Root morphology and biomechanical characteristics of high altitude alpine plant species and their potential application in soil stabilization

DOI:10.1016/j.ecoleng.2017.05.048

Root morphology and biomechanical characteristics of high altitude alpine plant species and their potential application in soil stabilization

C. Hudek[1,2*], C. J. Sturrock[3], B. S. Atkinson[3], S. Stanchi[1], M. Freppaz[1]

[1]University of Torino, DISAFA, Largo Paolo Braccini, 2, 10095 Grugliasco (TO), Italy

[2]T2M, Marie Curie Cofund Fellow

[3]Hounsfield Facility, School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Nr Loughborough, LE12 5RD, UK

*chudek@unito.it

Abstract

Glacial forefields are young, poorly developed soils with highly unstable soil conditions. Root system contribution to soil stabilization is a well-known phenomenon. Identifying the functional traits and root morphology of pioneer vegetation that establish on forefields can lead us to useful information regarding the practical application of plants in land restoration of high altitude mountain sites.

This study aims to gather information on the root morphology and biomechanical characteristics of the 10 most dominant pioneer plant species of the forefield of Lys Glacier (NW Italian Alps).

X-ray Computed Tomography (X-ray CT) was used to visualize and quantify non-destructively the root architecture of the studied species. Samples were then cored directly from the forefield. Data on root traits such as total root length, rooting depth, root diameter, root length density and number of roots in relation to diameter classes as well as plant height were determined and compared between species. Roots were also tested for their tensile strength resistance.

X-ray CT technology allowed us to visualize the 3D root architecture of species intact in their natural soil system. X-ray CT technology provided a visual representation of root–soil contact and information on the exact position, orientation and elongation of the root system in the soil core. Root architecture showed high variability among the studied species. For all species the majority of roots consisted of roots smaller than 0.5 mm in diameter. There were also considerable differences found in root diameter and total root length although these were not statistically significant. However, significant differences were found in rooting depth, root length density, plant height and root tensile strength between species and life forms. In all cases root tensile strength decreased with increasing root diameter. The highest tensile strength was recorded for graminoids such as *Luzula spicata* (L.) DC. and *Poa laxa* Haenke and the lowest for *Epilobium fleischeri* Hochst.

The differences in root properties among the studied species highlight the diverse adaptive and survival strategies plants employ to establish on and thrive in the harsh and unstable soil conditions of a glacier forefield. The data determined and discovered in this study could provide a significant contribution to a database that allow those who are working in land restoration and preservation of high altitude mountain sites to employ native species in a more efficient, effective and informed manner.

Keywords: alpine species; glacier forefield; root phenotyping; soil stabilization; X-ray CT

1. Introduction

Glaciers in alpine regions are affected by climate change twice as much as the global average with respect to other ecosystems (Bradley et al., 2014) which results in accelerated glacial retreat. Retreating glaciers expose young soils that are low in nutrients (carbon and nitrogen) (Bradley et al., 2014; Lazzaro et al., 2010) and highly unstable (Matthews, 1999). Mass wasting and erosion processes are common in these forefields creating an inhospitable environment for plant colonization. Vegetation establishment on glacier forefields requires species with strong adaptive strategies and with high stress and disturbance tolerances (Robbins and Matthews, 2009). In spite of the harsh environment, vegetation cover increases quickly (Matthews, 1999) due to the rapid colonization of pioneer species. Pioneer species can grow quickly on nitrogen poor soils due to their high reproduction capacity and photosynthetic activity, (Stöcklin and Bäumler, 1996) and tolerance against abiotic stresses e.g., extreme temperatures, ultraviolet radiation, atmospheric pressure, shortage of mineral nutrients (Jones and Henry, 2003 Körner, 2003; Stöcklin et al., 2009).

Successful colonization and establishment of alpine species on glacial forefields may provide important information on the practical aspects of land reclamation and habitat restoration (Robbins and Matthews, 2009). Root traits (architectural, morphological, physiological and biotic) play an important role in the physical and even though the present study will not discuss further, the chemical development of young soils (Bardgett et al., 2014; Massaccesi et al., 2015) bringing about increased structural stability in the forefield (Bardgett et al.,2014) and decreasing the frequency and severity of any mass wasting and erosion processes. The biomechanical characteristics of roots such as tensile strength is a useful parameter for the quantification of the reinforcement potential; in particular for quantifying the added soil cohesion provided by plant roots. Determining the tensile strength of roots and their distribution in the soil profile can provide information on the increased shear strength of the soil provided by root reinforcement which can also determine plants' resilience to solifluction, frequently occurring in a periglacial environment (Jonasson and Callaghan, 1992). Quantitative data on root traits and architecture is one of the most significant variables considered when plants are evaluated for soil stabilization (Stokes et al., 2009). However data on root traits of alpine species remains scarce (Hu et al., 2013; Jonasson and Callaghan, 1992; Nagelmüller et al., 2016; Onipchenko 2014; Pohl et al., 2011; Zoller and Lenzin, 2006) which limits our understanding of the role these plants can play in root-soil interactions on the forefield.

Traditional techniques applied to examine the root system such as rhizotron or mini rhizotron, the use of paper pouches, synthetic soil media are all limited by the visual tracking of roots and/or creating an artificial environment that can lead to distorted/deceptive results. Destructive root phenotyping methods can also produce misleading results (Mooney et al., 2012) as they involve the separation of roots from the soil media meaning the relationship of the roots to the soil and to each other can no longer be observed (Pierrer et al., 2005). Additionally, repeated analysis on the same root system over time cannot be carried out e.g., dynamics of root growth or derivation of root demography (Koebernick et al., 2014).

Non-destructive imaging techniques such as Neutron Radiography, Magnetic Resonance Imaging (MRI) and X-ray Computed Tomography (X-ray CT) have been effectively used in root phenotyping as they overcome the limitations of traditional techniques and able to provide results on intact root systems in undisturbed soil. Research involving modeling (e.g., Water Erosion Prediction Project (WEPP) or Chemicals, Runoff and Erosion from Agricultural Management Systems (CREAMS)) also benefits from the enhanced quality of numerical data on root traits provided by these state of the art techniques (Lobet et al., 2015; Tasser and Tappeiner, 2005).

X-ray CT has already been successfully employed in many studies focusing on plant roots (e.g., Aravena et al., 2011; Mooney et al., 2006; Pierret et al., 1999; Wantanabe et al., 1992) to obtain clear, 3D images of intact root systems in the soil without the paramagnetic (Materials that are attracted by an externally applied magnetic field and form internal, induced magnetic fields in the direction of the applied magnetic field. (Boundless, 2016)) impact on the image quality found in MRI (Mooney et al., 2012; Koebernick et al., 2014). Whilst the majority of X-ray CT studies have been carried out on agricultural species such as wheat (Jenneson et al., 1999; Gregory et al., 2003; Mooney et al., 2006), maize (Lontoc-Roy et al., 2006), soybean (Tollner et al., 1994), potato (Han et al., 2008) and tomato (Tracy et al., 2012), a few studies can be found on tree roots (Pierret et al., 1999; Kaestner et al., 2006; Paya et al., 2015) and grasses (Pfeifer et al., 2015). As yet, no research has been carried out on the root architecture of alpine species under natural soil conditions using the X-ray CT.

In the majority of these studies, sieved, pre-prepared low organic content soils were used as the plant growth matrix, as the greater amount of organic particles can make root differentiation from soil particles more difficult, hampering root segmentation (process of partitioning a digital image into multiple segments). Moreover, the moisture distribution within undisturbed soil is more inconsistent which may also complicate the image segmentation process due to variations in image grayscale range of the roots under investigation (Pfeifer et al., 2015). While there have been a number of studies on the relationship between the natural soil matrix and the roots that permeate it, these studies have tended to focus on aspects of soil architecture rather than the architecture of the root (e.g., soil macropores, soil pore space) (e.g., Hu et al., 2016; Kuka et al., 2013).

The aim of the present study is to investigate and compare the root architecture and root traits of the ten most dominant pioneer plant species of the forefield of Lys Glacier (NW Italian Alps) in their natural soil system by producing accurate 3D images of their root system using X-ray CT. The value of the X-ray CT is verified by comparing the obtained results with other commonly employed techniques. Moreover, root tensile strength measurements will be made to understand the biomechanical role of the plant species on soil stabilisation. The retrieved information is discussed in the light of the potential future use of the studied species for slope soil reinforcement.

2. Materials and methods

2.1 Study site

Plant sampling was carried out on the recently deglaciated forefield of the Lys Glacier in the Aosta Valley (North West Italy). The glacial till was deposited in 2004 at an altitude of 2300 m above sea level on a bedrock of granitic gneiss and paragneiss belonging to the Monte Rosa nappe (D'Amico et al., 2014). The climate is alpine subatlantic with a mean annual rainfall of 1200 mm. The mean annual air temperature is -1 °C (Mercalli, 2003) with a winter temperature below -4 °C on average. The sampling site is south facing with a soil texture of loamy sand and an udic moisture regime (Soil Survey Staff, 2010). The chemical properties of the soil at the study site correspond to a slightly acidic soil (pH 5.8 - 6.7) with very low amounts of total nitrogen (TN) and total organic carbon (TOC) (0.002-0.017 g kg^{-1} and 0.018-0.217 g kg^{-1} respectively) with available phosphorus (P) of 1.3-4.7 mg g^{-1}. Pioneer alpine plants, mostly graminoid and forb species colonize the site (e.g., *Epilobium fleischeri* Hochst., *Linaria alpina* (L.) Mill., *Trisetum distichophyllum* (Vill.) P. Beauve.), a detailed vegetation survey of the moraine can be found in D'Amico et al. (2014).

2.2 Sampling approach

The ten most common plant species of the forefield were selected. These were sampled between August and September 2015; *E. fleischeri*, *T. distichophyllum*, *Trifolium pallescens* Schreb., *Luzula spicata* (L.) DC., *Silene exscapa* All., *Minuartia recurva* (All.) Schinz and Thell., *Festuca halleri* All. *Poa laxa* Haenke, *Salix helvetica* Vill. and *Leucanthemopsis alpina* (L.) Heyw (Table1). A total of 60 soil columns, (i.e. 6 columns per species) were excavated. During sampling, special care was taken to avoid individuals with any visible neighbouring plant effects (Gaudet and Keddy, 1988) and to keep plant size as equal as possible for all 60 samples. One sample from each species was cored 10 samples in total) with their own PVC cylinder (maximum sample height of 20 cm x diameter of 7.4 cm). After coring, the ten soil columns were carefully secured and placed in plastic bags and transported to the laboratory. In the laboratory the cored samples were placed in a climate chamber until the X-ray CT tests were undertaken. The climate chamber was set to provide conditions so as to delay root decay using a photoperiod of 14 hours, a relative humidity of 65 % and temperatures of 15 °C by day and 10 °C by night.

