# Effects of Freezing Preservation and Loading Rate on Root Mechanical Properties of Four Hyrcanian Forest Tree Species

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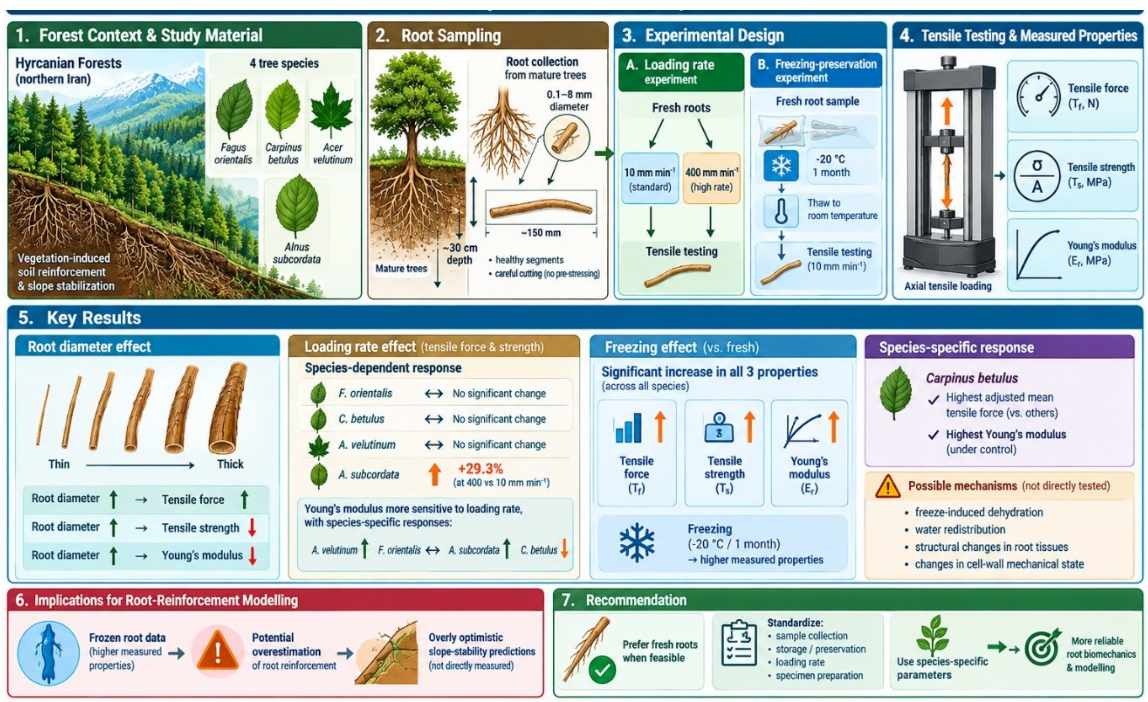
## GRAPHICAL ABSTRACT



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Root biomechanical properties, including tensile strength, tensile force, and Young's modulus, are fundamental parameters governing vegetation-induced soil reinforcement. However, the effects of sample preservation and loading rate on these properties remain insufficiently understood, limiting the reliability and comparability of root biomechanical data. This study investigated the influence of loading rate and freezing preservation on the mechanical properties of roots from four dominant Hyrcanian forest tree species. Fresh roots were tested at two loading rates, and an additional experiment evaluated the effect of one month of frozen storage before tensile testing. Freezing significantly increased tensile force, tensile strength, and Young's modulus, while also improving the consistency of diameter–property relationships. These increases most likely resulted from structural and moisture-related changes induced by freezing rather than reflecting the natural mechanical behavior of roots, indicating that frozen samples may overestimate root reinforcement capacity. In contrast, loading-rate effects were generally limited and species-dependent. Root diameter increased tensile force but was generally associated with lower tensile strength and Young's modulus. These findings highlight the importance of standardized testing protocols to improve the reliability of root biomechanical data and the accuracy of soil bioengineering applications.

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*Keywords: Root biomechanics; Shallow landslide; Soil bioengineering; Strain-rate dependence; Tensile properties*

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## INTRODUCTION

Landslides are a widespread global hazard, affecting an estimated 3.7 million km² of land and placing nearly 300 million people at risk (Nasir *et al.* 2025). Among different kinds of landslides, shallow ones, characterized by the downslope movement of the upper soil layer overlying bedrock, generally with depths less than 2 m (Zhu *et al.* 2025), are the most common and destructive slope-related hazards worldwide (Murgia *et al.* 2022). Their increasing frequency threatens human lives, damages infrastructure, degrades ecosystems, and accelerates soil erosion and the loss of fertile land resources (Navarro Hevia *et al.* 2016; Ran *et al.* 2018). Because shallow landslides are controlled by the combined effects of topography, geological conditions, hydrology, and vegetation cover (Murgia *et al.*

2022), effective and sustainable mitigation strategies are essential for reducing both their environmental and socio-economic impacts.

Conventional slope stabilization has traditionally relied on civil engineering approaches, commonly referred to as *grey solutions*. However, increasing environmental concerns and the demand for sustainable land management have promoted the development of vegetation-based or *green solutions* as environmentally friendly alternatives (Ni *et al.* 2018; Ng *et al.* 2022; Li *et al.* 2023; Chen *et al.* 2024). Recent studies have highlighted the strong influence of vegetation cover on soil erosion and landslide risk in steep forest ecosystems, emphasizing biological interventions, known as *green solutions,* as an important component of sustainable slope-management strategies (Giadrossich *et al.* 2013; Thanh *et al.* 2025; Keybondori *et al.* 2025; Kang *et al.* 2026). Unlike engineered structures that primarily provide mechanical support, vegetation improves slope stability through complementary hydrological (Kang *et al.* 2026) and mechanical mechanisms (Keybondori *et al.* 2025; Zhu *et al.* 2025). Hydrologically, plants reduce soil moisture by root water uptake, evapotranspiration, and rainfall interception by the canopy (McGuire *et al.* 2016; Nsengiyumva *et al.* 2018). Mechanically, root systems reinforce the soil matrix, increase soil cohesion, and improve resistance to shear failure, which is widely regarded as the dominant mechanism responsible for vegetation-induced slope stabilization (Fata *et al.* 2021; Mehtab *et al.* 2021).

