylvatica* with subsequent data treatment through principal component and cluster analysis yielded specific diameter sizes for fine root sub-classes having better resolution of the corresponding branching orders, and a more coherent relationship with the values of annual production and turnover rate.
- **Context** Fine root traits are poorly understood, impeding an accurate representation of terrestrial biogeochemical models. Traditionally used, arbitrary diameter thresholds lead to a misestimation of fine root traits such as branching order, environmental relationship, annual production, and turnover rate.
- **Aims** Here, we present, as modification of the traditional method, an integrated approach to segregate, at high-resolution, fine root populations of *Fagus sylvatica* into new diameter sub-classes that better correspond with the traits mentioned above.
- **Methods** Samples, collected with a sequential soil coring method, were subjected to a micrometric image analysis, and resultant data were treated with principal component and cluster analysis.
- **Results** Results showed that fine roots were distributed into diameter-size sub-classes (0–0.3 mm, 0.3–1 mm, and 1–2 mm) different from those determined by traditional methods (0–0.5 mm, 0.5–1 mm, and 1–2 mm). New sub-classes provided a better resolution of the corresponding branching-orders, and the values of annual production and turnover rate were more coherent with diameter class and soil depth. Moreover, new sub-classes provided a more precise match with soil temperature than traditional methods.
- **Conclusion** Our method may help to unveil fine root dynamics and development, reduce data analysis time, and make the diameter-based classification more precise and trustworthy even in the case of non-intact samples.

Keywords Branching order · Fine root production · Fine root turnover rate · Soil temperature · Root diameter · PCA

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Contribution of the co-authors AM and MT equally contributed to the study concept and design and to data collection and relative interpretation. AM outlined and wrote the article, dealt with manuscript process, improvements, and revisions. MT carried the data process and analysis and revised the first version of the manuscript draft. BG applied the chemometrics method to the data, contributed to the interpretation, and drafted the related part of the manuscript. GSS and DC conceived and supervised the research in all works aspects, gave important intellectual content to the article, and revised it critically.

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1 Introduction

Fine roots are commonly defined as a single pool of ephemeral roots having a diameter below 2 mm (Cannon 1949; Böhm 1979; Zobel and Waisel 2010; Rewald et al. 2014; McCormack et al. 2015; Laliberté 2017). Although fine roots represent a small part of the total tree root biomass, they significantly influence the biogeochemical processes in terrestrial ecosystems, particularly playing an important role in soil carbon cycling rates (Nadelhoffer and Raich 1992; Hendrick and Pregitzer 1993; Jackson et al. 1997; Röderstein et al. 2005). Indeed, fine roots rarely represent more than 5% of total tree biomass, but their annual production amounts to 33–67% of the total annual net primary production in most ecosystems (Joslin and Henderson 1982; McLaugherty et al. 1982; Jackson et al. 1997; Matamala et al. 2003; McCormack et al. 2015). Moreover, fine roots are the most sensitive and dynamic component within the overall root system. They respond

rapidly to variations in the rooting environment (Hendrick and Pregitzer 1992; Barlow 2010; Montagnoli et al. 2012a, b, 2014) and are a good indicator of forest adaptation to climate change (Eissenstat et al. 2000, 2013; Brunner et al. 2015; Montagnoli et al. 2016). In addition, fine roots are essential for high rates of seedling survival and robust growth after planting (Montagnoli et al. 2018).

In an effort to improve the understanding of fine root development and dynamics, many authors have classified fine roots by sub-classes rather than the 2-mm macro-category customarily used. Root orders along the branching hierarchy of roots < 2 mm in diameter encompass a diversity of forms and functions (Long et al. 2013; Iversen et al. 2017). In various studies with different species, fine root traits such as respiration (Di Iorio et al. 2015), production, lifespan, and decomposition rate (Gill and Jackson 2000; Guo et al. 2008, McCormack et al. 2012; Sun et al. 2013; Van Do et al. 2016), carbon, starch, and nitrogen concentration (Terzaghi et al. 2013, 2016), and relationship with soil temperature and water content (Montagnoli et al. 2012a, 2014) vary seasonally. Moreover, this seasonal fine root growth is of different magnitude and type (i.e., longitudinal and radial) depending on the diameter class considered. Yet, in turn, seasonal changes in water availability (Amendola et al. 2017) and nutrient concentration (Zobel et al. 2007) change the mean diameter size of the fine root population. These studies support the assertion of Pregitzer (2002) that the traditional view of treating all fine roots of trees as a homogeneous population is deeply flawed. The portion of the fine root pool on which measurements are made ranges from individual root tips to small root branches to all roots below a fixed diameter threshold (Comas et al. 2002; Pregitzer et al. 2002; Guo et al. 2008; Holdaway et al. 2011). Furthermore, branching order and root diameter are important components of root-system architecture. However, the relationship between the branching order and the root diameter remains uncertain, with contradictory results even within the same plant species (Wu et al. 2016). Thus, determining to what extent these differences are due to plant species diversity or environmental influence is difficult to discern (Wu et al. 2016). Hence, the variable manner in which fine roots are classified and the rigid frame composed by diameter thresholds in which roots are traditionally segregated may lead to a misestimation of fine root dynamics and development. The lack of a suitable method to avoid this misestimation has hindered a more accurate modelling of root development as well as of root processes in terrestrial biosphere (Jackson et al. 2000; McCormack et al. 2015; Smithwick et al. 2014; Warren et al. 2015; Wu et al. 2016).

The shortcomings of the current diameter-based approach are widely recognized. Within the root science community is an increasing desire to develop, and widely adopt, an

approach able to provide precise measurements regardless of biological variability. Several investigators have examined alternatives; for example, Rewald et al. (2014) demonstrated that the use of an extended root ordering (i.e., order and color) might explain the variance of respiration twice as well as root diameter or root-order classes alone. In a recent review, McCormack et al. (2015) aimed to redefine fine root classification based on order and function; indeed, recent work has found that root orders can be grouped into modules with similar function (Xia et al. 2010). In a desert system, assessing life span and structure of ephemeral root modules of different functional groups can simplify modelling fine root traits (Liu et al. 2016).

However, methodologies able to classify roots while including their high intrinsic variability are time-consuming and/or not operationally feasible (McCormack et al. 2015). In this study, we first hypothesized that developing a new methodology, including a high-resolution image analysis and a robust statistical methods, would segregate the fine root population into new diameter sub-classes different from the classical ones. Second, we hypothesized that the new classification would better correspond to the actual diameter distribution and thus should provide a better fit with fine root traits. To test our hypotheses, we first subjected fine root samples to a high-definition image analysis. Resulting morphological data were then subject to principal component analysis (PCA) and cluster analysis (CA) to segregate new diameter sub-classes. Finally, fine root topology, annual production, and turnover rate, as well as relationship with soil depth and temperature were measured using traditional diameter sub-classes and our new method. For the topological analysis, grouping of diameter sub-classes within branching order was used as criteria to assess which segregation method provided a better fit. In the case of fine root traits and environmental relationship, significance of the regression analysis was tested.

2 Materials and methods

2.1 Site descriptions and characteristics

We selected three differently managed beech (*Fagus sylvatica* L.) forest stands. The first stand was a 40-year-old coppice. Thinning treatments were applied to the second stand in 1994 and the third stand in 2004 to convert them from coppice to high forest. These stands were located in the Telo stream watershed in the Lombardy Alps (Intelvi Valley, northwestern Italy, 45° 59' 36" N, 9° 07' 40" E), approximately 1160 to 1200 m above sea level between Lakes Como and Lugano. This area is characterized by a sub-continental climate, with a mean annual precipitation of 1600 mm mainly concentrated in two main periods (April–May and October–November), and a mean annual temperature of 10–11 °C. More detailed

information about the characteristics of these stands was provided in Montagnoli et al. (2012b).