The remaining five replicates of each species (a total of 50) were excavated with a trowel. The 50 soil columns containing the root system of the individuals were placed in plastic bags, transported to the laboratory and stored at 3.5 °C until measurements were undertaken (Bast et al., 2015).

Table1.

Species	Common name	Life form	Succession	Family
Epilobium fleischeri Hochst.	Alpine willowherb	Forb	Early	Omagraceae
Trisetum distichophyllum (Vill.) P.Beauve.	Tufted hairgrass	Graminoid	Early	Poaceae
Trifolium pallescens Schreb.	Pale clover	Forb	Early	Fabaceae
Luzula spicata (L.) DC.	Spiked woodrush	Graminoid	Mid	Juncaceae
Silene exscapa All.	Moss campion	Forb	Mid	Caryophyllaceae
Minuartia recurva (All.) Schinz and Thell.	Recurved sandwort	Forb	Late	Caryophyllaceae
Festuca halleri All.	Haller's Fescue	Graminoid	Late	Poaceae
Poa laxa Haenke	Banff Bluegrass	Graminoid	Ubiquitous	Poaceae
Salix helvetica Vill.	Swiss willow	Dwarf shrub	Ubiquitous	Salicaceae
Leucanthemopsis alpina (L.) Heyw.	Alpine Moon Daisy	Forb	Ubiquitous	Asteraceae

2.3 Non-destructive root phenotyping

The cored samples from the PVC cylinder were scanned using a Phoenix V|TOME|X M 240 high resolution X-ray CT system (GE Sensing and Inspection Technologies, Wunstorf, Germany). The scanning parameters (Table 2) were optimized to allow balance between a large field of view and a high resolution. Due to the height of the cylinder (20 cm) two separate scans (upper and lower part of the sample) were made to cover and image the entire sample. Each sub-scan was then reconstructed using DatosRec software (GE Sensing and Inspection Technologies, Wunstorf, Germany) and then manually combined in VG Studio MAX v2.2 (Volume Graphics GmbH, Heidelberg, Germany) and exported as a single 3D volumetric dataset. To distinguish the root system from the soil material image processing techniques were applied. Roots were segmented from the reconstructed CT data by using the region growing method (Gregory et al., 2003) in VG Studio MAX v2.2. Quantification of 3D root traits was undertaken using RooTrak software (Mairhofer et al., 2012). RooTrak was able to provide quantitative data on the root volume (total mass of the root system; mm^3), root area (root area in direct contact with the soil; mm^2), the root system's maximum vertical and horizontal length (mm) as well as the convex hull (the region of soil explored by the root system; mm^3) (Mairhofer et al., 2015).

Table 1 Scanning parameters for X-ray CT.

2.4 Destructive root phenotyping

Following X-ray CT scanning, the roots were extracted from the soil column by carefully cleaning the soil matrix from the roots with a water jet under a sieve mesh to retain remnants of roots that may come loose during the cleaning process. The washed roots were then placed into a 15 % ethanol solution and stored at 3.5 ºC. Then the root systems were scanned with a flatbed scanner (EPSON Expression 11000XL). The images from scanning had a 600 dpi resolution and were used for two dimensional image analysis. This was with the aim to compare the CT scanned results with the results of a, traditional technique (Paez-Garcia et al., 2015). Root traits such as total root length, average root diameter, and the root system’s maximum vertical and horizontal length were considered for analysis.

The remaining 50 plant samples (five replicates of each species were followed the same cleaning, storing and scanning method as before . All 2D scanned images were analyzed with the WinRHIZO 2013e and ImageJ software. The data collected on root traits were total root length, root length distribution (%) in different diameter classes, average root diameter, root length density, rooting depth and total plant height. Additionally plant height was measured according to the standardized measurement of plant functional traits (Pérez-Harguindeguy et al., 2013).

2.5 Root tensile strength

Root tensile strength tests were performed to determine root resistance to breaking under tension (Bischetti et al., 2005; Pohl et al., 2011). The complete root system, kept in a 15 % ethanol solution was first cut into individual root segments. Randomly selected undamaged roots with the widest available range of diameters were then selected for testing. Before testing, root diameter at three points of the root segment were measured with a digital caliper to obtain the average root diameter of the individual root sample. This is necessary as the exact position of root rupture is unknown before testing.

Root tensile strength were measured in the laboratory using an electromechanical universal testing machine, MTS Criterion, Model 43 (MTS Systems, Eden Prairie, MN, USA). Plant roots were secured between clamps at both ends. The clamps consist of two metal discs (washers) covered with drafting tape holding the roots in place. The speed reduction of the device was maintained at a steady 10 mm min^{-1} as it was suggested in other studies (Bischetti et al., 2005; Bordoni et al., 2016; De Baets et al., 2008; Yang et al., 2016) and the tensile force was measured by a load cell (500N) connected to a computer to record the results. Roots broke when they could no longer resist tensile force. Measurement results were excluded from data analysis when root rupture occurred near the clamp. Measurement considered to be successful when the rupture occurred in the middle of the root section

2.6 Statistical analysis

In the present study comparative data analysis on root traits between the non-destructive and destructive technique was only respected when comparing the maximum vertical and horizontal length of the root system due to the lack of data available on very fine roots (< 0.5 mm) on the 3D images.

Results obtained from X-ray CT scanning (RooTrak) on the root system's maximum vertical and the maximum horizontal length were compared with results obtained from the destructive method (ImageJ) by applying Pearson's correlation test. Once the normality and homogeneity of variance were verified a one-way analysis of variance (ANOVA) was used to detect differences in the measured root properties (root length density, total root length, mean root diameter, rooting depth, root length distribution within diameter classes) and plant height among the studied species. In cases when significant differences were found between the groups, the Tukey post hoc test was run to detect where the differences occurred between the groups.

The relationship between root tensile strength and root diameter was evaluated by fitting a regression curve (power law equation). Analysis of covariance (ANCOVA) was performed to compare tensile strength results between the 10 studied species and to take root diameter into consideration as a covariant. Both tensile strength and root diameter values were log transformed before the analysis. All assumptions were tested before carrying out ANCOVA (linearity, homogeneity and normality) . All statistical analysis was carried out using the statistical software SPSS Statistics 22 (IBM SPSS, 2013).

3. Results

3.1 Non-destructive root phenotyping

X-ray CT was successfully used to reveal the 3D root architecture of the studied 10 species. Tap roots and thicker lateral roots (diameter >0.5 mm) were identified in all cases while individual examples of thinner lateral roots (diameter < 0.5 mm) were only identified for *S. helvetica*, *P. laxa*, *L. spicata* and *F. halleri,* (diameters of 0.35, 0.35, 0.25 and 0.25 mm, respectively). Even though it was not possible to extract the entire root system, a visual representation of root–soil contact in the undisturbed position, orientation and elongation of the core root system was possible. It should be noted that due to the size limitation of the PVC cylinder and the difficulty of identifying root position when coring, the tap root and/or lateral roots were cut off by the edge of the cylinder therefore the max vertical and horizontal root length in the present study is approximate and should only be taken into consideration as part of data validation for RooTrak.

The maximum vertical and horizontal root length data obtained from the 3D images were underestimated by an average of 42% and overestimated by 26% respectively when compared to measured data with ImageJ. The results from the Pearson's correlation tests between RooTrak and ImageJ showed a week positive correlation ($r = 0.57$, $p = 0.084$) for maximum vertical root length (Figure 3a) and a very week negative correlation ($r = -0.38$, $p = 0.275$) for the maximum horizontal root length (Figure 3b). Because the p-values are greater than the significance level of 0.05, there is inconclusive evidence about the significance of the association between the variables.

The highest root volume, root area and convex hull (Table 3) were all recorded for *T. pallescens* (1530 mm^3, 7752 mm^2, 505384 mm^3 respectively). The lowest root volume was recorded for *M. recurva* and *P. laxa* (144 and 150 mm^3 respectively) while the lowest value of root area (1146, 1547 and 1677 mm^2) and convex hull (24117, 45612, 60237 mm^3) was recorded for *S. helvetica, P. laxa* and *M. recurva* respectively. Results from *F. halleri* and *L. spicata* were excluded from the comparison as it was difficult to identify and segment the high number of fine (< 0.25 mm), overlapping roots and in many cases it was not possible at all. Therefore including the results of *F. halleri* and *L. spicata* would have caused misleading overall results.

Table 3. Values of root traits analyzed with RooTrak (volume, area, maximum vertical and horizontal length of the root system, convex hull), ImageJ (maximum

vertical and horizontal length of the root system) and WinRHIZO (total root length and average root diameter) of the X-ray CT scanned samples.

The highest total root length was recorded for *T. distichophyllum*, *L. spicata* and *S. exscapa* (192.7, 100.3 and 95.3 m respectively) and the lowest for *P. laxa* and *F. halleri* (10.5 and 20.7 m respectively). The rest of the species results fell between the values of 50.5 and 62.2 m (Table 3).

Average root diameter ranged between 0.16 and 0.31 mm. The lowest root diameters were recorded for *L. spicata* and *E. fleischeri* (0.16 mm and 0.17 mm respectively) and the highest for *P. laxa* and *T. pallescens* (0.31 mm and 0.30 mm respectively) (Table 3).

Figure 3 *a.,* Linear correlation between RooTrak and ImageJ data on the maximum vertical and *b.,* horizontal root length for the 10 studied alpine species.

The overall root architecture for each species displayed considerable variation (Figure 1 a-j). To determine and differentiate root system architecture between the species the root type classification established by Lichtenegger and Kutschera, (1991) was applied:

E. fleischeri showed a dominant pole root system with strong horizontal root spreading indicating the intense clonal growth of the plant. *T. pallescens* showed a cone shape and *S. exscapa* a wider cone shape upward extended root type. *S. helvetica* and *M. recurva* had a discoid shaped root system due to the shallow depth of rooting but large lateral spreading. *P. laxa*, *F. halleri* and *L. spicata* all showed a cone shape downwards dilated root type while *L. alpine* had an umbrella shaped and *T. distichophyllum* a cylindrical shaped root type.

Figure1. Root architecture of the 10 studied pioneer alpine species detected by X-ray CT scanning. Scale bars: a., 35 mm, b., 25 mm, c., 40 mm, d., 15 mm, e., 10 mm, f., 15 mm, g., 15 mm, h., 30 mm, i., 45 mm, j., 20 mm.