The mechanical contribution of vegetation is primarily determined by the reinforcing capacity of root systems (Stokes 2002), which generally increases with plant growth and root development, thereby enhancing long-term slope stability (Gabet and Dunne 2003). As roots penetrate the soil, they form a composite soil–root matrix that enhances particle interlocking and increases soil shear strength (Stokes *et al.* 2009; Masi *et al.* 2021). Root density, spatial distribution, architecture, and mechanical properties therefore strongly influence vegetation-mediated slope stabilization (Ji *et al.* 2012; Abdi *et al.* 2019; Lee *et al.* 2021; Rossi *et al.* 2022), with root mechanical properties serving as key input parameters in root reinforcement models and soil bioengineering applications (Liang *et al.* 2017). Reliable quantification of root biomechanical behavior is consequently essential for assessing the contribution of vegetation to slope stabilization.

Root biomechanical behavior is commonly characterized by tensile force, tensile strength, and Young's modulus (Stokes 2002; Mao *et al.* 2012; Zhu *et al.* 2025). Tensile force represents the maximum force required for root failure, whereas tensile strength accounts for cross-sectional area and enables comparisons among roots of different diameters (Genet *et al.* 2005; Abdi *et al.* 2014). The Young's modulus that is of interest here characterizes root tissue stiffness and resistance to elastic deformation under tensile loading (Mao *et al.* 2018). In soil bioengineering studies, Young's modulus refers specifically to the tensile Young's modulus measured in the axial (longitudinal) direction of the root (*i.e.* axial tensile Young's modulus). These parameters provide the mechanical basis for understanding root behavior and parameterizing models of vegetation-induced soil reinforcement. Root mechanical properties are influenced by biological, environmental, and methodological factors, including species, diameter, anatomy, chemical composition, moisture content, and environmental conditions (Hales *et al.* 2013; Hales and Miniat 2017; Giadrossich *et al.* 2017; Zhu *et al.* 2025). Consequently, methodological consistency is essential for obtaining reliable and comparable biomechanical measurements.

Several protocols have been recommended to improve measurement reproducibility, including storing freshly collected roots in 15% alcohol solution (Bischetti *et al.* 2005; Hales *et al.* 2013; Abdi *et al.* 2014), applying a loading rate of 10 mm $min^{-1}$ during tensile testing (Bischetti *et al.* 2005; Vergani *et al.* 2014; Yang *et al.* 2016; Abdi 2018), and limiting the interval between sampling and testing to approximately one week (Bischetti *et al.* 2005). Nevertheless, substantial variation persists in sample preparation, storage conditions, root dimensions, testing equipment, and loading rates among studies (Hales *et al.* 2009; Lim *et al.* 2011; Giadrossich *et al.* 2017). These methodological inconsistencies can limit comparability among studies and introduce systematic bias into reported root mechanical properties.

Among these methodological factors, post-harvest sample preservation is particularly important because root tissues may undergo biological and physicochemical changes after sampling. Delays between field sampling and laboratory testing can promote tissue degradation, including cellulose decomposition, and reduce mechanical resistance (Hales *et al.* 2013). Therefore, appropriate preservation is essential for minimizing deterioration while maintaining the original mechanical properties of roots.

Several preservation methods have been evaluated, but their effects remain inconsistent. Bischetti *et al.* (2005) found no significant differences in tensile strength among oven drying followed by rehydration, freezing in plastic bags, and storage in 15% alcohol. Conversely, Hales *et al.* (2013) reported that drying and subsequent rewetting can significantly alter root biomechanical properties. Freezing is widely used as a practical preservation method, particularly when large numbers of samples must be processed (Freschet *et al.* 2021), yet its effects on root mechanical behavior remain insufficiently resolved.

Loading rate is another methodological factor that may affect measured root mechanical properties. Although 10 mm $min^{-1}$ is widely used (Zhang *et al.* 2012; Abdi and Deljouei 2019; Hu *et al.* 2024), tensile tests have been conducted at rates ranging from 0.8 to 400 mm $min^{-1}$ (e.g., Cofie and Koolen 2001; Comino and Druetta 2010; Giadrossich *et al.* 2016; Liang *et al.* 2017). Given the viscoelastic behavior of root tissues, loading rate may influence mechanical responses and represent different deformation conditions associated with slow and rapid slope failures. However, only a few studies have explicitly examined loading-rate effects on root mechanical properties (Cofie *et al.* 2000; Cofie and Koolen 2001; Zhang *et al.* 2012), and available evidence remains limited to a small number of species. Thus, it remains unclear whether loading-rate effects represent a general biomechanical response or are species-dependent.

More broadly, the reliability of root reinforcement models depends on the accuracy and comparability of the mechanical properties used to parameterize them. While biological sources of variation such as species, root diameter, and moisture content have received considerable attention, methodological factors such as preservation and loading rate remain less studied. Consequently, differences among studies may reflect not only biological variation but also experimental procedures, potentially introducing systematic bias into biomechanical datasets and affecting predictions of vegetation-induced soil reinforcement.