2.2 Fine root sampling and digitalization

Fine roots (diameter < 2 mm) were collected by the soil core sampling method (Vogt and Persson 1991) during two growing seasons. From April to October 2007, a number of three 30-cm-deep cores (8 cm diameter) were sampled about every 40 days on 7 sampling dates in three stands (63 total) using a manual auger (Eijkelkamp Agrisearch Equipment: Plant Root Sampling model P1.30). Afterward, from May 2008 to October 2008 and in April 2009, a number of eight 30-cm-deep cores (4 cm diameter) were collected about every 30 days on eight sampling dates in three stands (192 total) using a motor-driven portable core sampler. These latter 30-cm cores were divided into three 10-cm samples by depth for a total of 576 fine root samples (Montagnoli et al. 2012b). The three soil cores per each stand in the case of 2007, and the two soil cores per plot in the case of 2008–2009, were considered as replicates, pooled, and treated as one. The samples were cleaned from soil residues by soaking in water prior to the root extraction procedure using a sieve with a 300- μm mesh. We examined roots at the stereomicroscope (Nikon SMZ 800) and only beech fine roots were considered for analysis. Living (biomass) and dead roots (necromass) were distinguished under the stereomicroscope by inspecting color, texture, and shape (Vogt and Persson 1991). All root samples were scanned at the resolution of 400 dpi with a calibrated flatbed scanner coupled to a lighting system for image acquisition (Epson Expression 10,000 XL). Images were analyzed by WinRhizo Pro V. 2007d (Regent Instruments Inc. Quebec) in order to separate with higher accuracy fine ($d < 2$ mm) and coarse ($d > 2$ mm) roots. The morphological analysis was carried for the fine root fraction only, while coarse roots were not considered for this study.

2.3 Soil temperature

Measurements of soil temperature (ST; $^{\circ}\text{C}$) were taken by Checktemp 1 thermometer with an NTC thermistor sensor (Hanna Instruments; ± 0.3 $^{\circ}\text{C}$). For each stand, soil temperature was measured at each sampling date and proximal to the sampling point, at three soil depths (5, 15, and 25 cm).

2.4 A new method for image processing and data analysis

To test our hypotheses, we employed a new, integrated, three-step method to process a posteriori fine root images obtained through a sequential soil core technique. The first step was a micrometric image analysis of fine root samples obtained from one or more growing seasons. The second step was data

arrangement and processing. The final step was data modeling through principal component analysis (PCA) and cluster analysis (CA) to define species-specific diameter class thresholds.

2.5 Micrometric image analysis

To identify the smallest root diameter, an image of fine root samples was processed by WinRhizo (Pro V. 2007d software, Regent Instruments Inc., Quebec, Canada). Then, for the micrometric image analysis (μIA), we used a threshold diameter approximately 25% that of the smallest root diameter observed. For our samples, we selected a 50- μm -diameter unit because our smallest observed root diameter was 0.19 mm. In the WinRhizo analysis, missing values of some diameter classes occur when the pixel size is greater than that of the selected diameter threshold. Therefore, to avoid mismeasurements and obtain a pixel size smaller than the selected 50- μm -diameter unit, fine root images were resized to higher resolution (from 400 dpi to 800 dpi) and pixel size reduced from 63.50 μm to 31.75 μm . This resulted in a continuous diameter size distribution of the fine root samples (i.e., no dimensional gaps). After the WinRhizo analysis was performed, each fine root segment (Fig. 1a), naturally characterized by differences in thickening along the root axis, was divided by the progressive increase of micrometer-diameter unit each highlighted by different colors (Fig. 1b). Moreover, we associated quantitative information of root length with each diameter unit (Fig. 1c).

2.6 Data arrangement and processing

Fine root length data were arranged in tabular form with samples (objects) in rows and variables in columns for calculation (Fig. 2a). Because samples were collected in replicates as previously described, an average for each sample (for each root diameter) was calculated. We considered 50- μm fine root diameter intervals as variables. We quantified the abundance of fine roots as the total fine root length measured for each diameter interval. The fine root length for each diameter interval was expressed as a percentage of the total fine root length found for each average sample (Fig. 2b). Data (average and percentage transformation) were then arranged into two tables. The first data matrix contained 30-cm-deep samples collected in 2007 from the three forest stands, which yielded a table having dimensions of 21 samples \times 39 variables. The second data matrix contained samples collected in 2008 from the three forest stands at three different soil depths, which showed dimensions of 63 samples \times 39 variables. Then, in order to compare results obtained from different sampling year, soil depth, and forest stands, autoscaling was performed according to the sampling sites. In particular, autoscaling is

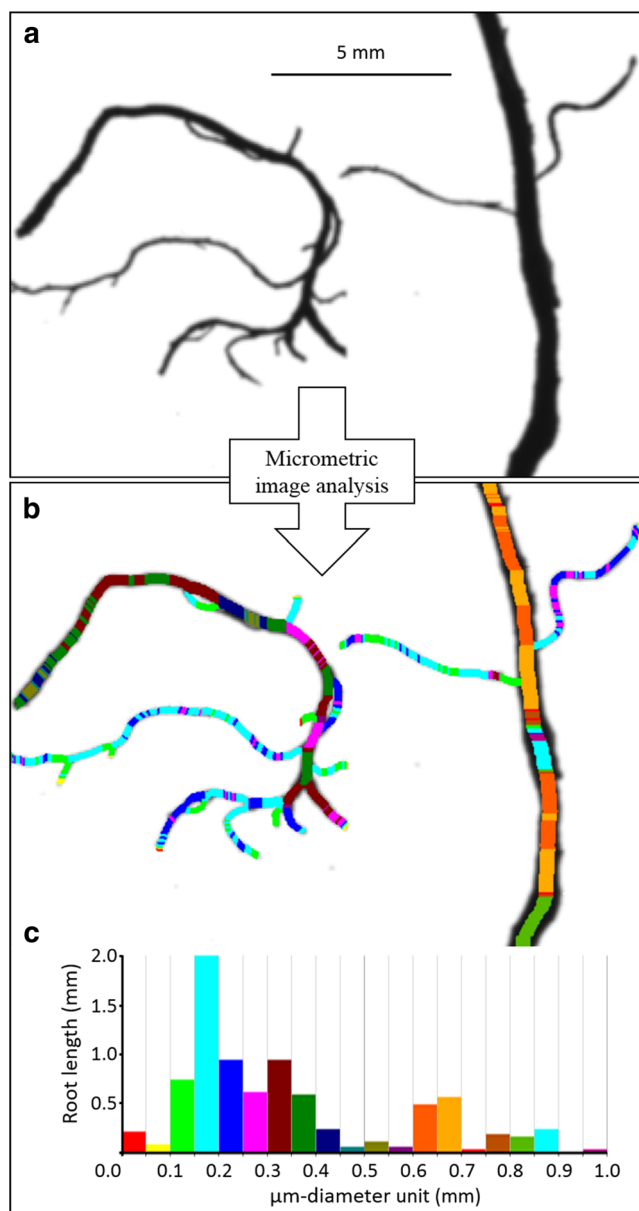


Fig. 1 Scanned image of *Fagus sylvatica* fine root segments, obtained by sequential coring technique (a). The scanned image analyzed by WinRhizo software with progressive increments of 50-µm-diameter units highlighted by different colors (b). Color histogram representing the data of fine root length (mm) for each 50-µm-diameter unit (c)

a column transformation that provides all variables the same opportunity to influence the estimation of PCA components as described in Eq. (1):

$$x_{ij}^{autoscale} = \frac{x_{ij}^{original} - \bar{x}_j}{S_j} \tag{1}$$

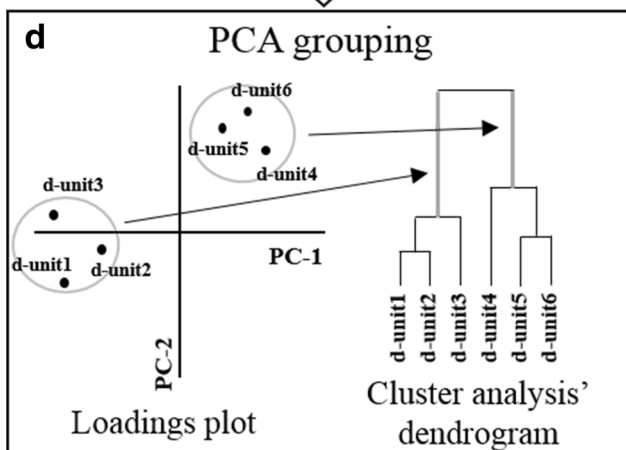
where $x_{ij}^{original}$ is the original measurement, \bar{x}_j is the average of the column for each forest stand, and S_j is the standard deviation of the column for each forest stand (Fig. 2c). Finally, PCA and CA were employed.