Figure 2 *a.,* Image of the core root system *b.,* the core root system in relation to the soil matrix and *c.,* the washed entire root system of *T. pallescens*. Scale bar a., 45 mm b., 40 mm and c., the ruler uses cm.

The natural soil matrix showed a great variation in terms of soil structure among the cored samples. Figure 5 a-c shows examples of the structural diversity of the samples. The soil matrix in Figure 5 a., indicates a deposition of glacial till with little reorganization due to slope processes as Figure 5 b., and c., are fluvio-glacial and lake depositions with visible silt and sand layers.

Figure 5 Examples of the grayscale CT images of the soil matrices *a.,* glacial till with *T. distichophyllum b.,* and *c.,* fluvio-glacial and lake depositions.

3.2 Destructive root phenotyping

Root length density results varied greatly among the studied species (9.3–85 cm cm^{-3}). The lowest density was recorded for *E. fleisheri*, *M. recurva* and *T. pallescens*, with 9.3, 29 and 33 cm cm^{-3} respectively and the highest was recorded for *T.*

distichophyllum and *L. spicata* with 85 and 81 cm cm^{-3} respectively. There was significant difference found in root length density among the species (F (9, 22) = 4.78, p <0.001). Post-hoc comparisons using the Tukey HSD test indicated that root length density differed significantly (p <0.05) between *E. fleisheri* and *L. spicata*, *T. distichophyllum*, *S. helvetica* and *F. halleri* as well as between *M. recurva* and *L. spicata*. There was no statistically significant difference in root length density between the other species. However, the difference between *T. pallescens* and *L. spicata* showed a substantial trend toward significance (p=0.078) as well as between *M. recurva* and *T. distichophyllum* (p=0.062). Specifically, the results suggest that out of the ten studied species, only *E. fleisheri's* and *M. recurva*'s root system resulted in a significantly lower root length density when compared to the majority of the studied plants. It should be noted that in most but not all cases, higher root length density was found among the graminoid (*L. spicata, T. distichophyllum, F. halleri*) and the dwarf shrub (*S. helvetica*) species.

Total root length results (Table 4) showed no significant differences between the species (F (9, 39) =1.07, p=0.417) even though the mean results showed moderate variability among them (75.3–368.5 m). The shortest length was recorded for *E. fleischeri*, and *S. exscapa*, with 75.3 and 106.2 m respectively and the highest for *L. alpina* and *S. helvetica* with 368.5 and 342.3 m respectively.

Table 4 Plant height (mm), rooting depth (mm) measured with ImageJ, total root length (m), mean root diameter (mm) and root length density (cm cm-3) of the 10 studied alpine species measured with WinRhizo.

Table 5 shows the root length distribution in different diameter classes (%). Eight out of ten species had their highest root count (57-36 %) in the diameter class 0<L<=0.1 mm with the exception of *T. distichophyllum* and *S. helvetica* which had it at 0.1<L<=0.2 (41 %) and 0.2<L<=0.3 mm (37 %) respectively. *T. pallescens* and *S. helvetica* also had roots larger than 2 mm in diameter as the other species rarely exceeded 1 mm in diameter.

Table 5 Root length distribution (%) of the 10 pioneer alpine plants in relation to different diameter classes (mm).

Figure

The mean root diameter results (Table 3) also showed no significant differences between the species (F (9, 22) =1.78, p=0.129) values. The results ranged between 0.21 mm and 0.47 mm. The lowest mean root diameter was recorded for *T. distichophyllum* with 0.21 mm and the highest for *T. pallescens* with 0.47 mm.

Rooting depth results (Figure 6), determined by ImageJ showed considerable variation among the species, ranging from 9 to 19.7 cm. The deepest penetrating root system was recorded for *E. fleischeri* and the shallowest for *S. helvetica*. A one-way ANOVA was used to compare the rooting depth results between the 10 species which showed significant difference at F (9, 38) = 2.38, p <0.03. The Tukey HSD test indicated that *E. fleischeri* had a significantly (p <0.05) longer rooting depth than *S. helvetica* and *F. halleri*.

Plant height also varied between the species, ranging from 15 to 65 cm. The highest plant height was recorded for *E. fleischeri* and the lowest for *M. recurva*. There was significant differences found at $F(9, 29) = 57.73$, $P < 0.000$ between the studied species.

Figure 6 Plant height (cm) and rooting depth (cm) of the 10 studied alpine plant species.

3.3 Root tensile strength

There was a great variation in the tensile strength results among the studied species (Table 5). The highest mean tensile strength was found at the graminoid and shrub species ranging between 138-86 MPa and the lowest among the forbs ranging between 60-29 MPa. The results showed that graminoid species have comparable tensile strength results to the dwarf shrub *S. helvetica*. When the significant differences were tested between the studied species taken root diameter into consideration as a covariate the results showed significant differences between the studied species at F()=, p<0.

Tensile strength and the related root diameter values were plotted (Figure 7) to show the relationship between root tensile strength and root diameter which confirmed the power law relationship meaning that with increasing root diameter root tensile strength decreased.

Table 6 Life forms, the number of samples (n) tested, the range of root diameters (mm), root tensile strength (MPa) values, scale factor (α) rate of strength decrease (β) and the goodness of fit (R^2) of the 10 studied alpine species.

Table 7 ANOVA table with multiple comparisons of root tensile strength (MPa) between the studied plant species.

Figure 7 The relationship between root tensile strength (MPa) and root diameter (mm) for the 10 studied alpine species

4. Discussion

4.1 Non-destructive root phenotyping

The X-ray CT scanning has provided the first ever 3D images of the intact core root system of 10 different pioneer alpine plant species in their natural soil matrix. Visual information on the vertical and horizontal spreading as well as the rooting angle and branching of thicker roots in connection to the soil matrix were visible and could be important information when determining the significance of the root system on soil reinforcement in future studies (e.g. the resistance of the root system to uprooting or its protective role against shallow landsliding). During the use of X-ray CT several challenges and limitations were discovered; The following aspects made it difficult to decide on the scanning parameters: There was a limited amount (Stöckli and Bäumler, 1996; Pohl et al., 2011) or no data available on the root traits of the studied species prior to testing. They also had varying characteristics in terms of life form, family (Körner, 2003; Pignatti, 2003; Broglio and Poggio, 2008) and succession (Damico et al., 2014; Stöcklin and Bäumler, 1996) indicating different root architecture and anatomy. Additionally they had never been subject to study with current state of the art phenotyping techniques. The samples were cored from their natural habitat in a heterogenic soil matrix and the soil absorbed a high level of the X-rays resulting in prohibitively long scans to achieve the necessary beam penetration. The tracking of individual roots during segmentation was extremely difficult as the heterogenic soil matrix made it difficult to differentiate roots from other organic particles in the soil (Figure 4 a, b, and c). Additionally the root system contained vast amounts of overlapping roots and neighbouring plant roots were invariably cored together with the test sample even when, from the surface, samples appeared free from any neighbouring plant effects.

Roots with a diameter >0.5 mm are visible on the 3D images, these thicker roots allow us to estimate the location of thinner roots (Stokes et al., 2009). Not being able to detect the thinner roots on the present 3D images was not due to the limitations of the X-ray CT technology, rather the issue of resolution, sample size and the heterogenic soil matrix. In general, in homogeneous background the minimum resolution should be set twice as high as the cored sample is long in millimeters and set even higher if the background is heterogenic (Kaestner et al., 2006). A higher resolution setting however would have resulted in a prohibitively prolonged scanning and segmenting time. The method suggested by Kaestner et al. (2006) was successful at detecting roots with a diameter <0.5 mm in homogeneous background however roots in heterogeneous soil matrix (Figure 4 a-c) remained challenging. Cored samples of reduced length and diameter may have allowed for the detection and segmentation of the finer roots within the system but the compromise would be the smaller PVC cylinders would not have been suitable for sampling the species from the field without causing damage i.e. preventing disturbed soil conditions within the sample. A factor to possibly bear in mind for future work conducted on alpine species with fine root systems would be to take two sets of cores when assessing the different scales in root architecture.

Interestingly, although it was not possible to segment using the available software; many of the fine roots were often visible to the naked eye when manually scrolling through the greyscale images providing a unique insight into the complexity of these alpine species.

4.2. Analysis of root architecture and root traits

S. exscapa and *T. pallescens* both have a dominant tap root morphology with a large number of tillers. Their tap root and thicker lateral roots are often found growing through cracks in the bedrock thereby anchoring the plant and stabilizing the soil from shallow landsliding. The number of lateral roots and the diversity of their branching angles resulting in a larger shear zone indicate an increased soil stability (Abe and Ziemer, 1991). Both *S. exscapa* and *T. pallescens* have dense, fine root networks that can play an important role in reducing soil erosion. Root nodules are clearly visible on the roots of *T. pallescens* reflecting the existing association the plant has with symbiotic nitrogen-fixing bacteria (Holzmann and Haselwandter, 1988).

S. helvetica also has a dominant taproot morphology with the potential of growing through cracks in the bedrock though it has a shallower rooting depth than *S. exscapa* or *T. pallescens*. *S. helvetica* has a large lateral spread in the upper soil layer with a dense fine root network which can provide increased support in soil erosion control and horizontal anchoring.

Due to the uniform length of the umbrella shaped root system of *L. alpina* it can be easily uprooted (cit), therefore, its potential as soil reinforcement is greatly limited although it is capable of trapping a significant amount of soil due to its dense fine root network (Hudek et al., 2017) and reducing soil erosion.

The dominant pole type of root system of *E. fleischeri* showed the greatest rooting depth with intensive rhizome spreading. The main feature of the plant's strategy is rapid colonization of open space through wide lateral clonal spreading (Stöckli and Bäumler, 1996) which is a typical strategy for early successional plants such as *Hieracium staticifolium* All., *Achillea moschata* (Wulfen) or *Cerastium pedunculatum* Gaudin (Stöckli and Bäumler, 1996). Its root system does not have notable anchoring properties, its survival strategy relies on an elaborate network of rhizome spreading, widely spaced ramets and rapid colonization (Alpandino, 2011). In this way the plant is able to quickly overcome diverse mass wasting processes. Additionally its short and fragile fine root (<1mm) network is unclearly able to provide additional soil stabilization (Bischetti et al., 2009) even though plant biomass and allometry are stated being a significant element when plants are evaluated for soil-root reinforcement (Gonzalez-Ollauri and Mickovski, 2016). In general the function of these roots is limited to water and nutrient uptake to support plant growth (Stokes et al., 2009; Tasser and Tappeiner, 2005).