To address these methodological uncertainties, this study investigated the mechanical behavior of roots from four dominant broadleaf tree species in the Hyrcanian forests (*Fagus orientalis*, *Carpinus betulus*, *Acer velutinum*, and *Alnus subcordata*) using tensile tests at two loading rates (10 and 400 mm $min^{-1}$). The effect of freezing preservation prior to testing was also assessed to determine whether this widely used storage method alters root biomechanical properties.

We hypothesized that (i) freezing preservation modifies root mechanical properties through structural and moisture-related changes during storage, (ii) loading-rate effects are species-dependent because of differences in root anatomy and viscoelastic behavior, and (iii) both factors affect the relationships between root diameter and mechanical properties. These methodological effects may contribute to variability among previously reported biomechanical measurements.

Because root mechanical properties are key inputs for root reinforcement models used in soil bioengineering and slope-stability analyses, identifying methodological effects is essential for improving the reliability and comparability of biomechanical data. Such improvements can support more robust predictions of vegetation-induced soil reinforcement and nature-based slope stabilization.

## EXPERIMENTAL

### Study Site

The Hyrcanian vegetation zone, also known as the Caspian forest, is a temperate forest belt extending along the northern slopes of the Alborz Mountain range in northern Iran, covering approximately 1.9 million hectares. This forest ecosystem is characterized by high biodiversity, including about 50 tree species and 80 shrub species, with broadleaved species being dominant (Marvie Mohadjer 2011). Although limited small patches of coniferous species have been introduced in the past decades, the natural composition of the forest remains predominantly hardwood (Berenji Tehrani *et al.* 2015). The main tree species include *Fagus orientalis*, *Carpinus betulus*, *Parrotia persica*, *Acer cappadocicum*, *Acer velutinum*, *Alnus glutinosa*, *Ulmus glabra*, and *Quercus castaneifolia* (Sagheb Talebi *et al.* 2014).

The present study was conducted in the Educational and Experimental Forest of the University of Tehran known as Kheyrud Forest, located between 51°32′ to 51°35′ E longitude and 36°34′ to 36°37′ N latitude, within the central part of the Hyrcanian forests. The forest covers an area of approximately 8,000 ha, with elevation ranging from 40 to 2,000 m above sea level. The climate is humid temperate, characterized by high relative humidity and a mean annual precipitation of approximately 1,200 mm.

Soils in the region are predominantly fine-grained with high clay and silt content. The underlying bedrock is primarily limestone, which, combined with the fine soil texture and climatic conditions, contributes to slope instability and the frequent occurrence of shallow landslides, particularly along forest roads after prolonged rainfall events (Abdi 2018).

Within this forest, the study was specifically conducted in the first district, known as Patom, covering an area of approximately 900 ha (Fig. 1). The elevation in this district ranges from 40 to 930 m above sea level, and the climate is humid with relatively cold winters. According to the Unified Soil Classification System (USCS), the soils are classified as CH, CL, and ML, underlain by limestone parent material. The mean slope of the area is approximately 46%, indicating highly susceptible conditions for shallow slope failures.

The vegetation communities in the Patom district exhibit a clear altitudinal zonation, ranging from low to high elevations as follows: *Tilio–Buxetum*, *Parrotio–Carpinetum*, *Querceto–Carpinetum*, *Fageto–Carpinetum*, *Fagetum orientalis*, and *Aceretum velutini–Alnetum subcordatae*. Shallow landslides are frequently observed along road networks within this district, occasionally disrupting transportation routes and indicating high slope sensitivity under hydrological loading.