a Raw data matrix

Sample	µm-diameter units (length data, cm)					
	d-unit1	d-unit2	d-unit3	d-unit4	d-unit5	d-unit6
Sample 1						
Sample 2						
Sample 3						
...						
Sample n						

b Expression of data in percentage

c Autoscaling



e Fine-root diameter sub-classes
 Class I: (d-unit1 + d-unit2 + d-unit3)
 Class II: (d-unit4 + d-unit5 + d-unit6)

f Elaborated data matrix

Sample	Root length		Root mass		Date	Plot	Etc.
	Class I	Class II	Class I	Class II			
Sample 1							
Sample 2							
Sample 3							
...							
Sample n							

2.7 Data modelling: PCA and CA

PCA, a bilinear model that extracts information from multi-variate data transforming the original variables into new

◀ **Fig. 2** Theoretical framework of the model. Fine root length data were arranged in tabular form with samples (objects) in rows and variables in columns (a). Fine root length for each diameter interval was expressed as a percentage of the total fine root length found for each average sample (b). Autoscaling was performed according to the different sites (c). PCA employment resulted in graphed score and loading plots used as input for the CA calculation with the generation of a dendrogram (d). New diameter sub-classes are obtained by summing values of the diameter units falling within each diameter sub-class (e). Fine root length and mass may be calculated according to the new sub-classes (f)

variables called principal components (PCs), could be defined as a qualitative data visualization method. PCs are a linear combination of the original variables and carry independent information as they are orthogonal. To each component, a value of explained variance is associated. These values are complementary and in decreasing order where the first component has the higher value and the last component accounts for model noise. PCA calculation returns matrices of scores (object coordinates in the space described by the PCs) and loadings (directions of the original variables with respect to the PCs expressed as the cosine of the angles), which are represented in bi-dimensional graphs called score plots and loading plots. In the score plot, samples are depicted, and thus, groups, tendencies, and outliers can be visualized. The loading plot shows correlations between variables. In this study, we employed a PCA strategy both as an exploratory analysis and as a data reduction method. Score and loading plots were created and interpreted and loading plots were used as input for the CA calculation (Fig. 2d).

CA is defined as an unsupervised classification methodology. It allows finding groups between objects on the basis of their similarities with respect to specified characteristics. In order to confirm the presence of groups among the 50- μm diameter units obtained from PCA, loading values of the PCs yielding a higher significance were used as the algorithm input for the calculation. Thus, a new data matrix was arranged with 50- μm -diameter unit intervals as samples and loadings extracted from PCA as variables. We then used the method of Ward (1963), an agglomerative hierarchical clustering algorithm that uses an analysis of variance approach to evaluate the distances between clusters to calculate the distance between samples. Ward's method attempts to minimize the sum of squares of any two clusters that could be formed at each step of the analysis. A dendrogram based on the distance between samples was generated (Fig. 2d).

2.8 Fine root traits according to sub-classes

Fine root length and volume of two different diameter classifications, classical diameter sub-classes (0–0.5, 0.5–1.0, 1.0–2.0 mm; hereafter simply “classical sub-class”) and

newly defined sub-classes (hereafter simply “new sub-class”) according to the new method described above, were calculated by summing values of the 50- μm diameter units falling within each diameter sub-class (Fig. 2e). Fine root traits such as length (mm) and biomass (g) were calculated according to the new sub-classes (Fig. 2f). Fine root biomass values for the whole 0–2.0 mm diameter interval were considered as the actual dry weight. To calculate biomass values for each diameter sub-class, fine root volume (cm^3) was multiplied by different root density according to diameter classes considered. In particular, for the thinner fine root class (0–0.5 and 0–0.3 mm), root density was 0.35 g cm^{-3} calculated as the mean value of the range ($0.25\text{--}0.45 \text{ g cm}^{-3}$) published by Beyer et al. (2013). The root density for the intermediate and the thicker fine root classes (0.5–1 mm and 0.3–1 mm; 1–2 mm) corresponded respectively to the lowest and the mean values of the range ($0.49\text{--}0.78 \text{ g cm}^{-3}$) published by Hertel et al. (2013). Specific root length (SRL; m g^{-1}) was calculated as the length-to-mass ratio (L/M) of a root fragment (Ostonen et al. 2007). At this point, in order to test if the new sub-classes would better fit with root topology in comparison with the classical ones, classical and new sub-classes were applied to a scanned image of a 2-year-old beech seedling root system. Moreover, different fine root sub-classes were mathematically related to soil temperature to see the relationship with an environmental factor. Finally, conventional variables such as annual production and turnover rate were aligned with the classical and new root classifications. In particular, fine root production was estimated using the minimum–maximum method procedure. This method calculates, and sums in case of multimodal seasonal pattern, only significant differences between seasonal minimum and maximum fine root dry mass (live mass plus necromass) (Edwards and Harris 1977; McClaugherty et al. 1982; Hertel and Leuschner 2002; Montagnoli et al. 2014). Moreover, fine root turnover rates (year^{-1}) of live root biomass were calculated as annual root production ($\text{g m}^{-2} \text{ year}^{-1}$) divided by mean standing biomass (g m^{-2}) (Gill and Jackson 2000; Godbold et al. 2003; Montagnoli et al. 2014).

2.9 Statistical analysis

PCA and CA were performed using the software The Unscrambler X (version 10.4.1—CAMO). We assessed the effect of air temperature on fine root biomass using a linear regression function with SPSS 17.0 (SPSS Inc., Chicago IL, USA).

Data availability The datasets analyzed during the current study are available in the FRED (fine root ecology database) repository (<https://roots.ornl.gov/>).

3 Results

3.1 New fine root sub-classes defined by PCA and CA

The first two principal components (PC1 and PC2) accounting for 62% of the data variance (PC1 49%, PC2 13%) were assumed as the most principal components being related to the sought information and therefore considered for data interpretation. The sum of all remaining (13) PCs accounted for the 38% of the data variance. These remaining PCs were not considered for the data interpretation because they did not correspond to the data structure and, thus, did not include further relevant information to the data analysis.

The first PCA model was built on the raw data (data not autoscaled according to the sampling site). The score plot obtained by the first two PCs showed an influence of external factors such as sampling year (2007, 2008; Fig. 3a), soil depth (cm; 0–10, 10–20, 20–30, 0–30; Fig. 3b), and forest stand characteristics (CpS, CvS 04, CvS 94; Fig. 3c). After autoscaling transformation, the score plot depicted according to the external factors (Fig. 3d–f) showed that samples lie

homogeneously around the model center, highlighting that, at this level of analysis, the PCs' construction is no longer affected by these factors. The loading values for each PC (Fig. 4a, b) showed a distribution of micrometer-diameter units into groups. The first PC (Fig. 4a) segregated the finest root diameters, which showed positive values on this axis, from the larger ones that lie in the negative part of the axis. The second PC (Fig. 4b) showed a further segregation within the larger root diameters. The segregation of fine root diameter sub-classes emerged qualitatively from the loading plot. When CA calculation was applied, the emergence of three main clusters defined the corresponding new sub-classes: 0–0.3, 0.3–1.0, and 1.0–2.0 mm (Fig. 4c).