T. distichofillum also uses horizontal spreading through clonal growth as a strategy for rapid colonization but with shorter distance between ramets (Alpandino, 2011). It also has a dense lateral root system with moderate rooting depth and a high percentage of fine and very fine roots throughout the entire root system. This can make the plant more resilient to uprooting and at the same time, through the elaborate network of rhizome spreading, able to overcome diverse mass wasting processes (Körner, 2003). Its dense fine and very fine roots trap soil providing erosion control. *P. laxa* is a plant with clumped clonal growth form with short distance between ramets. *F. halleri* and *L. spicata* both form compact tussocks with a dense fibrous root system. This phalanx type of clonal growth results in a slow horizontal spreading (Alpandino, 2011). These types of root morphology can make the plants extremely resilient to uprooting and a potentially effective plant in erosion control.

The root architecture of the species showed a wide range of root types dictated by genetic characteristics (Gray and Sotir, 1996) and environmental factors e.g., nutrient availability or soil temperature (Nagelmüller et al., 2016; Khan et al., 2016). Root plasticity too has effects on root architecture, it is essential in coping with and overcoming stress (Bardgett et al., 2014; Poorter et al., 2012; Stöcklin and Bäumler, 1996) as well as strengthening the resilience of pioneer species to the harsh environmental conditions.

Even though *E. fleischeri* had a significantly higher rooting depth compared to the other species, in general, rooting depth was uniformly shallow which is in line with previous findings (Lichtenegger, 1996; Jonasson and Callaghan, 1992; Pohl et al., 2011) on alpine species. This is influenced by two main controlling environmental factors; soil temperature and water availability (Lichtenegger, 1996; Körner, 2003). Alpine vegetation in general have a shallower rooting system than species from lowlands as at high altitudes with increasing soil depth, soil temperature and water fluctuations decrease at a higher rate than in the lowlands (Lichtenegger, 1996). This also can reflect on root distribution within the different soil horizons, indicating that the high root density in the upper soil layer quickly decreases with increasing soil depth (Lichtenegger, 1996).

Root length density has a great influence on soil stability (Bardgett et al., 2014; Stokes et al., 2009) by altering the hydrological properties of the soil and increasing the resistance of the roots for disruptive forces. All studied species had a large amount of fine and very fine roots which is common in alpine species (Körner, 2003; Pohl et al., 2011). In general, fine and very fine roots have a rapid turnover supplying a large amount of carbon to the soil and increasing the organic content of the soil. Together with the physical and chemical contribution they gradually increase the aggregate stability of the soil which reduces the susceptibility of the soil to erosion processes (Pohl et al., 2011; Hudek et al., 2017). Additionally, both live and dead roots provide potential preferential flow paths in hillslopes, securing the stability of the soil by reducing pore water pressure (Ghestem et al., 2011). On the other hand, bypass flow can lead to perched water tables, saturating the soil that can develop positive pore-water pressure that could trigger landslides (Ghestem et al., 2011). Glacier forefields are nutrient limited soils; fine and very fine roots (< 0.5 mm) however, provide strong symbiotic links between the plant and the fungus systems and it has been proven that mycorrhizal fungi increases the water and nutrient uptake of the plant (Smith and Read, 2008) and promotes root growth (Ola et al., 2015) which also influences RLD (Bast et al., 2014; Graf and Frei 2013; Tisdall, 1991). The dense fine root system of the studied species is also able to mechanically bind the soil particles thereby contributing to increased soil stabilization (Pohl et al., 2011; Norris et al., 2008).

In the present study the total root length values showed non-significant difference between the species and life forms while the highest values were recorded among

the graminoid species as was with the work of Pohl et al. (2011) though in the present study the measured values greatly exceed those of Pohl et al. (2011). This can be attributed to the fact that at the sampling site of Pohl et al., (2011) sampling was carried out on managed ski slopes where soil compaction inhibits root growth (Nagel et al., 2012; Pfeifer et al., 2014) while in the case of our study on the recently deglaciated forefield, sampling was performed on a site relatively free from human interference and soil compaction was not an inhibiting factor for root growth.
Under natural conditions species grow together creating a complex underground root network/structure due to the diversity of root types, enlarging the protective role of plants on soil stabilization at different levels and soil layers (Pohl et al., 2009; Reubens et al., 2007). Plant richness should therefore be encouraged when plants are considered for soil conservation purposes such as land reclamation.

4.3. Root tensile strength

The tensile strength results of the present study were 3-7 times higher than those found in literature data on the same alpine species (*L. spicata, L.alpina*) (Pohl et al., 2011) and other alpine and arctic graminoid and forb species (Pohl et al., 2011, Jonasson and Callaghan, 1992). Root tensile strength is mainly effected by the genetic properties of the plant (Gray and Sotir, 1996) while additional factors such as age (Reubens et al, 2007), ecological conditions and management practices(Bischetti et al., 2009) can result in varying tensile strength values for the same species. Gonzallez-Ollauri et al. (2017) highlighted that root tensile strength can vary with changes in root moisture content which closely links to soil moisture content (i.e. dry roots have a lower level of tensile strength compare to roots with optimum root moisture). Root diameter has direct influence on root tensile strength as root tensile strength is calculated by the ratio between the breaking force (N) and the root cross section area (mm^2) which depends on root diameter (Bischetti et al., 2016). In general, fine and medium size roots (in diameter 0.01-10.00 mm) have higher values of tensile strength compared to roots with a larger diameter (> 10.00 mm). Larger sized roots act primarily as individual anchors mobilising only a small amount of their tensile strength before slipping through the soil (Bischetti et al., 2005). However, fine and medium sized roots can mobilize their entire tensile strength and due to their higher surface area, have superior resistance to uprooting (Gray and Sotir, 1996). In the present study the diameter of the tested roots ranged between 0.03 mm and 1.66 mm, these values are smaller than what is found in the literature data which can be one of the explanation for the considerably higher tensile strength results. Additionally the samples in Pohl et al. (2011) were collected from a managed ski slope which confirms results observed by Bischett et al. (2009) that ecological conditions and management can alter tensile strength.

It was possible to demonstrate the significant relationship between tensile strength and root diameter the plotted tensile strength results can demonstrate the power law relationship and can be used to make comparisons between species.

5. Conclusions

This study aimed to provide information on root morphology and root traits on pioneer alpine species from a recently deglaciated site in the Italian Alps with the view to determine the plants’ efficiency in soil stabilization. To provide unique visual 3D data on the root architecture of a wide variety of alpine pioneer species under intact natural soil conditions, we applied a state of the art non-destructive plant phenotyping technique, X-ray CT. This is the first study that uses the X-ray CT technique to image the root system of alpine plants undisturbed in their natural alpine soil matrix.

Results showed great variation in global root architecture between the studied species. X-ray CT could successfully identify roots >0.25, 0.35 mm in diameter at the resolution used for scanning. With complementary use of destructive phenotyping techniques, quantitative data on root traits and the plants biomechanical characteristic allowed us to determine species' efficiency in soil stabilization. The high tensile strength results of graminoid and the dwarf shrub species combined with a dense elaborate root morphology, provide many anchoring points and enhanced plant resilience to solifluction in a periglacial environment. While forbs longer, anchoring root system with lower but comparable tensile strength to the garminoid and dwarf shrub species, could advocate their suitability as protection against shallow landsliding. With the exception of one or two species (*E. fleischeri, M. recurva*) all studied plants play an important role in soil erosion control due to their dense elaborate fine and very fine root system.

Acknowledgements

This research was enabled by the Transnational Access capacities of the European Plant Phenotyping Network (EPPN, grant agreement no. 284443) funded by the FP7 Research Infrastructures Programme of the European Union. As well as receiving funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 609402 - 2020 researchers: Train to Move (T2M). The Hounsfield Facility received funding from European Research Council (Futureroots Project), Biotechnology and Biological Sciences Research Council of the United Kingdom and The Wolfson Foundation.

The authors wish to thank Alessio Cislaghi, Enricho Chiaradia and Gian Battista Bischetti for the access to and support with the tensile testing machine and Michele Lonati for his help on the identification of graminoid species.

References

Abe, K., Ziemer, R.R., 1991. Effect of tree roots on a shear zone: modeling reinforced shear stress. Canadian Journal of Forest Research 21, 1012-1019.

Alpandino, 2011. Alpandino by Institute of Botany, University of Basel, Switzerland. https://www.alpandino.org

Aravena, J.E., Berli, M., Ghezzehei, T.A., Tyler, S.W., 2011. Effects of root-induced compaction on rhizosphere hydraulic properties X-ray microtomography imaging and numerical simulations. EnvSci & Tech, 45, 425–431.

Bardgett, R.D., Mommer, L., De Vries, F.T., 2014. Going underground: root traits as drivers of ecosystem processes. Trends in Ecology and Evolution, 29, 12, 692-699.

Bast, A., Wilcke, W., Graf, F., Lüscher, P., Gärtner, H., 2015. A simplified and rapid technique to determine an aggregate stability coefficient in coarse-grained soils. Catena, 127, 170-176.

Bischetti, G.B., Bassanelli, C., Chiaradia, E.A., Minotta, G., Vergani, C., 2016. The effect of gap openings on soil reinforcement in two conifer stands in northern Italy. Forest Ecology and Management, 359, 286–299.

Bischetti, G.B., Chiaradia, E.A., Epis, T., Morlotti, E., 2009. Root cohesion of forest species in Italian Alps. Plant and Soil, 324, 71-89.

Bischetti, G.B., Chiaradia, E.A., Simonato, T., Speziali, B., Vitali, B., Vullo, P., Zocco, A., 2005. Root strength and root area ratio of forest species in Lombardy (Northern Italy). Plant and Soil, 278, 11–22

Bordoni, M., Meisina, C., Vercesi, A., Bischetti, G.B., Chiaradia, E.A., Vergani, C., Chersich, S., Valentino, R., Bittelli, M., Comolli, R., Persichillo, M.G., Cislaghi, A., 2016. Quantifying the contribution of grapevine roots to soil mechanical reinforcement in an area susceptible to shallow landslides. Soil and Tillage, 163, pp. 195-206.

Boundless. "Diamagnetism and Paramagnetism." *Boundless Chemistry* Boundless, 08 Aug. 2016. Retrieved 03 Mar. 2017. https://www.boundless.com/chemistry/textbooks/boundless-chemistry-textbook/periodic-properties-8/electron-configuration-68/diamagnetism-and-paramagnetism-320-10520/

Bradley, J.A., Singarayer, J.S., Anesio, A.M., 2014. Microbial community dynamics in the forefield of glaciers. Peoc. R. Soc.B 281: 20140882.