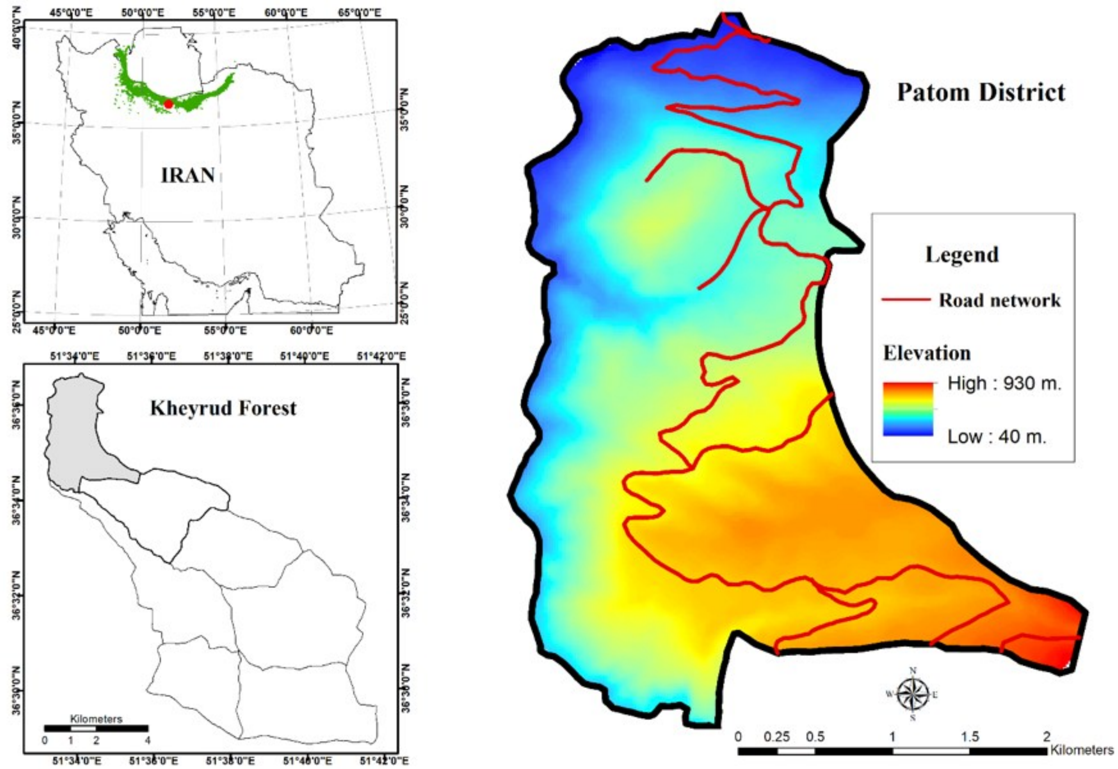



**Fig. 1.** Location of the study area in the Kheyrud Forest and Patom District, Hyrcanian Forests, northern Iran

## Root Sampling

Root samples were collected in November 2024 from the first district of Kheyrud Forest. Due to limitations in the available budget and equipment for fully excavating root systems of mature trees in Iran, detailed information on the root systems of these species is still lacking. However, field observations of windthrown trees indicate that root development is generally limited to a depth of approximately 2 to 3 m, while the diameter of horizontal spread of roots extends to about 10 to 15% of tree height.

Four dominant and widespread broadleaf tree species were investigated: beech (*Fagus orientalis*), hornbeam (*Carpinus betulus*), maple (*Acer velutinum*), and alder (*Alnus subcordata*). These species are commonly found along forest road verges and can significantly contribute to stabilizing slopes adjacent to roads. For each species, five mature trees were randomly selected within a relatively homogeneous forest area on a uniform slope with similar aspect and gradient to minimize environmental variability. Because the forest is natural and uneven-aged, tree ages were unavailable. Instead, only healthy, straight trees of comparable size (DBH= 40 to 60 cm), with intact crowns and undamaged and healthy trunks, were selected for the study.

Root samples were collected by excavating soil adjacent to tree trunks to a depth of approximately 30 cm (Cofie and Koolen 2001; Abdi 2014). The approximate range of root diameters in the collected specimens was 0.1 to 8 mm. To avoid pre-stressing effects, roots were carefully cut using sharp scissors rather than being manually pulled from the soil.

The collected roots were thoroughly washed to remove adhering soil particles and then sealed in plastic bags. To minimize fungal and bacterial contamination, samples were treated with a 15% alcohol solution and stored at 4 °C prior to testing (Bischetti *et al.* 2005; Abdi 2018).

## Root Preservation Treatments

The collected root samples were randomly divided into two groups while maintaining a similar distribution of root diameters between treatments.

The first group was subjected to tensile testing immediately after transfer to the laboratory at two loading rates, 10 and 400 mm min$^{-1}$ (Cofie and Koolen 2001; Zhang *et al.* 2012). A loading rate of 10 mm min$^{-1}$ was chosen because it is the most widely used rate in root tensile studies, providing a standard reference for comparison with previous research. In contrast, 400 mm min$^{-1}$ was selected as the highest loading rate identified in the available literature, thereby providing a substantially elevated loading condition for assessing potential strain-rate effects. The use of these contrasting rates enabled us to evaluate whether root mechanical responses change between a conventional and a high loading-rate condition.

To evaluate the effect of freezing, samples in the second group were sealed in plastic bags without water (Freschet *et al.* 2021) and stored at −20 °C for one month (Loades 2010). Following removal from the freezer, the frozen samples were allowed to thaw to room temperature under laboratory conditions. Tensile tests were subsequently conducted at a loading rate of 10 mm min$^{-1}$, corresponding to the most commonly used testing rate reported in the literature (Abdi 2018).

## Tensile Testing Procedure and Root Mechanical Properties

Healthy root segments approximately 150 mm in length were selected for tensile testing (Abdi, 2014; Leung *et al.* 2015). Root diameter was measured at three points along the middle section of each sample using a digital caliper, and the average value was considered the representative diameter.

Mechanical tests were performed using a Universal Testing Machine (SANTAM Co./SMT-5, Tehran, Iran) with a force measurement precision of ± 0.001 N and a displacement accuracy of ± 0.0001 mm. Root samples were securely fixed between the machine jaws prior to testing. Samples that ruptured near the clamps were considered invalid and excluded from further analysis (Ji *et al.* 2012; Zhang *et al.* 2012). At least 43 root samples with diameters ranging from 0.14 to approximately 6 mm were tested for each treatment.

The maximum force required to rupture each root sample was recorded as the tensile force ($T_f$, N). Tensile strength ($T_s$, MPa) was calculated by dividing the tensile force by the root cross-sectional area (mm²) (De Baets *et al.* 2008). The relationships between root tensile force ($T_f$), tensile strength ($T_s$), and root diameter ($d$) were described using power-law equations (Eq. 1 and Eq. 2) (Gray and Sotir 1996),

$$T_f = \alpha_1 . d^{\beta_1} \tag{1}$$

$$T_s = \alpha_2 . d^{\beta_2} \tag{2}$$

where α and β are empirical coefficients related to species characteristics.

Young's modulus ($E_r$) was determined from the linear elastic portion of the stress–strain curve obtained during tensile testing (Boldrin et al. 2017; Lee *et al.* 2020). The modulus of elasticity was calculated using Eq. 3,

$$E_r = \frac{FL_0}{\pi\,(\frac{d^2}{4})\Delta L} \tag{3}$$

where $F$ is the applied force within the elastic deformation range (N); $L_0$ is the initial length of the root sample before testing (mm); $\Delta L$ is the change in root length (mm); and $d$ is the diameter of root sample (mm).

## Statistical Analysis

The normality of the data was assessed using the Kolmogorov–Smirnov test. The response variables were tensile force, tensile strength, and Young's modulus. Several regression models, including linear, exponential, and power models, were evaluated based on the Akaike Information Criterion (AIC) and residual distribution patterns. The results indicated that the log-transformed power model provided the best fit to the data.