3.2 Fine root branching order

Figure 5a shows a portion of an intact, 2-year-old beech seedling root system characterized by five topological orders (according to the centripetal-segmented ordering system, by Berntson 1997 and references therein). When the classical (Fig. 5b) and the new (Fig. 5c) sub-class classifications were

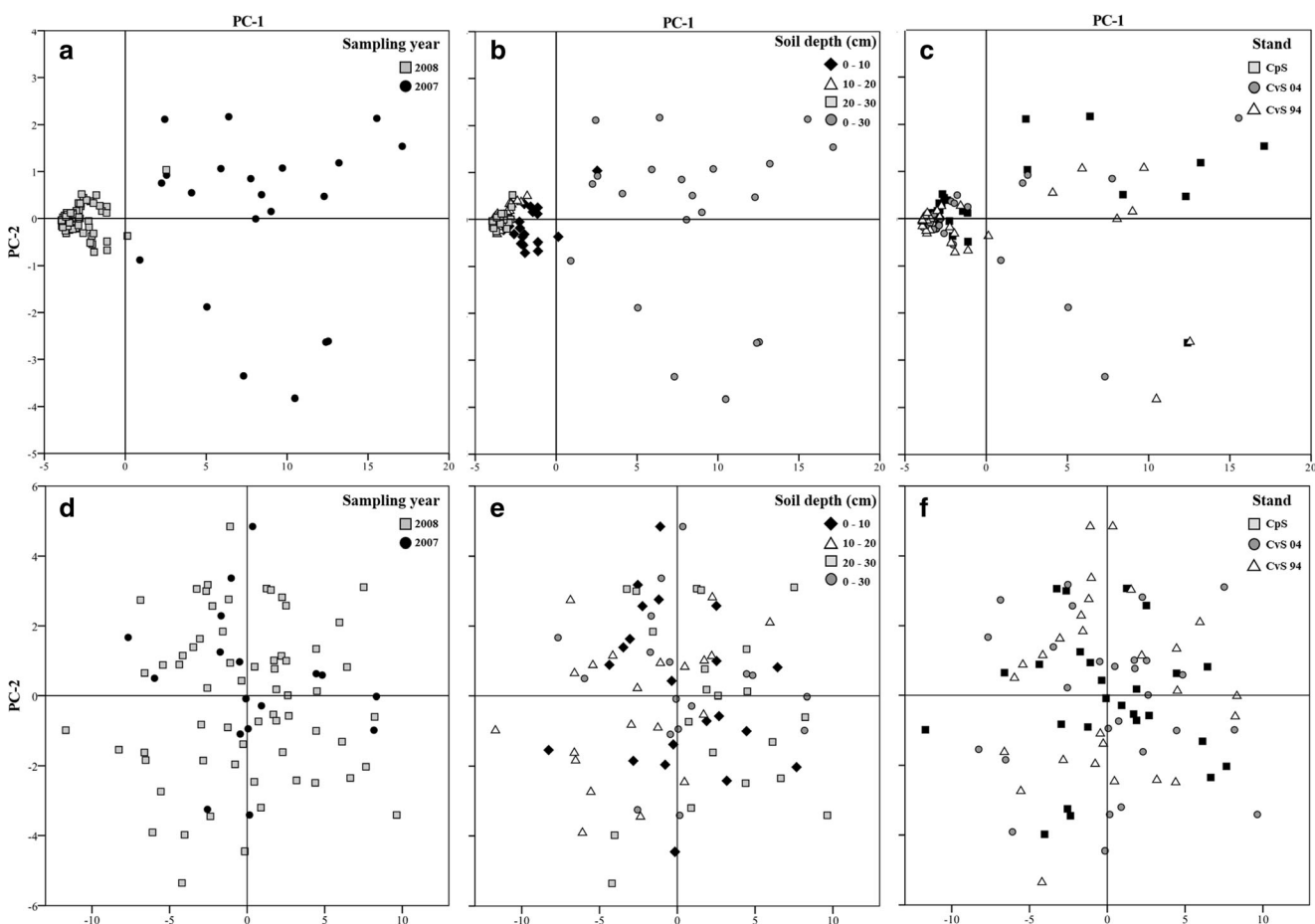


Fig. 3 Score values before (a–c) and after (d–f) autoscaling transformation representing fine root length obtained by PC1 and PC2 and depicted according to a, d sampling year (2007, 2008), b, e soil depth

(0–10, 10–20, 20–30, 0–30 cm), and c, f forest stand (40-year-old coppice–CpS; Conversion to high forest 2004–CvS 04; Conversion to high forest 1994–CvS 94)