Broglio, M., Bovio, M., Poggio, L., 2008. Guida alla flora della Valle d'Aosta.

D'Amico, M.E., Freppaz, M., Filippa, G., Zanini, E., 2014. Vegetation influence on soil formation rate in a proglacial chronosequence (Lys Glacier, NW Italian Alps).CATENA, 113, 122-137.

Gaudet, C.L., Keddy, P.A., 1988. A comparative approach to predicting competitive ability from plant traits. Nature, 334, 242–3

Ghestem, M., Sidle, R.C., Stokes, A., 2011. The influence of plant root system on subsurface flow: Implications for slope stability. BioScience, 61, 869-879.

Gonzalez-Ollauri, A., Mickovski, S.B., 2016. Using the root spread information of pioneer plants to quantify their mitigation potential against shallow landslides and erosion in temperate humid climates. Ecological Engineering, 95, 302-315.

Gonzalez-Ollauri, A., Mickovski, S.B., 2017. Plant-soil reinforcement response under different soil hydrological regimes. Geoderma, 285, 141-150.

Gregory, P.J., Hutchinson, D.J., Read, D.B., Jenneson, P.M., Gilboy, W.B., Morton, E.J., 2003. Non-invasive imaging of roots with high resolution X-Ray micro-tomography. Plant Soil, 255, 351–359.

Graf, F., Frei, M., 2013. Soil aggregate stability related to soil density, root length, and mycorrhiza using site-specific *Alnus ncana* and *Melanogaster variegatus*s l.. Ecological Engineering, 57, 314-323.

Grey, D.H., Sotir, R.B., 1996.Biotechnical and soil bioengineering slope stabilization. In: A practical guide for erosion control. Willey, John & Sons, New York, pp. 1-105.

Han, L., Dutilleul, P., Prasher, S.O., Beaulieu, C., Smith, D.L., 2008. Assessment of common scab-induced pathogen effects onpotato underground organs via computed tomography scanning. Phytopathology, 98, 1118–1125.

Holzmann, H., Haselwandter, K., 1988. Contribution of nitrogen fixation to nitrogen nutrition in an alpine sedge community (Caricetum curvulae). Oecologia, 76, 298-302. doi: 10.1007/BF00379967

Hu, X., Brierley, G., Zhu, H., Li, G., Fu, J., Mao, X., Yu, Q., Qiao, N., 2013. An exploratory analysis of vegetation strategies to reduce shallow landslide activity on loess hillslopes, Northeast Qinghai-Tibet Plateau, China. J. Mt. Sci., 10, 668–686.

Hu, X., Li, Z.C., Li, X.Y., Liu, L.Y., 2016. Quantification of soil macropores under alpine vegetation using computed tomography in the Qinghai Lake Watershed, NE Qinghai–Tibet Plateau. Geoderma, 264,244-251.

Hudek, C., Stanchi, S., D'Amico, M., Freppaz, M., (submitted) Quantifying the contribution of the root system of alpine vegetation in the soil aggregate stability of moraine.

IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

Jenneson, P.M., Gilboy, W.B., Morton, E.J., Luggar, R.D., Gregory, P.J., Hutchinson, D., 1999. Optimisation of X-ray microtomography for the in situ study of the development of plant roots. 1999 Ieee Nuclear Science Symposium Conference Record, 1–3, 429–432.

Jonasson, S., and Callaghan, T.V., 1992. Root Mechanical Properties Related to Disturbed and Stressed Habitats in the Arctic. The New Phytologist 122, 179-186.

Jones, G.A. and Henry, G.H.R., 2003. Primary plant succession on recently deglaciated terrain in the Canadian High Arctic. Journal of Biogeography, 30, 277–296. doi:10.1046/j.1365-2699.2003.00818.x

Kaestner, A., Schneebeli, M., Graf, F., 2006. Visualizing three-dimensional root networks using computed tomography. Geoderma, 136, 459–469.

Khan, M.A., Gemenet, D.C., Villordon, A., 2016. Root System Architecture and Abiotic Stress Tolerance: Current Knowledge in Root and Tuber Crops. Front. Plant Sci., 7, Article 1584.

Koebernick, N., Weller, U., Huber, K., Schlüter, S., Vogel, H.J., Jahn, R., Vereecken,H., Vetterlein, D., 2014. *In situ* visualization and quantification of three-dimensional root system architecture and growth using x-ray computed tomography. Vadose Zone, 13, 1-10.

Körner, C., 2003. Alpine plant life Functional plant ecology of high mountain ecosystems. Springer-Verlag Berlin.

Kuka, K., Illerhaus, B., C.A., Fox, Joschko, M., 2013. X-ray Computed Microtomography for the Study of the Soil–Root Relationship in Grassland Soils. Vadose Zone Journal, 12, 1-10.

Lazzaro, A., Franchini, A.G., Brankatschk, R., Zeyer, J., 2010. Pioneer communities in the forefields of retreating glaciers: how microbes adapt to a challenging environment. In: Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology (Edi.Mendes-Villas, A.) Formatex, Badajoz, Spain.

Lichtenegger, E., 1996. Root distribution in some alpine plants. Acta Phytogeogr. Suec., 81, 76-82.

Lichtenegger, E., and Kutschera-Nitter, L., 1991. Spatial root types. In Edited by McMichael, B.L., and Persson, H. Development in agricultural and managed forests ecology 24. Plant roots and their environment. Proceedings of an ISRR symposium Elsevier Science Publisher B.V. Amsterdam, Netherlands.

Lobet, G., Pound, M.P., Diener, J., Pradal, C., Drayer, X., Godin, C., Javaux, M., Leitner, D., Maunier, F., Nancy, P., Pridmore, T.P., Schnepf, A., 2015. Root system markup language: toward a unified root architecture description language. Plant Physiology, 167, 617-627.

Lontoc-Roy, M., Dutilleul, P., Prasher, S.O., Han, L., Brouillet, T., Smith, D.L., 2006. Advances in the acquisition and analysis of CT scan data to isolate a crop root system from the soil medium and quantify root system complexity in 3-Dspace. Geoderma, 137, 231–241.

Mairhofer, S., Sturrock, C., Wells, D.M., Bennett, M.J., Mooney, S.J., Pridmore, T.P., 2015. On the evaluation of methods for the recovery of plant root systems from X-ray computed tomography images. Functional Plant Biology, 42, 460–470.

Mairhofer, S., Zappala, S., Tracy, S.R., Sturrock, C., Bennett, M., Mooney, S.J., Pridmore, T., 2012. RooTrak: Automated Recovery of Three-Dimensional Plant Root Architecture in Soil from X-Ray Microcomputed Tomography Images Using Visual Tracking. Plant Physiology, 158, 561–569.

Massaccesi, L., Benucci, G.M.N., Gigliotti, G., Cocco, S., Corti, G., Agnelli, A., 2015. Rhizosphere effect of three plant species of environment under periglacial conditions (Majella massif, central Italy). Soil Biology and Biochemistry, 89,184-195.

Matthews, J.A., 1999. Disturbance regimes and ecosystem response on recently-deglaciated terrain. In Ecosystems of Disturbed Ground, Walker LR (ed.). Elsevier: Amsterdam; 17–37.

Mercalli, L., 2003.Atlanteclimaticdella Valle d'Aosta. Societa Meteorologica Italia, Torino 405.

Mooney, S.J., Morris, C., Berry, P.M., 2006. Visualization and quantification of the effects of cereal root lodging on three-dimensional soil macrostructure using X-ray Computed Tomography. Soil Sci, 171, 706–718.

Mooney, S.J., Pridmore, T.P., Helliwell, J., Bennett, M.J., 2012. Developing X-ray Computed Tomography to non-invasively image 3-D root systems architecture in soil. Plant Soil, 352, 1-22.

Nagel, K.A., Putz, A., Gilmer, F., Heinz, K., Fischbach, A., Pfeifer, J., Faget, M., Blossfeld, S., Ernst, M., Dimaki, C., Kastenholz, B., Kleinert, A.K., Galinski, A., Scharr, H., Fiorani, F., Schurr, U., 2012. GROWSCREEN-Rhizo is a novel phenotyping robot enabling simultaneous measurements of root and shoot growth for plants grown in soil-filled rhizotrons. Functional Plant Biology, 39, 891-904. http://dx.doi.org/10.1071/FP12023

Nagelmüller, S., Hiltbrunner, E., Körner, C., 2016. Critically low soil temperatures for root growth and root morphology in three alpine plant species. Alp Botany 126, 11–21.

Norris, J.E., Stokes, A., Mickovski, S.B., Cammeraat, E., Van Beek, R., Nicoll, B.C., Achim, A., 2008. Slope stability and erosion control: ecotechnological solutions. Springer, Dordrecht.

Ola, A., Dodd, I.C., Quinton, J.N., 2015. Can we manipulate root system architecture to control soil erosion? SOIL, 1, 603-612.

Onipchenko, V.G., Kipkeev, A.M., Makarov, M.I., Kozhevnikova, A.D., Ivanov, V.B., Soudzilovskaia, N.A., Tekeev, D.K., Salpagarova, F.S., Werger, M.J.A., Cornelissen, J.H.C., 2014. Digging deep to open the white black box of snow root phenology. Ecol Res, 29, 529–534.

Paez-Garcia, A., Motes, C.M., Scheible, W.-R., Chen, R., Blancaflor, E.B., Monteros, M.J., 2015. Root Traits and Phenotyping Strategies for Plant Improvement. Plants, 4, 334–355.

Paya, A.M., Silverberg, J., Padgett, J., Bauerle, T.L., 2015. X-ray computed tomography uncovers root-root interactions: quantifying spatial relationships between interacting root systems in three dimensions. Frontiers in Plant Science, 6, 274.

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., de Vos, A.C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., ter Steege, H., van der Heijden, M.G.A., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M.V., Conti, G., Staver, A.C., Aquino S., Cornelissen, J.H.C., 2013. New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of Botany, 61, 167–234.

Pierret, J.S., Prasher, S.O., Kantzas, A., Langford, C., 1999. Three-dimensional quantification of macropore networks in undisturbed soil cores. Soil Sci. Soc. Am. J, 63, 1530–1543.

Pierret, A., Moran, C., Doussan, C., 2005. Conventional detection methodology is limiting our ability to understand the roles and functions of fine roots. New Phytol, 166, 967–980.