Root samples from all trees were pooled and stored at 4 °C prior to treatment assignment, making it impossible to track individual tree identity; therefore, a standard ANCOVA was employed rather than a mixed-effects model. Durbin-Watson test, with no evidence of significant autocorrelation or clustering indicating a violation of independence. The assumption of homogeneity of regression slopes was assessed by testing covariate-by-factor interactions (root diameter × species, root diameter × storage treatment, and root diameter × loading rate). As all interaction terms were non-significant, the assumption was satisfied, and they were removed from the final model.

Variable selection was performed using likelihood ratio tests ($\alpha = 0.05$) in combination with AIC values. The significance of coefficients in the final models was assessed using the Wald test ($p < 0.05$). Model performance was evaluated using the coefficient of determination ($R^2$).

Analysis of covariance (ANCOVA) was used to examine the effects of species, storage treatment, and loading rate on the measured mechanical properties while accounting for root diameter as a covariate. For ANCOVA-based mean comparisons, estimated marginal means (EMMs) were calculated from the fitted models while accounting for root diameter as a covariate. EMMs represent model-based estimates of the response for each factor level at a common reference value of root diameter, and therefore differ from the arithmetic means calculated directly from the observed data. All statistical analyses were performed using R software version 4.2.2.

# RESULTS

## Loading-rate Experiment

To evaluate the influence of loading rate on root mechanical behavior, tensile tests were conducted at two loading rates (10 and 400 mm $min^{-1}$) using fresh root samples. The effects of loading rate on tensile force, tensile strength, and Young's modulus are presented below.

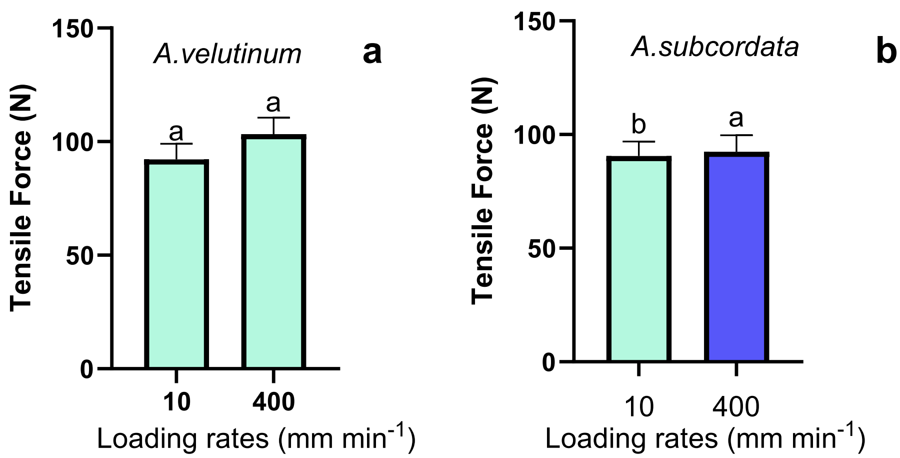
## Tensile Force

According to the descriptive statistics presented in Table 1, *C. betulus* exhibited the highest estimated marginal mean tensile force at a loading rate of 10 mm min$^{-1}$ (106.4 N), whereas *F. orientalis* showed the lowest value (85.6 N). At a loading rate of 400 mm min$^{-1}$, *C. betulus* exhibited the highest estimated marginal mean tensile force (105.4 N), while *F. orientalis* showed the lowest value (89.1 N).

**Table 1.** Descriptive Statistics and Estimated Marginal Means (EMMs) of Root Tensile Force (N) at Loading Rates of 10 and 400 mm min$^{-1}$

| Species | Loading Rate (mm min$^{-1}$) | Min | Max | EMMs | α | β |
|---|---|---|---|---|---|---|
| *A. velutinum* | 10 | 4 | 368.0 | 92.21 | 15.89 | 1.676 |
| | 400 | 4.5 | 393.8 | 103.31 | 19.054 | 1.584 |
| *A. subcordata* | 10 | 1.2 | 372.3 | 90.61 | 15.16 | 1.72 |
| | 400 | 12.6 | 328.1 | 92.38 | 21.37 | 1.50 |
| *F. orientalis* | 10 | 2.9 | 326.3 | 85.66 | 17.86 | 1.59 |
| | 400 | 10.4 | 428.3 | 89.09 | 18.96 | 1.57 |
| *C. betulus* | 10 | 3.2 | 720.5 | 106.45 | 22.26 | 1.62 |
| | 400 | 2 | 489.8 | 105.42 | 19.73 | 1.75 |

The ANCOVA results indicated that root diameter significantly affected tensile force in all studied species ($p < 0.001$). However, loading rate had no significant effect on tensile force in *F. orientalis*, *C. betulus*, and *A. velutinum* ($p > 0.05$). In contrast, *A. subcordata* exhibited significantly greater tensile force at 400 mm min$^{-1}$ compared with 10 mm min$^{-1}$ ($p < 0.001$) (Fig. 2).