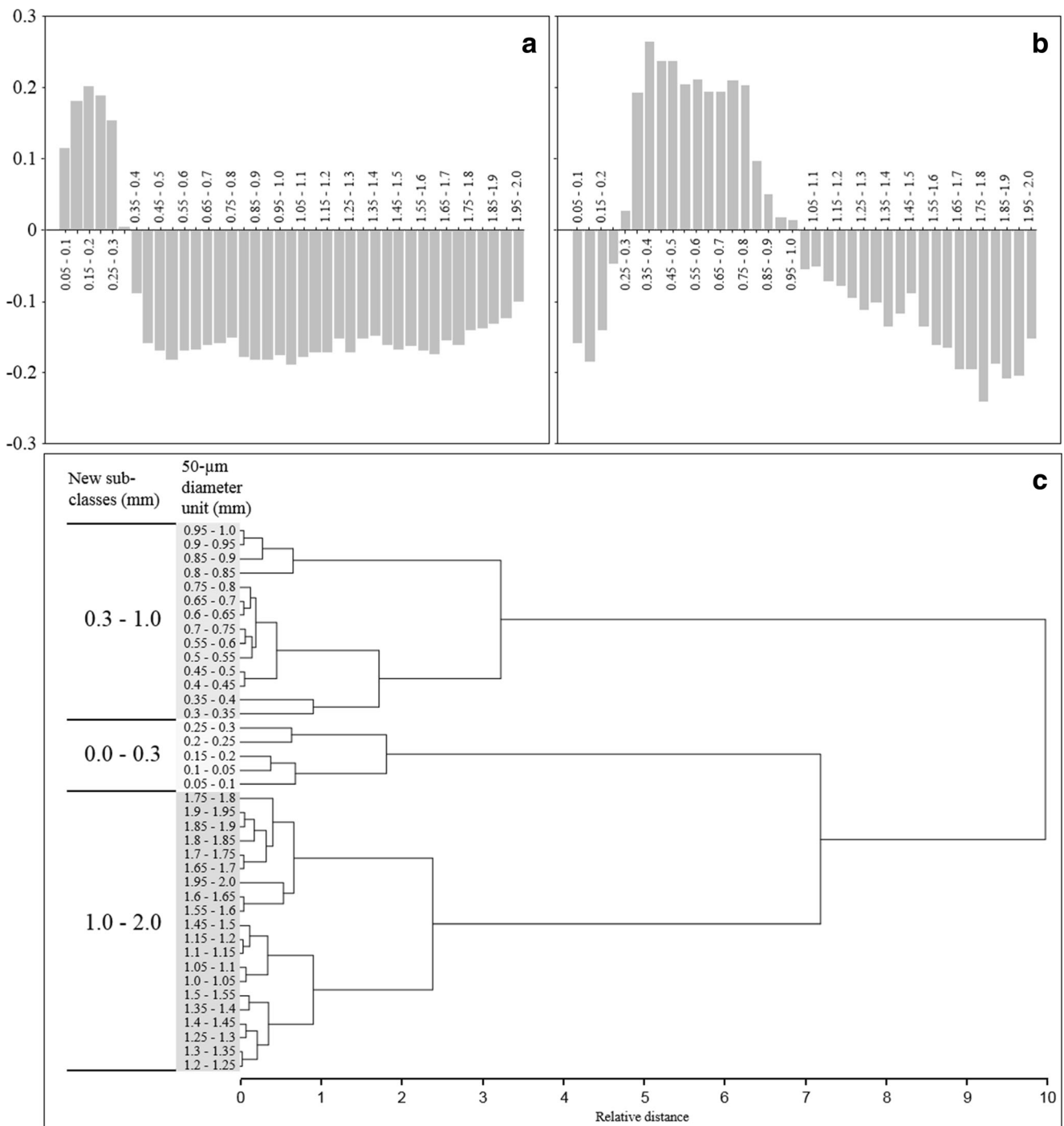


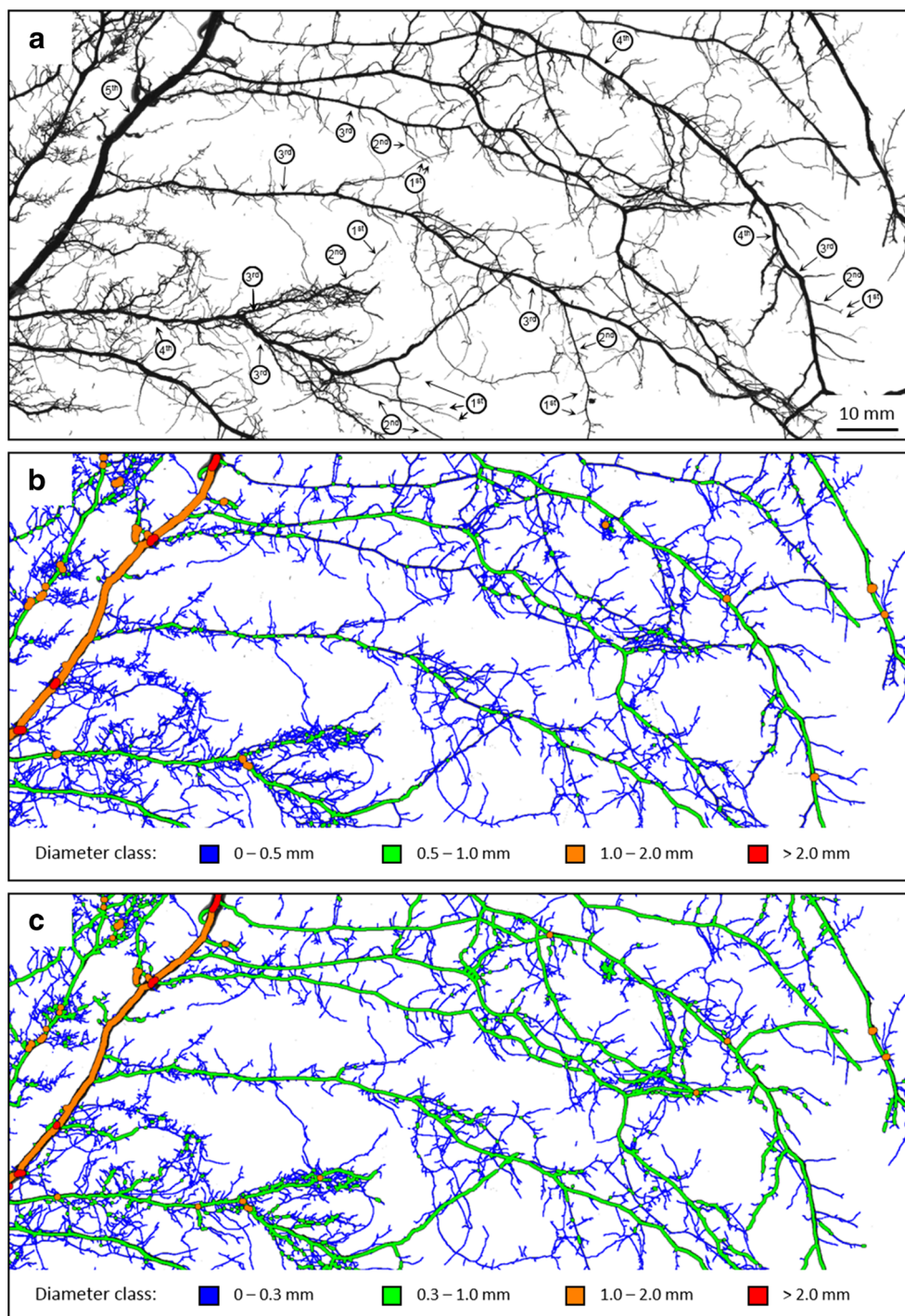
Fig. 4 The loading values for PC-1 (a) and PC-2 (b) showing a distribution of micrometer-diameter units into groups. Dendrogram showing the clustering of the new sub-classes: 0–0.3, 0.3–1.0, and 1.0–2.0 mm (c)

applied to scanned images of the fine root system, differences in diameter sub-class were observed for each root order. In particular, in the case of classical classification, third- and fourth-order roots repeatedly occurred within the 0–0.5 and 0.5–1.0 mm sub-classes, respectively (Fig. 5b). In contrast, using the new classification approach, first- and second-order roots were 0–0.3 mm, third- and fourth-order roots were 0.3–1.0 mm, and fifth-order roots were 1.0–2 mm (Fig. 5c).

3.3 Fine root relationship with soil temperature

Correlation between mean live fine root length of each sub-class and soil temperature showed a significant relationship ($p < 0.05$) only for the smallest sub-class for both the classical (0–0.5 mm) and new (0–0.3 mm) classifications (Table 1). For the upper soil layers (0–10), relationship significance was stronger for the new sub-classes ($p = 0.021$) than with the

Fig. 5 Scanned image of a portion of an intact, 2-year-old beech seedling root system characterized by five topological orders numbered according to the centripetal-segmented ordering system (a). Classical (b) and new (c) diameter-size sub-class classifications were applied to the scanned images of the fine root system. Different colors indicate different diameter sub-class



classical ($p = 0.035$). Moreover, in the case of the deepest soil layers (10–20 and 20–30 cm), the relationship was significant only in the case of the new sub-class (Table 1).

3.4 Fine root traits

Mean live fine root biomass, length, and annual production for all the soil depths investigated showed that the 0–0.3 mm new sub-class had values nearly two- to threefold

lower than values measured for the corresponding 0–0.5 mm classical sub-class (Table 2). In contrast, when considering the second sub-class (new, 0.3–1.0 mm; classical, 0.5–1.0 mm), values were higher for the new sub-class (Table 2). Values of SRL measured at all three soil depths for both new sub-classes (0–0.3 mm and 0.3–1.0 mm) were twofold higher than values measured for the corresponding classical sub-classes (0–0.5 mm and 0.5–1) (Table 2). Because the third sub-class was the same

Table 1 Comparison of regression line coefficients of the relationship between fine root length and soil temperatures according to different diameter sub-classes (classical and new). Data refer to three soil depths between April and November 2008

Soil depth (cm)	Classical sub-class (mm)	R^2	P	New sub-class (mm)	R^2	P
0–10	0.0–0.5	<i>.230</i>	<i>.035</i>	0.0–0.3	<i>.260</i>	<i>.021</i>
	0.5–1.0	.030	.826	0.3–1.0	.520	.331
	1.0–2.0	.030	.489	1.0–2.0	.030	.489
10–20	0.0–0.5	.120	.329	0.0–0.3	<i>.250</i>	<i>.020</i>
	0.5–1.0	.120	.329	0.3–1.0	.130	.114
	1.0–2.0	.005	.348	1.0–2.0	.005	.348
20–30	0.0–0.5	.150	.080	0.0–0.3	<i>.220</i>	<i>.031</i>
	0.5–1.0	.190	.145	0.3–1.0	.012	.314
	1.0–2.0	.110	.345	1.0–2.0	.110	.345

Italicized values highlight significant correlations ($P < 0.05$)

regardless of classification method, no differences were detected for any fine root trait considered. In the case of fine root turnover, both smallest sub-classes (0–0.3; 0–0.5 mm) showed the highest turnover rate in comparison with the larger sub-classes. In general, for the first two sub-classes, fine root turnover was higher for the new sub-classes than the classical. For all soil depths investigated and when new sub-classes were considered, values showed an inverse relationship with fine root diameter. In contrast, fine root turnover for classical sub-classes did not follow a regular pattern with fine root diameter.

4 Discussion

Although fine root (diameter < 2 mm) dynamics has a central role in the global carbon budget (Norby and Jackson, 2000; Hendricks et al. 2006; Brunner et al. 2013; Laliberté 2017), fine root traits such as mass, length, lifespan, and annual production, as well as the relationship with environmental drivers are poorly known compared to aboveground compartments (Brunner et al. 2013; Lehtonen et al. 2015). In addition, root system architecture as the result of developmental process is scarcely understood because key traits such as branching/root

Table 2 Mean live fine root biomass (FRB), mean live fine root length (FRL), annual fine root production (FRP), fine root turnover rate (FRT), and specific root length (SRL) calculated for classical and new sub-classes at different soil depth (0–10, 10–20, and 20–30 cm)