Pignatti, S., 2003. Flora D'Italia. Vol.3. Edagricole S.r.l., Bologna.

Pfeifer, J., Kirchgessner, N., Colombi, T., Walter, A., 2015. Rapid phenotyping of crop root systems in undisturbed field soil using X-ray computed tomography. Plant Methods, 11, 41.

Pfeifer, J., Faget, M., Walter, A., Blossfeld, S., Fiorani, F., Schurr, U., Nagel, K.A., 2014. Spring barley shows dynamic compensatory root and shoot growth responses when exposed to localised soil compaction and fertilisation. Functional Plant Biology,41, 581-597.

Pohl, M., Alig, D., Körner, C., Rixen, C., 2009. Higher plant diversity enhances soil stability in disturbed alpine ecosystems. Plant Soil, 324, 91–102.

Pohl, M., Stroude, R., Buttler, A., Rixen, C., 2011. Functional traits and root morphology of alpine plants. Annals of Botany, 108, 537–545, doi:10.1093/aob/mcr169

Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P., Mommer, L., 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist. 193, 30-50.

Reubens, B., Poesen, J., Danjon, F., Geudens, G., Muys, B., 2007. The role of fine and coarse roots in shallow slope stability and soil erosion control with a focus on root system architecture: a review. Trees, 21, 385–402.

Robbins, J.A., Matthews, J.A., 2009. Pioneer vegetation on glacier forefields in southern Norway: emerging communities? Journal of Vegetation Science, 20, 889-902.

Sati, S.P., Sundriyal, Y.P., 2007. Role of some species in slope instability. Himalayan Geol., 28, 75-78.

Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis. Academic Press, Cambridge.

Soil Survay Staff, 2010. Keys to soil Taxonomy. United States Department of Agriculture, Eleventh edition. Natural Resources Conservation Services.

Stokes, A., Atger, C., Bengough, A.G., Fourcaud, T., Sidle, R.C., 2009. Desirable plant root traits for protecting natural and engineered slopes against landslides. Plant Soil, 324, 1-30.

Stöcklin, J., Bäumler, E., 1996. Seed rain, seedling establishment and clonal growth strategies on a glacier foreland. Journal of Vegetation Science., 9, 45-56.

Stöcklin, J., Kuss, P., Pluess, A.R., 2009. Genetic diversity, phenotypic variation and local adaptation in the alpine landscape: case studies with alpine plant species. Bot.Helv.119, 125-133.

Tasser, E., Tappeiner, U., 2005. New model to predict rooting in diverse plant community compositions. Ecological Modelling, 185, 195-211.

Tisdall, J.M., 1991. Fungal hyphae and structural stability of soil. Australian Journal of Soil Research, 29, 729-743.

Tollner, E.W., Rasmseur, E.L., Murphy, C., 1994.Techniquesand approaches for documenting plant root development with X-ray computed tomography. In Tomography of soil water-root processes. Edited by Anderson, S.H., and Hopmans, J.W., SSSA Special Publication, 36, Madison,Wis. 115–133.

Tracy, S.R., Black, C.R., Roberts, J.A., Sturrock, C., Mairhofer, S., Craigon, J., Mooney, S.J., 2012. Quantifying the impact of soil compaction on root system architecture in tomato (Solanum lycopersicum) by X-ray micro-computed tomography. Ann. Bot., 110, 511-519.

Wantanabe, K., Mandang, T., Tojo, S., Ai, F., Huang, B.K., 1992. Non-destructive root-zone analysis with X-ray CT scanner. Paper 923018. American Society of Agricultural Engineers. St Joseph, MI, USA

Yang, Y., Chen, L., Li, N., & Zhang, Q., 2016. Effect of Root Moisture Content and Diameter on Root Tensile Properties. *PLoS ONE*, *11*, e0151791.

Zoller, H., and Lenzin, H., 2006. Composed cushions and coexistence with neighbouring species promoting the persistence of *Eritrichium nanum* in high alpine vegetation. Bot. Helv., 116, 31–40.

Table and Figure Captions

Table 1

Table 2 Scanning parameters for X-ray CT.

Table 3 Values of root traits analyzed with RooTrak (volume, area, maximum vertical and horizontal length of the root system, convex hull), ImageJ (maximum vertical and horizontal length of the root system) and WinRHIZO (total root length and average root diameter) of the X-ray CT scanned samples.

Table 4 Plant height (mm), rooting depth (mm) measured with ImageJ, total root length (m), mean root diameter (mm) and root length density (cm cm^{-3}) of the 10 studied alpine species measured with WinRHIZO.

Table 5 Root length distribution (%) of the 10 pioneer alpine plants in relation to different diameter classes (mm).

Table 6 Life forms, the number of samples (n) tested, the range of root diameters (mm), root tensile strength (MPa) values, scale factor (α) rate of strength decrease (β) and the goodness of fit (R^2) of the 10 studied alpine species.

Table 7 ANOVA table with multiple comparisons of root tensile strength (MPa) between the studied plant species.

Figure1 a - j Root architecture of the 10 studied pioneer alpine species detected by X-ray CT scanning. a., *E. fleischeri*; b., *F. halleri*; c., *L. alpine*; d., *L. spicata*; e., *M. recurva*; f., *P. laxa*; g., *S. helvetica*; h., *S. exscapa*; i., *T. pallescens*; j., *T. distichophyllum*; Scale bars: a., 35 mm, b., 25 mm, c., 40 mm, d., 15 mm, e., 10 mm, f., 15 mm, g., 15 mm, h., 30 mm, i., 45 mm, j., 20 mm.

Figure 2 *a.,* Image of the core root system *b.,* the core root system in relation to the soil matrix and *c.,* the washed entire root system of *Trifolium pallescens*. Scale bar a., 45 mm b., 40 mm and c., the ruler uses cm.

Figure 3 *a.,* Linear correlation between RooTrak and ImageJ data on the maximum vertical and *b.,* horizontal root length for the 10 studied alpine species.

Figure 4

Figure 5 Examples of the grayscale CT images of the soil matrices *a.,* glacial till with *T. distichophyllum b.,* and *c.,* fluvio-glacial and lake depositions.

Figure 6 Plant height (cm) and rooting depth (cm) of the 10 studied alpine plant species.

Figure 7 The relationship between root tensile strength (MPa) and root diameter (mm) for the 10 studied alpine species.

Table 1

Species	Common name	Life form	Succession	Family
Epilobium fleischeri Hochst.	Alpine willowherb	Forb	Early	Omagraceae
Trisetum distichophyllum (Vill.) P.Beauve.	Tufted hairgrass	Graminoid	Early	Poaceae
Trifolium pallescens Schreb.	Pale clover	Forb	Early	Fabaceae
Luzula spicata (L.) DC.	Spiked woodrush	Graminoid	Mid	Juncaceae
Silene exscapa All.	Moss campion	Forb	Mid	Caryophyllaceae
Minuartia recurva (All.) Schinz and Thell.	Recurved sandwort	Forb	Late	Caryophyllaceae
Festuca halleri All.	Haller's Fescue	Graminoid	Late	Poaceae
Poa laxa Haenke	Banff Bluegrass	Graminoid	Ubiquitous	Poaceae
Salix helvetica Vill.	Swiss willow	Dwarf shrub	Ubiquitous	Salicaceae
Leucanthemopsis alpina (L.) Heyw.	Alpine Moon Daisy	Forb	Ubiquitous	Asteraceae

Table 2 Scanning parameters for X-ray CT.

Voltage (kV)	Current (μA)	Number of projections	Exposure time (ms)	Resolution (μm)	Signal averaging	Total scanning time
180	160	2160	250	54	4/1	2h17min

Table 3 Values of root traits analyzed with RooTrak (volume, area, maximum vertical and horizontal length of the root system, convex hull), ImageJ (maximum vertical and horizontal length of the root system) and WinRHIZO (total root length and average root diameter) of the X-ray CT scanned samples.

Plant species	Root type	RooTrak					ImageJ	
		Volume (mm^3) (total mass of the root system)	Area (mm^2) (root area in direct contact with the soil)	Depth (mm) (root system's maximum vertical distance)	Width (mm) (root system's maximum horizontal distance)	Convex hull (mm^2) (region of soil explored by the root system)	Vertical length (mm)	Horizonta[l] length (mm)
T. distichophyllum	Cylindrical	353	3399	63	68	65774	75	70
E. fleischeri	Pole	967	3711	105	65	90931	115	70
T. pallescens	Cone↑	1530	7752	132	72	505364	225	70
S. exscapa	Cone↑	385	2383	102	70	357053	173	69
L. spicata	Cone↓	306	2106	39	71	27046	137	70
F. halleri	Cone↓	828	5866	67	71	60318	107	55
M. recurva	Discoid	144	1677	44	68	60237	164	50
P. laxa	Cone↓	150	1547	33	72	45612	119	34
L. alpina	Umbrella	542	4666	126	72	224012	141	69
S. helvetica	Discoid	435	1146	35	73	24117	49	39

Table 4 Plant height (mm), rooting depth (mm) measured with ImageJ, total root length (m), mean root diameter (mm) and root length density (cm cm^{-3}) of the 10 studied alpine species measured with WinRHIZO.

Plant species	**Plant height (mm)**	**Rooting depth (mm)**	**Total root length (m)**	**Mean root diameter (mm)**	**Root length density (cm cm^{-3})**
T. distichophyllum	50	133	336.9	0.21	85
E. fleischeri	65	197	75.3	0.23	9
T. pallescens	47	133	197.6	0.47	33
S. exscapa	20	153	106.2	0.33	49
L. spicata	30	117	202.1	0.22	81
F. halleri	32	101	297.8	0.35	59
M. recurva	15	118	135.9	0.32	29
P. laxa	51	119	210.1	0.28	47
L. alpina	20	127	368.5	0.26	53
S. helvetica	25	90	342.3	0.27	68

Table 5 Root length distribution (%) of the 10 pioneer alpine plants in relation to different diameter classes (mm).

	0<L<0.1	**0.1<L<0.2**	**0.2<L<0.3**	**0.3<L<0.4**	**0.4<L<0.5**	**0.5<L<0.75**	**0.7**
T. distichophyllum	33	41	15	5	2	1	
T. pallescens	49	19	10	5	4	6	
S. exscapa	42	30	12	5	3	4	
L. spicata	57	27	9	3	1	1	
F. halleri	37	22	15	8	5	7	
M. recurva	36	30	13	6	3	5	
P. laxa	49	19	12	6	4	5	
L. alpina	36	29	14	7	5	5	
S. helvetica	9	25	37	6	4	4	

Table 6 Life forms, the number of samples (n) tested, the range of root diameters (mm), root tensile strength (MPa) values, scale factor (α) rate of strength decrease (β) and the goodness of fit (R^2) of the 10 studied alpine species.