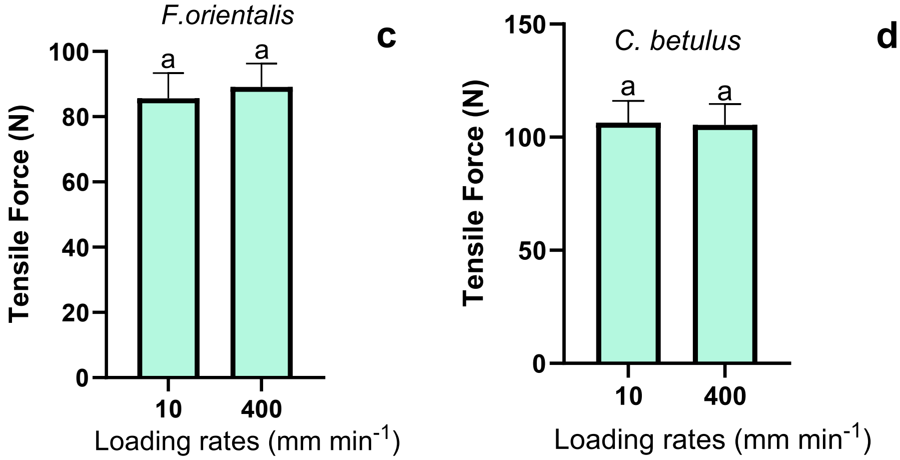



**Fig. 2.** Estimated marginal means (EMMs) of root tensile force (N) at loading rates of 10 and 400 mm min$^{-1}$ in: (a) *A. velutinum*, (b) *A. subcordata*, (c) *F. orientalis*, and (d) *C. betulus*; Different letters indicate significant differences between treatments

In all four species, tensile force increased with increasing root diameter following a positive power-law relationship. This trend was consistent at both loading rates (Fig. 3).

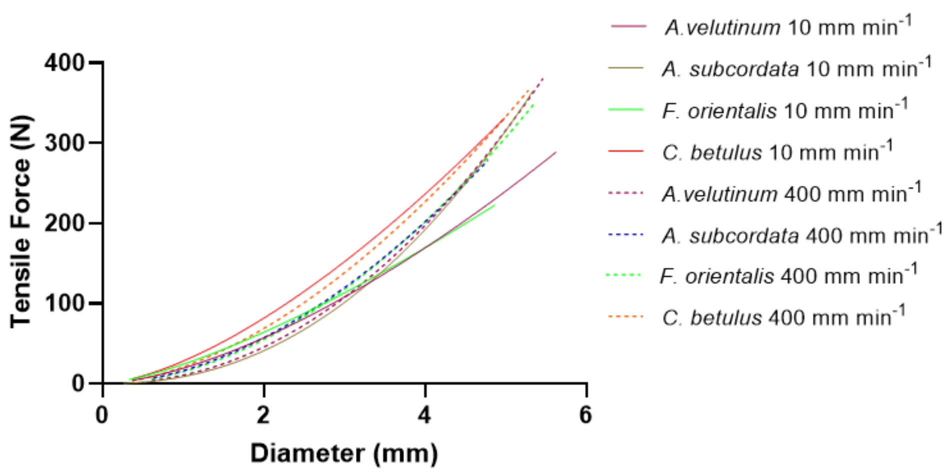



**Fig. 3.** Relationship between root tensile Force (N) and root diameter (mm) in the studied species at two loading rates

## Tensile Strength

Based on the descriptive statistics presented in Table 2, *C. betulus* exhibited the highest estimated marginal mean tensile strength at loading rates of 10 and 400 mm min$^{-1}$. In contrast, *A. velutinum* showed the lowest tensile strength at 10 and 400 mm min$^{-1}$, rates.

**Table 2.** Descriptive Statistics and Estimated Marginal Means (EMMs) of Root Tensile Strength (MPa)

| Species | Loading Rate (mm min$^{-1}$) | Min | Max | EMMs | α | β |
|---|---|---|---|---|---|---|
| *A. velutinum* | 10 | 7.28 | 37.42 | 16.97 | 19.34 | -0.27 |
| | 400 | 4.04 | 47.21 | 19.02 | 22.54 | -0.37 |
| *A. subcordata* | 10 | 4.95 | 36.91 | 17.49 | 19. 29 | -0.28 |
| | 400 | 10.55 | 45.89 | 22.52 | 27.84 | -0.44 |
| *F. orientalis* | 10 | 4.87 | 56.20 | 20.10 | 22.75 | -0.4 |
| | 400 | 5.51 | 47.58 | 19.31 | 24.60 | -0.4 |
| *C. betulus* | 10 | 6.14 | 43.07 | 24.90 | 28.37 | -0.37 |
| | 400 | 8.2 | 40.17 | 24.80 | 27.3 | -0.2 |

The ANCOVA revealed that root diameter significantly affected tensile strength in all studied species ($p < 0.05$). However, loading rate did not significantly affect tensile strength in *F. orientalis*, *C. betulus*, or *A. velutinum* ($p > 0.05$). In contrast, tensile strength in *A. subcordata* differed significantly between the two loading rates, with higher values observed at 400 mm min$^{-1}$ ($p < 0.001$), (Fig. 4).