Soil depth (cm)		Sub-class 0–1.0 mm				Sub-class 1.0–2.0 mm		0–2.0 mm
		Classical segregation method		New segregation method		Common to classical and new segregation methods	Total	
		0–0.5	0.5–1.0	0–0.3	0.3–1.0			
0–10	FRB (g m^{-2})	16.2 ± 1.3	30.0 ± 2.8	5.8 ± 0.5	52.3 ± 2.2	55.3 ± 4.5	98.5 ± 5.3	
	FRL (m m^{-2})	856 ± 82	170 ± 11	590 ± 63	437 ± 32	65 ± 6	1092 ± 58	
	FRP (g m^{-2})	14.8	14.7	6.7	30.6	27.9	84.0	
	FRT (year^{-1})	0.91	0.49	1.16	0.59	0.50	0.67	
	SRL (m g^{-1})	53.0 ± 1.0	5.0 ± 0.1	101.2 ± 1.0	8.8 ± 0.1	1.10 ± 0.02	13.7 ± 0.6	
10–20	FRB (g m^{-2})	6.1 ± 0.5	13.6 ± 1.3	2.1 ± 0.3	22.5 ± 2.4	28.8 ± 3.2	39.7 ± 2.7	
	FRL (m m^{-2})	317 ± 29	77 ± 8	213 ± 21	181 ± 17	33 ± 5	427 ± 25	
	FRP (g m^{-2})	7.9	11.7	2.8	22.3	27.7	60.8	
	FRT (year^{-1})	1.31	0.86	1.32	0.99	0.96	0.87	
	SRL (m g^{-1})	52.6 ± 1.0	5.2 ± 0.1	101.8 ± 1.1	9.0 ± 0.2	1.11 ± 0.03	15.5 ± 0.9	
20–30	FRB (g m^{-2})	5.2 ± 0.5	9.0 ± 1.0	2.0 ± 0.3	16.0 ± 2.0	16.7 ± 2.4	26.5 ± 2.3	
	FRL (m m^{-2})	283 ± 29	49 ± 6	201 ± 21	131 ± 15	18 ± 3	350 ± 25	
	FRP (g m^{-2})	5.6	9.3	2.6	15.8	12.4	31.1	
	FRT (year^{-1})	1.07	1.03	1.33	0.99	0.74	0.76	
	SRL (g m^{-1})	58.6 ± 1.6	5.6 ± 0.1	105.5 ± 1.3	10.1 ± 0.3	1.18 ± 0.03	21.0 ± 1.1	

FRB and FRL values are the mean of 96 samples ± SE

diameter remain uncertain (Wu et al. 2016). Therefore, defining fine roots with arbitrary diameter thresholds has not helped clarify fine root dynamics and development (Iversen 2014; McCormack et al. 2015) because the fine root pool has to be approached as a heterogeneous group (McCormack et al. 2015; Iversen et al. 2017). Moreover, although not considered in the present analysis, the assumption of a constant diameter along the root axes may lead to a significant misestimation of root traits (Rose 2017). The lack of a powerful method for a precise identification of order- and diameter-based thresholds of these different pools is a central, vexing problem; resolution of these uncertainties would significantly aid the identification of fine root form, and its impact on dynamics and development (McClaugherty et al. 1982; Montagnoli et al. 2014; Wu et al. 2016).

Here, we presented an integrated modification of the traditional method for the species-specific, high-resolution identification of fine root sub-classes. When the high-resolution method was applied, we found that the fine root population of beech segregates differently, for two out of three diameter sub-classes, from those obtained by the classical method—this supports our first hypothesis. Furthermore, our model showed that fine root length was influenced by external factors such as sampling season, soil depth, and stand characteristics as demonstrated in previous works (Montagnoli et al. 2012; 2014). However, after data transformation, the model was able to show the inherent dimensional information independently from the external factors. This finding, deriving from our data treatment, concurs with the conceptual model proposed by Pierret et al. (2016) where root growth has a genetically pre-set developmental scheme, which is then modulated from local biophysical conditions. In addition, Kong et al. (2014) found that, different from root architecture, diameter-related root traits were highly conserved phylogenetically. This was also supported recently by Liese et al. (2017) who found a significant phylogenetic signal for root diameter trait as results of the analysis of 14 traits of 13 temperate tree species of Central Europe. Therefore, many species have the capacity to substantially alter architectural traits, senior to root topology and distribution (Lynch 1995; Iversen et al. 2014; Kong et al. 2014; Liese et al. 2017), as these largely change with the availability of soil resources (Weemstra et al. 2016). Morphological traits of fine roots may also respond to soil resource availability with thicker fine roots (i.e., radial growth) that live longer and conserve valuable resources (Weemstra et al. 2016), or increasing length values of very fine roots (i.e., longitudinal growth and/or production of new thinner fine roots) in relation to water shortage and high temperature (Montagnoli et al. 2012a, 2014). This may imply the variation in the branching pattern (Liese et al. 2017) and the root tip density (Iversen et al. 2014), as well as root distribution (Lynch 1995). Not less important, other factors such as ectomycorrhizal community structure and soil characteristics

(i.e., organic matter, nitrogen content, bulk density), although not considered in this work, have a strong direct influence on fine root branching order and morphology (Ostonen et al. 2009; Freschet et al. 2017; Razaq et al. 2017).

In our model, the changing of thresholds that characterize the first two sub-classes resulted in a better fit with all measured fine root traits, supporting our second hypothesis. This is reasonable because the thicker part (i.e., 0.3–0.5 mm) of the fine root belonging to the first classical sub-class (0–0.5 mm) was included in the second new sub-class (0.3–1.0 mm). The topological analysis revealed, when new sub-classes generated by our method were applied, a more precise grouping in branching order with the smallest diameter sub-classes (0–0.3 and 0.3–1.0 mm) in comparison to the classical method. Although to be defined as site-specific, this result concurs with what was experimentally found by Beyer et al. (2013), where roots of beech saplings of the first- and second-order occurred with diameters less than 0.3 mm. In addition, the mean value of SRL found in Beyer et al. (2013) for the first- and second-root order is quite consistent with our SRL values when the smaller new diameter sub-class was considered (0–0.3 mm). As already mentioned above, this improved resolution of relationship between branching order and fine root diameter afforded by our model can help improve understanding of fine root development and architecture (Wu et al. 2016). Moreover, the development of more automated image-based procedures provides the advantage of reducing the time-consuming nature of data collection. Indeed, although applied to samples of *Fagus sylvatica* only, our integrated method showed that such measurements could be easily applied to each experimental species, for a large number of non-intact samples (i.e., soil core technique).

When fine root biomass, length, and productivity were analyzed in relation to sub-classes, we found lower values in the first-order subclass and higher values in the second-order subclass derived by our method compared with the classical approach, providing important insight on the turnover rate of the corresponding branching-orders. In particular, fine root turnover rate was higher (i.e., faster) if new sub-classes were applied. The statistical significance of the regression analysis between fine root length and soil temperature was observed only for the smallest fine root sub-class using either the classical or the new classification. However, when the diameter thresholds outputting of our model were used, a higher significance of regression than the classical one was yielded. These findings are supported by previous work on beech (Montagnoli et al. 2014) that demonstrate how the growth of the very fine root fraction, mainly longitudinal type, is driven by soil temperature. Moreover, our findings are also consistent with Bjork et al. (2007) and Makita et al. (2011) who reported a morphological plasticity of roots especially in the finest fine root

fraction, which belongs to the first- and second branching-order, being the most sensitive to environmental factors (Montagnoli et al. 2012a, b; McCormack et al. 2015; Ostonen et al. 2017). Another interesting aspect that emerged is that at deeper soil layers, where relationships between fine roots and environmental cues are usually lower (Hendrick and Pregitzer 1996), our model yielded significant effects that the classical approach failed to detect. Thus, these findings make the assumed causal relationship between soil temperature and fine root elongation highly plausible and highlights that the application of our integrated method has the potential to unveil ecological mechanisms occurring in fine roots dynamics. We believe that one reason for the effectiveness of our model is that our data used for model construction only contains two main sources of variance. The quantitative source of variance is linked to the variability of the amount of roots found in each sample. The qualitative source of variance, because our dataset relied on the intra-annual dynamics of root architecture and branching order, is linked to the change of proportion between fine root sub-class pools due to seasonality (Konopka et al. 2005; Hartevelde et al. 2007; Brassard et al. 2009; Montagnoli et al. 2012a,b, 2014; Maeght et al. 2015). In this study, data transformation removed the quantitative variance while maintaining the qualitative variance to allow defining species-specific new fine root sub-classes. Therefore, we stress that for a model, it is fundamental to analyze data obtained from seasonal sampling and a specific plant species.