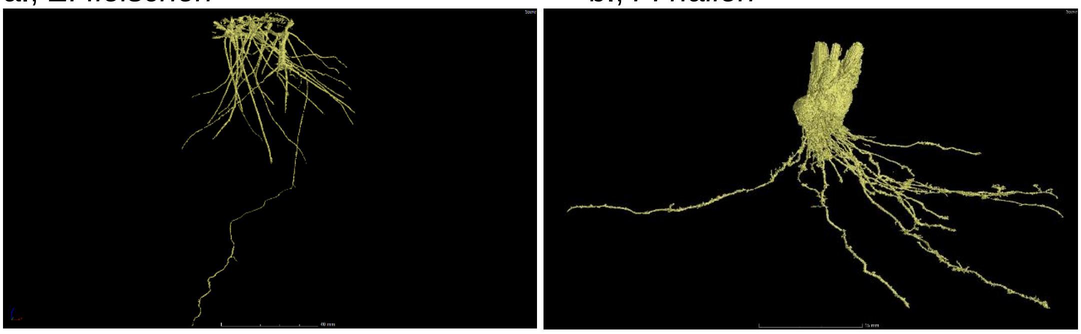
Species	Life form	n	*d* range (mm)	Mean Tr (MPa)	α	β	R^2	p
T. distichophyllum	Graminoid	30	0.05-1.15	86	23.26	0.62	0.56	<0.001
E. fleischeri	Forb	32	0.04-1.56	58	3.61	1.15	0.67	<0.001
T. pallescens	Forb	32	0.05-1.66	44	10.55	0.88	0.65	<0.001
S. exscapa	Forb	30	0.03-1.14	54	11.85	0.84	0.65	<0.001
L. spicata	Graminoid	30	0.03-0.37	138	9.54	1.01	0.71	<0.001
F. halleri	Graminoid	30	0.05-0.46	94	17.92	0.75	0.70	<0.001
M. recurva	Forb	30	0.03-0.35	60	6.24	1.11	0.78	<0.001

P. laxa	Graminoid	30	0.03-0.56	113	21.65	0.75	0.82	<0.001
L. alpina	Forb	32	0.05-0.59	29	8.67	0.75	0.71	<0.001
S. helvetica	Dwarf shrub	30	0.03-0.78	110	11.34	0.94	0.78	<0.001

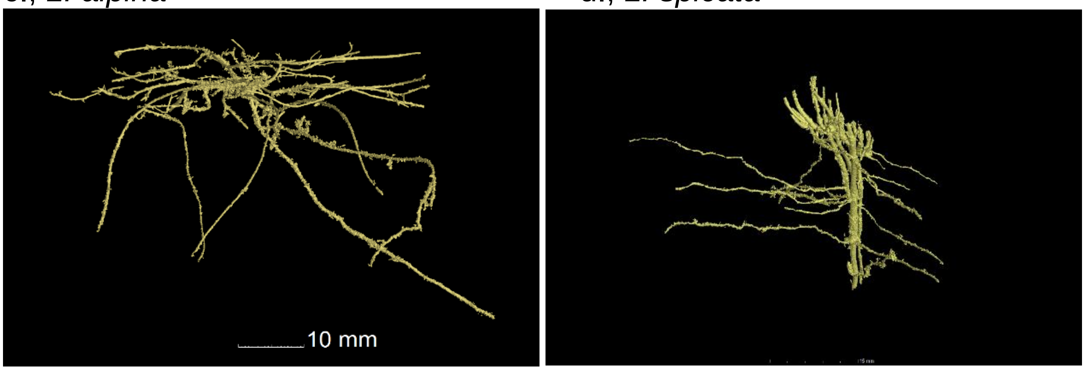
Table 7 ANOVA table with multiple comparisons of root tensile strength (MPa) between the studied plant species.

Figure1 a - j Root architecture of the 10 studied pioneer alpine species detected by X-ray CT scanning. a., *E. fleischeri*; b., *F. halleri*; c., *L. alpine*; d., *L. spicata*; e., *M. recurva*; f., *P. laxa*; g., *S. helvetica*; h., *S. exscapa*; i., *T. pallescens*; j., *T. distichophyllum*; Scale bars: a., 35 mm, b., 25 mm, c., 40 mm, d., 15 mm, e., 10 mm, f., 15 mm, g., 15 mm, h., 30 mm, i., 45 mm, j., 20 mm.

a., *E. fleischeri* b., *F. halleri*

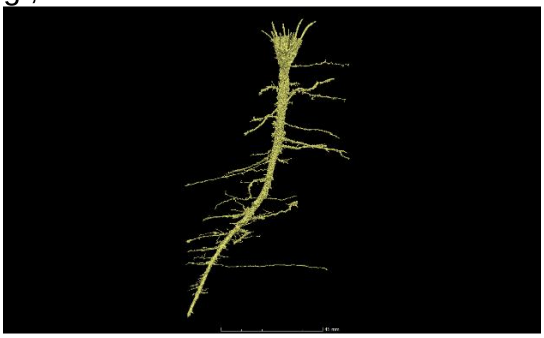


c., *L. alpina* d., *L. spicata*



e., *M. recurva* f., *P. laxa*

g., *S. helvetica* h., *S. exscapa*



i., *T. pallescens* j., *T. distichophyllum*

Figure 2 *a.,* Image of the cored root system *b.,* the core root system in relation to the soil matrix and *c.,* the washed entire root system of *Trifolium pallescens*. Scale bar a., 45 mm b., 40 mm and c., the ruler uses cm.

a., b., c.,

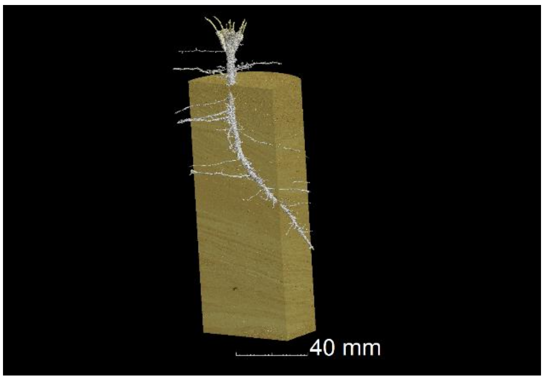


Figure 3 *a.,* Linear correlation between RooTrak and ImageJ data on the maximum vertical and *b.,* horizontal root length for the 10 studied alpine species.

a., b.,

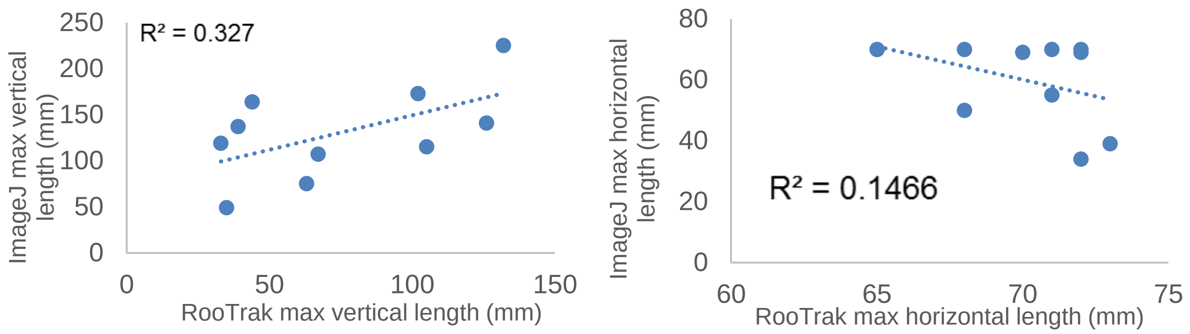


Figure 4

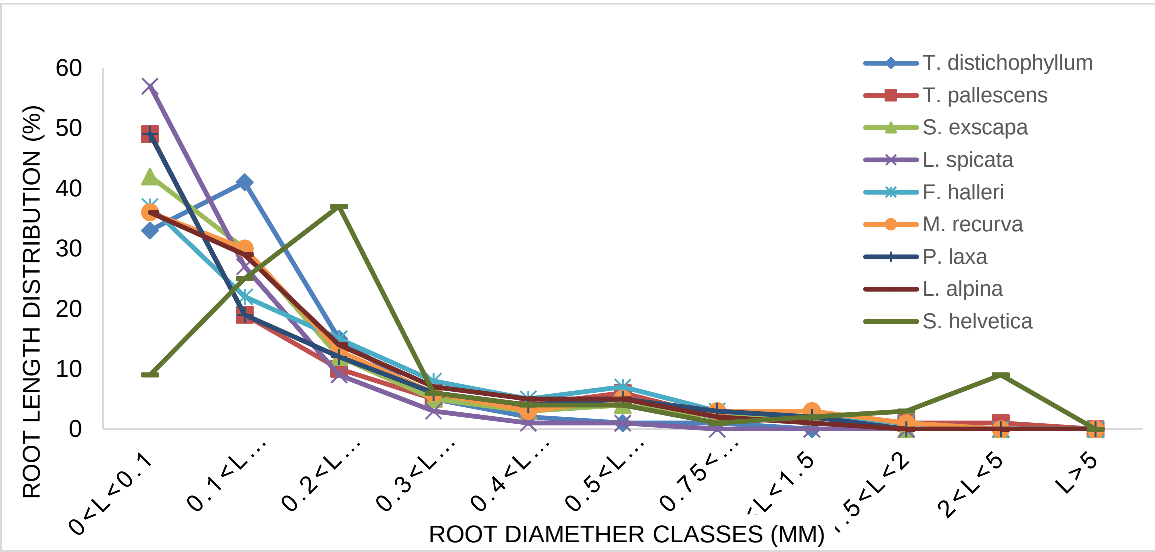
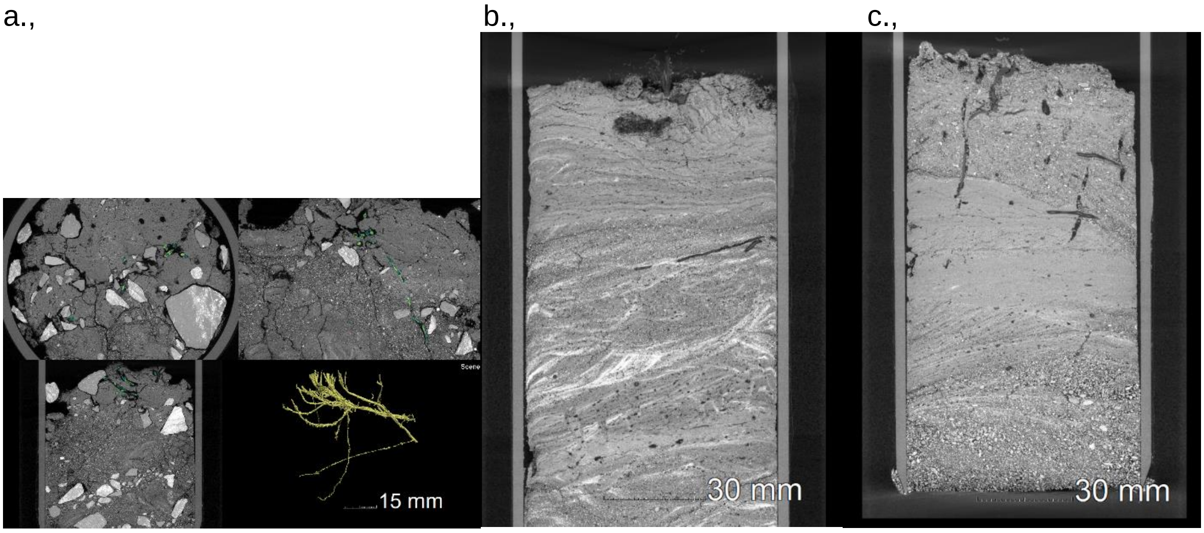