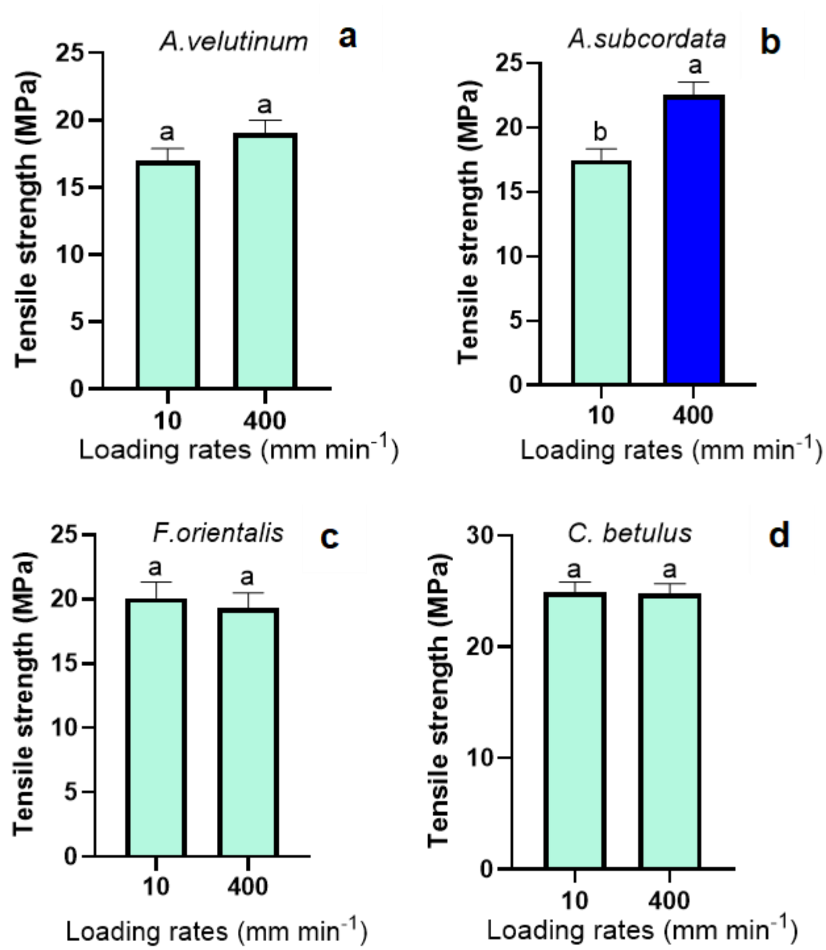



**Fig. 4.** Estimated marginal means (EMMs) of root tensile strength (MPa) at loading rates of 10 and 400 mm min$^{-1}$ in : (a) *A. velutinum*, (b) *A. subcordata*, (c) *F. orientalis*, and (d) *C. betulus*; Different letters indicate significant differences between treatments

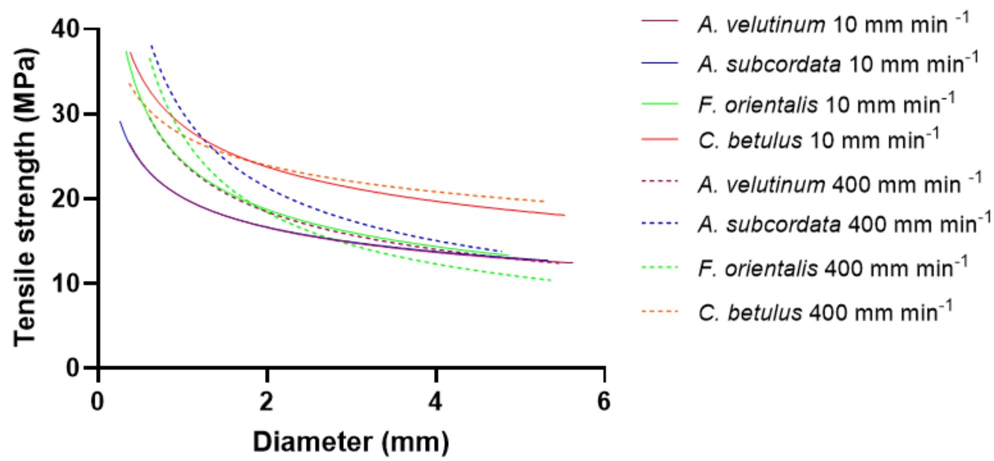



**Fig. 5.** Relationship between root tensile strength (MPa) and root diameter (mm) in the studied species at two loading rates

The results demonstrated a significant negative power-law relationship between root tensile strength and root diameter in all studied species, indicating that tensile strength decreased with increasing root diameter (Fig. 5).

## Young's Modulus

Table 3 summarizes the descriptive statistics of Young's modulus for the tested roots. *A. velutinum* exhibited the lowest estimated marginal mean Young's modulus at 10 mm min$^{-1}$ (275.7 MPa), whereas *C. betulus* showed the highest value (427.6 MPa) at the same loading rate.

**Table 3.** Descriptive Statistics and Estimated Marginal Means (EMMs) of Young's Modulus (MPa)

| Species | Loading Rate (mm min$^{-1}$) | Min | Max | EMMs | α | β |
|---|---|---|---|---|---|---|
| *A. velutinum* | 10 | 102.08 | 594.85 | 275.72 | 299.63 | -0.21 |
| | 400 | 160.22 | 849.86 | 397.36 | 436.97 | -0.2 |
| *A. subcordata* | 10 | 86.11 | 987.84 | 291.85 | 312.25 | -0.27 |
| | 400 | 172.31 | 595.21 | 374.83 | 402.75 | -0.27 |
| *F. orientalis* | 10 | 99.22 | 1270.65 | 393.28 | 337.33 | -0.28 |
| | 400 | 110.72 | 533.88 | 312.17 | 350.2 | -0.24 |
| *C. betulus* | 10 | 197.93 | 865.75 | 427.67 | 435.53 | -0.20 |
| | 400 | 97.52 | 687.83 | 338.68 | 355.67 | -0.26 |

The ANCOVA showed that loading rate significantly affected Young's modulus across the studied species ($F = 12.27$, $p < 0.001$). *Post hoc* analysis revealed that loading rate significantly affected Young's modulus in *C. betulus* ($F = 13.63$, $p < 0.001$), *A. velutinum* ($F = 30.77$, $p < 0.001$), and *A. subcordata* ($F = 8.66$, $p < 0.001$), whereas no significant effect was detected in *F. orientalis* ($p > 0.05$).

Comparisons of estimated marginal means of Young's modulus values indicated that *A. velutinum* and *A. subcordata* exhibited significantly greater Young's modulus values at 400 mm min$^{-1}$ than at 10 mm min$^{-1}$. In contrast, *C. betulus* exhibited higher Young's modulus values at the lower loading rate (Fig. 6).