5 Conclusion

Our species-specific integrated technique provided a better resolution than the classical approach to segregate fine root population of *Fagus sylvatica* into diameter classes. Thus, we were able to fit fine root diameters with branching order and dynamics in relation to environment (i.e., soil temperature and depth). Our model has the potential to reduce the time-consuming nature of data analysis, to unveil ecological mechanisms of fine root dynamics, and to make the diameter classification more precise and trustworthy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Amendola C, Montagnoli A, Terzaghi M, Trupiano D, Oliva F, Baronti S, Miglietta F, Chiatante D, Scippa GS (2017) Short-term effects of biochar on grapevine fine root dynamics and arbuscular mycorrhizae production. *Agric Ecosyst Environ* 239:236–245
- Barlow PW (2010) Plastic, inquisitive roots and intelligent plants in the light of some new vistas in plant biology. *Plant Biosyst* 144:396–407
- Berntson M (1997) Topological scaling and plant root system architecture: developmental and functional hierarchies. *New Phytol* 135: 621–634
- Beyer F, Hertel D, Leuschner C (2013) Fine root morphological and functional traits in *Fagus sylvatica* and *Fraxinus excelsior* saplings as dependent on species root order and competition. *Plant Soil* 373: 143–156
- Bjork RG, Majdi M, Klemedtsson L, Jonsson LL, Molau U (2007) Long-term warming effects on root morphology, root mass distribution, and microbial activity in two dry tundra plant communities in northern Sweden. *New Phytol* 176:862–873
- Böhm W (1979) *Methods of studying root systems*. Springer, Berlin
- Brassard BW, Chen HYH, Bergeron Y (2009) Influence of environmental variability on root dynamics in northern forests. *Crit Rev Plant Sci* 28:179–197
- Brunner I, Bakker MR, Björk RG, Hirano Y, Lukac M, Aranda X, Børja I, Eldhuset TD, Helmisaari HS, Jourdan C, Konôpka B, López BC, Miguel Pérez C, Persson H, Ostonen I (2013) Fine- root turnover rates of European forests revisited: an analysis of data from sequential coring and ingrowth cores. *Plant Soil* 362:357–372
- Brunner I, Herzog C, Dawes MA, Matthias A, Sperisen C (2015) How tree roots respond to drought. *Front Plant Sci* 6:547–563
- Cannon WA (1949) A tentative classification of root systems. *Ecology* 30:542–548
- Comas LH, Bouma TJ, Eissenstat DM (2002) Linking root traits to potential growth rate in six temperate tree species. *Oecologia* 132:34–43
- Di Iorio A, Giacomuzzi V, Chiatante D (2015) Acclimation of fine root respiration to soil warming involves starch deposition in very fine and fine roots: a case study in *Fagus sylvatica* saplings. *Physiol Plant* 156:294–310
- Edwards NT, Harris WF (1977) Carbon cycling in a mixed deciduous forest floor. *Ecology* 58:31–437
- Eissenstat DM, McCormack ML, Du Q (2013) Global change and root lifespan. In: Eshel A, Beeckman T (eds) *Plant Roots: The Hidden Half*. CRC Press, Boca Raton, pp 1–13
- Eissenstat DM, Wells CE, Yanai RD, Whitbeck JL (2000) Building roots in a changing environment: implications for root longevity. *New Phytol* 147:33–42
- Freschet GT, Valverde-Barrantes OJ, Tucker CM, Craine JM, McCormack ML, Violle C, Fort F, Blackwood CB, Urban-Mead KR, Iversen CM, Bonis A, Comas LH, Cornelissen JHC, Dong M, Guo D, Hobbie SE, Holdaway RJ, Kembel SW, Makita N, Onipchenko VG, Picon-Cochard C, Reich PB, de la Riva EG, Smith SW, Soudzilovskaia NA, Tjoelker MG, Wardle DA, Roumet C (2017) Climate, soil and plant functional types as drivers of global fine-root trait variation. *J Ecol* 105:1182–1196
- Gill AR, Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. *New Phytol* 147:13–31
- Godbold DL, Fritz HW, Jentschke G, Meesenburg H, Rademacher P (2003) Root turnover and root necromass accumulation of Norway spruce (*Picea abies*) are affected by soil acidity. *Tree Physiol* 23: 915–921
- Guo DL, Mitchell RJ, Withington JM, Fan P-P, Hendricks JJ (2008) Endogenous and exogenous controls of root lifespan, mortality