Figure 5 Examples of the grayscale CT images of the soil matrices *a.,* glacial till with *T. distichophyllum b.,* and *c.,* fluvio-glacial and lake depositions.

a., b., c.,



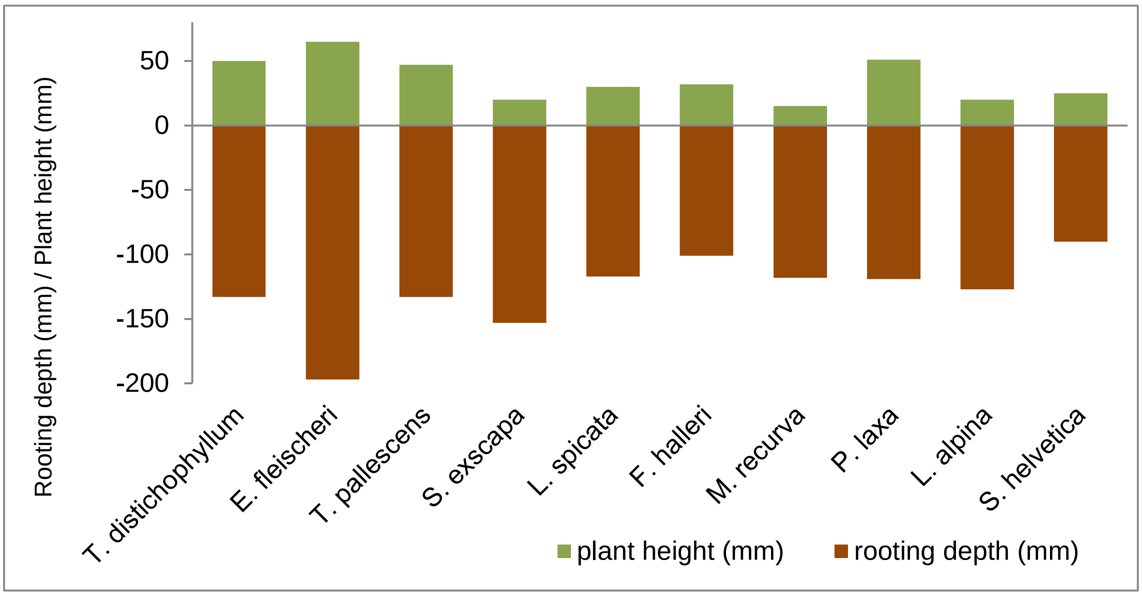
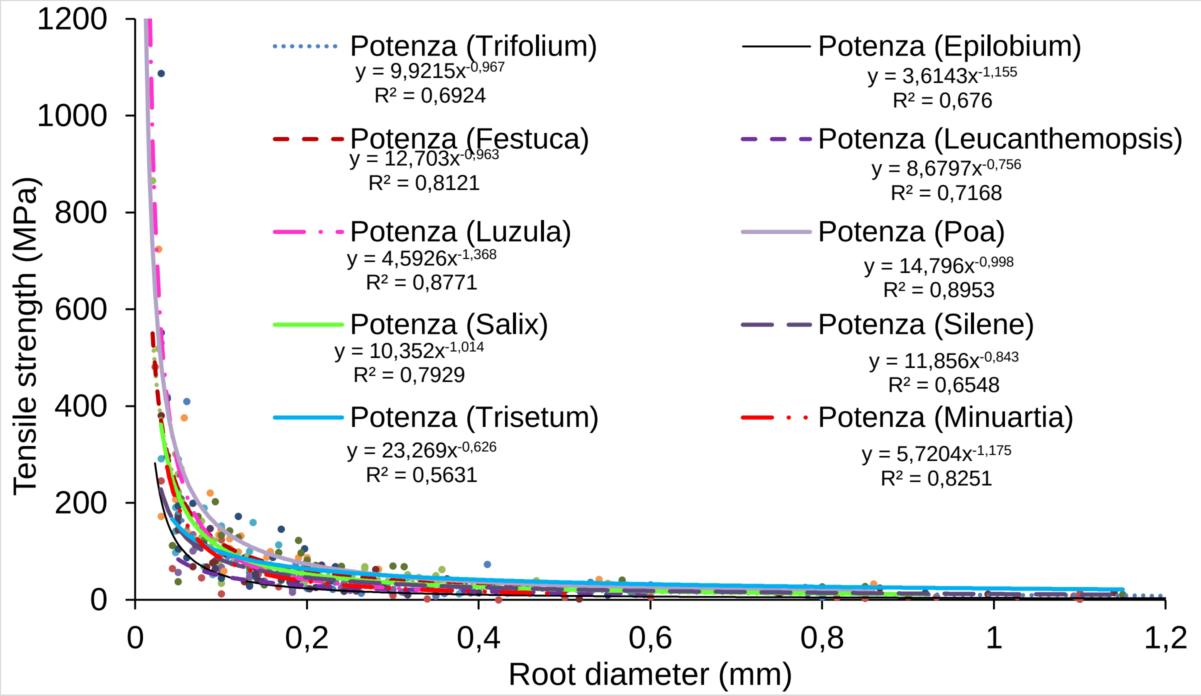
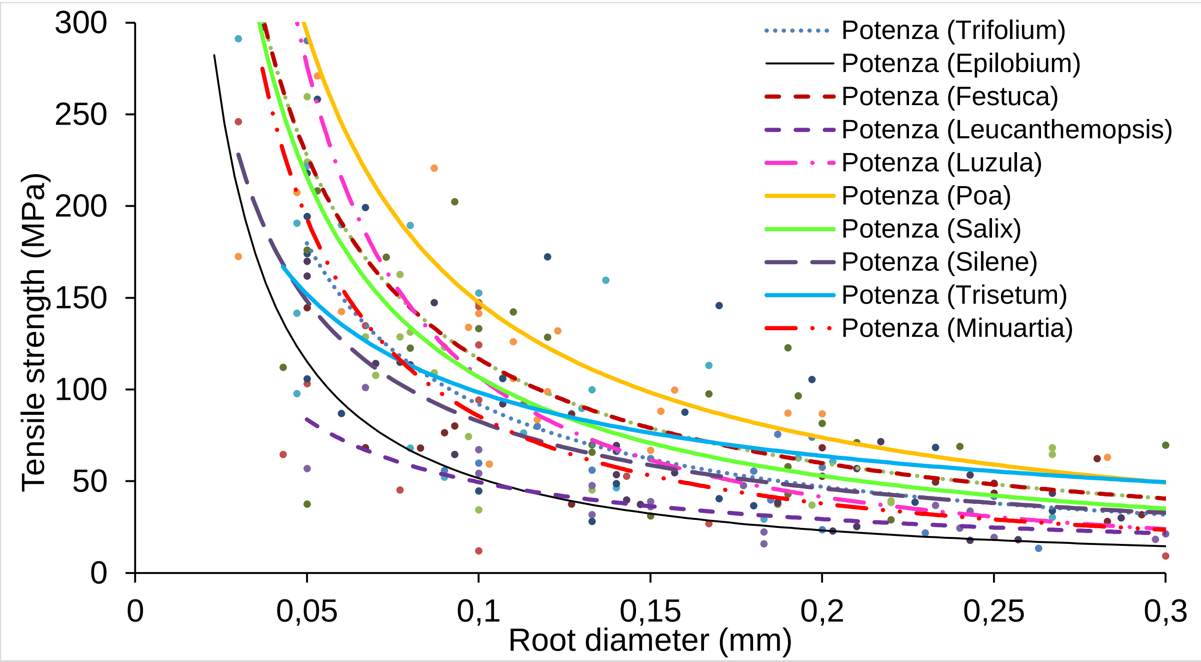


Figure 6 Plant height (cm) and rooting depth (cm) of the 10 studied alpine plant species.

Figure 7 The relationship between root tensile strength (MPa) and root diameter (mm) for the 10 studied alpine species


Tensile strength (MPa)
Root diameter (mm)
Potenza (Trifolium)
y = 9,9215x-0,967
R² = 0,6924
Potenza (Epilobium)
y = 3,6143x-1,155
R² = 0,676
Potenza (Festuca)
y = 12,703x-0,963
R² = 0,8121
Potenza (Leucanthemopsis)
y = 8,6797x-0,756
R² = 0,7168
Potenza (Luzula)
y = 4,5926x-1,368
R² = 0,8771
Potenza (Poa)
y = 14,796x-0,998
R² = 0,8953
Potenza (Salix)
y = 10,352x-1,014
R² = 0,7929
Potenza (Silene)
y = 11,856x-0,843
R² = 0,6548
Potenza (Trisetum)
y = 23,269x-0,626
R² = 0,5631
Potenza (Minuartia)
y = 5,7204x-1,175
R² = 0,8251


Tensile strength (MPa)
Root diameter (mm)
Potenza (Trifolium)
Potenza (Epilobium)
Potenza (Festuca)
Potenza (Leucanthemopsis)
Potenza (Luzula)
Potenza (Poa)
Potenza (Salix)
Potenza (Silene)
Potenza (Trisetum)
Potenza (Minuartia)