- and nitrogen flux in a longleaf pine forest: root branch order predominates. *J Ecol* 96:737–745
- Harteveld M, Hertel D, Wiens M, Leuschner C (2007) Spatial and temporal variability of fine root abundance and growth in tropical moist forests and agroforestry systems (Sulawesi, Indonesia). *Ecotropica* 13:111–120
- Hendricks JJ, Hendrick RL, Wilson CA, Mitchell RJ, Pecos SD, Guo D (2006) Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. *J Ecol* 94:40–57
- Hendrick RL, Pregitzer KS (1992) The demography of fine roots in a northern hardwood forest. *Ecology* 73:1094–1104
- Hendrick RL, Pregitzer KS (1993) The dynamics of fine-root length, biomass, and nitrogen content in two northern hardwood ecosystems. *Can J For Res* 23:2507–2520
- Hendrick RL, Pregitzer KS (1996) Temporal and depth-related patterns of fine root dynamics in northern hardwood forests. *J Ecol* 84:167–176
- Hertel D, Leuschner C (2002) A comparison of four different fine root production estimates with ecosystem carbon balance data in a *Fagus-Quercus* mixed forest. *Plant Soil* 239:237–251
- Hertel D, Strecker T, Muller-Haubold H, Leuschner C (2013) Fine root biomass and dynamics in beech forests across a precipitation gradient – is optimal resource partitioning theory applicable to water-limited mature trees? *J Ecol* 101:1183–1200
- Holdaway RJ, Richardson SJ, Dickie IA, Peltzer DA, Coomes DA (2011) Species- and community-level patterns in fine root traits along a 120 000-yr soil chronosequence in temperate rain forest. *J Ecol* 99: 954–963
- Iversen CM (2014) Using root form to improve our understanding of root function. *New Phytol* 203:707–709
- Jackson RB, Mooney HA, Schulze ED (1997) A global budget for fine root biomass, surface area, and nutrient contents. *Proc Natl Acad Sci U S A* 94:7362–7366
- Iversen CM, McCormack ML, Powell AS, Blackwood CB, Freschet GT, Kattge J, Roumet C, Stover DB, Soudzilovskaia NA, Valverde-Barrantes OJ, van Bodegom PM, Violle C (2017) A global fine-root ecology database to address below-ground challenges in plant ecology. *New Phytol* 215:15–26
- Jackson RB, Schenk HJ, Jobbagy EG, Canadell J, Colello GD, Dickinson RE, Field CB, Friedlingstein P, Heimann M, Hibbard K, Kicklighter DW, Kleidon A, Neilson RP, Parton WJ, Sala OE, Sykes MT (2000) Belowground consequences of vegetation change and their treatment in models. *Ecol Appl* 10:470–483
- Joslin JD, Henderson GS (1982) A test of the budget method: a revised approach to the measurement of fine root turnover. In: Muller RN (ed) Central hardwood Forest conference: proceedings of the fourth meeting, 1982. University of Kentucky, Lexington, pp 220–228
- Kong D, Ma C, Zhang Q, Li L, Chen X, Zeng H, Guo D (2014) Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytol* 203:863–872
- Konôpka B, Yuste JC, Janssens IA, Ceulemans R (2005) Comparison of fine root dynamics in scots pine and Pedunculate oak in sandy soil. *Plant Soil* 276:33–45
- Laliberté E (2017) Below-ground frontiers in trait-based plant ecology. *New Phytol* 213:1597–1603
- Lehtonen A, Palviainen M, Ojanen P, Kallioikoski T, Nöjd P, Kukkola M, Penttilä T, Mäkipää R, Leppälampi-Kujansuu J, Helmisaari H-S (2015) Modelling fine root biomass of boreal tree stands using site and stand variables. *Forest Ecol Manag* 359:361–369
- Liese R, Alings K, Meier IC (2017) Root branching is a leading root trait of the plant economics spectrum in temperate trees. *Front Plant Sci* 8:315–327
- Lynch J (1995) Root architecture and plant productivity. *Plant Physiol* 109:7–13
- Liu B, He J, Zeng F, Lei J, Arndt SK (2016) Life span and structure of ephemeral root modules of different functional groups from a desert system. *New Phytol* 211:103–112
- Long Y, Kong D, Chen Z, Zeng H (2013) Variation of the linkage of root function with root branch order. *PLoS One* 8:e57153
- Maeght J-L, Gonkhamdee S, Clément C, Na Ayutthaya SI, Stokes A, Pierret A (2015) Seasonal patterns of fine root production and turnover in a mature rubber tree (*Hevea brasiliensis* Müll. Arg.) stand – differentiation with soil depth and implications for soil carbon stocks. *Front Plant Sci* 6:1022–1033
- Makita N, Hirano Y, Mizoguchi T, Kominami Y, Dannoura M, Ishii H, Finer L, Kanazawa Y (2011) Very fine roots respond to soil depth: biomass allocation, morphology, and physiology in a broad-leaved temperate forest. *Ecol Res* 26:95–104
- Matamala R, Gonzalez-Meler MA, Jastrow JD, Norby RJ, Schlesinger WH (2003) Impact of fine root turnover on forest NPP and soil C sequestration potential. *Science* 302:1385–1387
- McLaugherty CA, Aber JD, Melillo JM (1982) The role of fine roots in the organic matter and nitrogen budgets of two forested ecosystems. *Ecology* 63:1481–1490
- McCormack ML, Adams TS, Smithwick EA, Eissenstat DM (2012) Predicting fine root lifespan from plant functional traits in temperate trees. *New Phytol* 195:823–831
- McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari HS, Hobbie EA, Iversen CM, Jackson RB, Leppälampi-Kujansuu J, Norby RJ, Phillips RP, Pregitzer KS, Pritchard SG, Rewald B, Zadworny M (2015) Redefining fine roots improves understanding of belowground contributions to terrestrial biosphere processes. *New Phytol* 207:505–518
- Montagnoli A, Di Iorio A, Terzaghi M, Trupiano D, Scippa GS, Chiatante D (2014) Influence of soil temperature and water content on fine root seasonal growth of European beech natural forest in Southern Alps. *Italy Eur J For Res* 133:957–968
- Montagnoli A, Dumroese RK, Terzaghi M, Pinto JR, Fulgato N, Scippa GS, Chiatante D (2018) Tree seedling response to LED spectra: implications for forest restoration. *Plant Biosyst* 152:515–523
- Montagnoli A, Terzaghi M, Baesso B, Santamaria R, Scippa GS, Chiatante D (2016) Drought and fire stress influence seedling competition in oak forests: fine-root dynamics as indicator of adaptation strategies to climate change. *Reforesta* 1:86–105
- Montagnoli A, Terzaghi M, Di Iorio A, Scippa GS, Chiatante D (2012a) Fine-root morphological and growth traits in a Turkey-oak stand in relation to seasonal changes in soil moisture in the Southern Apennines. *Italy Ecol Res* 27:1015–1025
- Montagnoli A, Terzaghi M, Di Iorio A, Scippa GS, Chiatante D (2012b) Fine-root seasonal pattern, production and turnover rate of European beech (*Fagus sylvatica* L.) stands in Italy Prealps: possible implications of coppice conversion to high forest. *Plant Biosyst* 146:1012–1022
- Nadelhoffer KJ, Raich JW (1992) Fine root production estimates and belowground carbon allocation in forest ecosystems. *Ecology* 73: 1139–1147
- Norby RJ, Jackson RB (2000) Root dynamics and global change: seeking an ecosystem perspective. *New Phytol* 147:3–12
- Ostonen I, Puttsepp U, Biel C, Alberton O, Bakker MR, Lohmus K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A, Vanguelova E, Weih M, Brunner I (2007) Specific root length as an indicator of environmental change. *Plant Biosyst* 141:426–442
- Ostonen I, Tedersoo L, Suvi T, Lohmus K (2009) Does a fungal species drive ectomycorrhizal root traits in *Alnus* spp.? *Can J For Res* 39: 1787–1796
- Ostonen I, Truu M, Helmisaari H-S, Lukac M, Borken W, Vanguelova E, Godbold DL, Lohmus K, Zang U, Tedersoo L, Preem J-K, Rosenvald K, Aosaar J, Armolaitis K, Frey J, Kabral N, Kukumägi M, Leppälampi-Kujansuu J, Lindroos A-J, Merilä P, Napa Ü, Nöjd P, Parts K, Uri V, Varik M, Truu J (2017) Adaptive root foraging strategies along a boreal-temperate forest gradient. *New Phytol* 215:977–991

- Pierret A, Maeght J-L, Clément C, Montoroï J-P, Hartmann C, Gonkhamdee S (2016) Understanding deep roots and their functions in ecosystems: an advocacy for more unconventional research. *Ann Bot* 118:621–635
- Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL (2002) Fine root architecture of nine North American trees. *Ecol Monogr* 72:293–309
- Razaq M, Salahuddin S H-I, Sher H, Zhang P (2017) Influence of biochar and nitrogen on fine root morphology, physiology, and chemistry of *Acer mono*. *Sci Rep* 7:5367
- Rewald B, Rechenmacher A, Godbold DL (2014) It's complicated: intraroot system variability of respiration and morphological traits in four deciduous tree species. *Plant Physiol* 166:736–745
- Röderstein M, Hertel D, Leuschner C (2005) Above- and below- ground litter production in three tropical montane forests in southern Ecuador. *J Trop Ecol* 21:483–492
- Rose L (2017) Pitfalls in root trait calculations: how ignoring diameter heterogeneity can lead to overestimation of functional traits. *Front Plant Sci* 8:898
- Smithwick EAH, Lucash MS, McCormack ML, Sivandran G (2014) Improving the representation of roots in terrestrial models. *Ecol Model* 291:193–204
- Sun T, Mao Z, Han Y (2013) Slow decomposition of very fine roots and some factors controlling the process: a 4-year experiment in four temperate tree species. *Plant Soil* 372:445–458
- Terzaghi M, Di Iorio A, Montagnoli A, Baesso B, Scippa GS, Chiatante D (2016) Forest canopy reduction stimulates xylem production and lowers carbon concentration in fine roots of European beech. *For Ecol Manag* 379:81–90
- Terzaghi M, Montagnoli A, Di Iorio A, Scippa GS, Chiatante D (2013) Fine-root carbon and nitrogen concentration of European beech (*Fagus sylvatica* L.) in Italy Prealps: possible implications of coppice conversion to high forest. *Front Plant Sci* 4:192–199
- Van Do T, Osawa A, Sato T (2016) Estimation of fine-root production using rates of diameter-dependent root mortality decomposition and thickening in forests. *Tree Physiol* 36:513–523
- Vogt KA, Persson H (1991) Measuring growth and development of roots. In: Lassoie JP, Hickenly TM (eds) *Techniques and approaches in forest tree ecophysiology*. CRC Press, Boca Raton, pp 477–501
- Ward JH (1963) Hierarchical grouping to optimize an objective function. *J Am Stat Assoc* 58:236–244
- Warren JM, Hanson PJ, Iversen CM, Kumar J, Walker AP, Wullschleger SD (2015) Root structural and functional dynamics in terrestrial biosphere models – evaluation and recommendations. *New Phytol* 205:59–78
- Weemstra M, Mommer L, Visser EJW, van Ruijven J, Kuyper TW, Mohren GMJ, Sterck FJ (2016) Towards a multidimensional root trait framework: a tree root review. *New Phytol* 211:1159–1169
- Wu Q, Pagès L, Wu J (2016) Relationships between root diameter, root length and root branching along lateral roots in adult, field-grown maize. *Ann Bot* 117:379–390
- Xia M, Guo D, Pregitzer KS (2010) Ephemeral root modules in *Fraxinus mandshurica*. *New Phytol* 188:1065–1074
- Zobel RW, Baligar VC, Kinraide TB (2007) Fine root diameters can change in response to changes in nutrient concentrations. *Plant Soil* 297:243–254
- Zobel RW, Waisel Y (2010) A plant root system architectural taxonomy: a framework for root nomenclature. *Plant Biosyst* 144:507–512