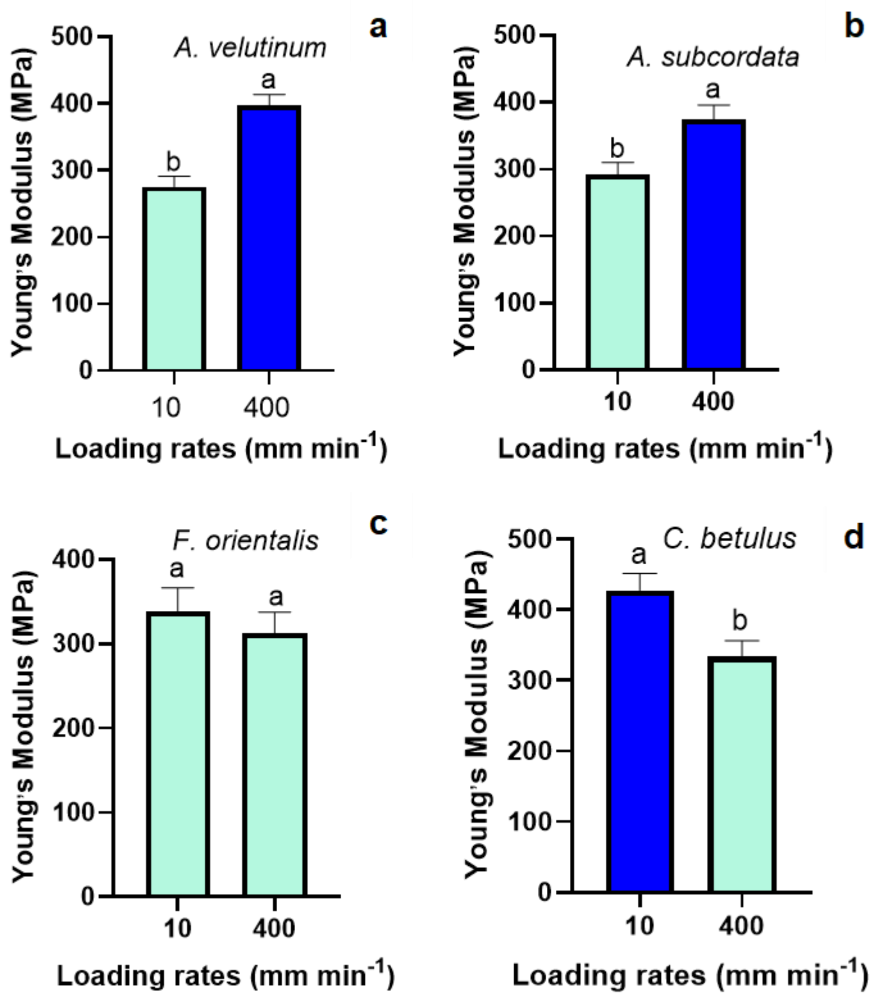



**Fig. 6.** Estimated marginal means (EMMs) of Young's modulus (MPa) at loading rates of 10 and 400 mm min$^{-1}$ in: (a) *A. velutinum*, (b) *A. subcordata*, (c) *F. orientalis*, and (d) *C. betulus*; Different letters indicate significant differences between treatments.

The results revealed a negative power-law relationship between Young's modulus and root diameter in all studied species, indicating that Young's modulus decreased with increasing root diameter (Fig. 7).

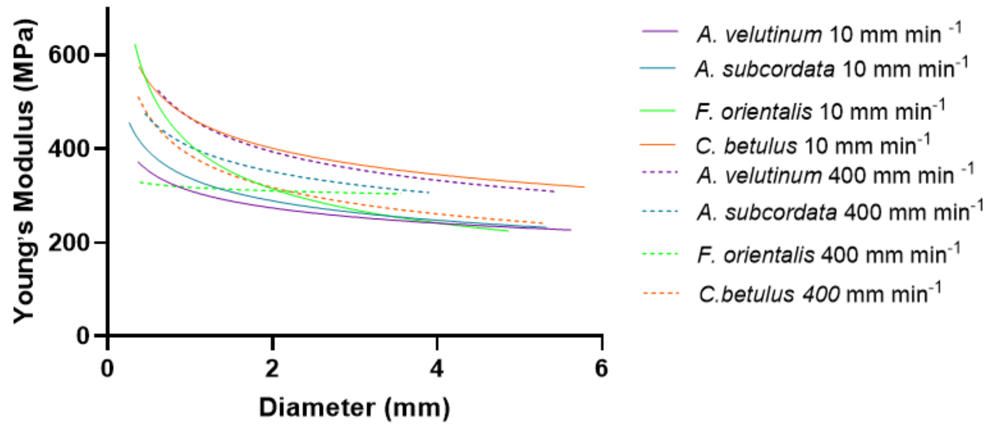



**Fig. 7.** Relationship between Young's modulus (MPa) and root diameter (mm) in the studied species at two loading rates

## Freezing Experiment

To determine whether freezing preservation affects root mechanical behavior, a subset of root samples was stored at −20 °C for one month and subsequently tested at the standard loading rate of 10 mm min$^{-1}$. The effects of freezing on the three mechanical properties are presented below.

## Tensile Force

Descriptive statistics of root tensile force are presented in Table 4. Among the studied species, *C. betulus* exhibited the highest estimated marginal mean tensile force under freezing treatment (132.7 N). The parameters α and β represent the size coefficient and the rate of increase in tensile force with increasing root diameter, respectively. Higher values of these parameters indicate greater root mechanical resistance.

**Table 4.** Descriptive Statistics and Estimated Marginal Means (EMMs) of Root Tensile Force (N) Under Control and Freezing Treatments

| Species | Treatment | Min | Max | EMMs | α | β |
|---|---|---|---|---|---|---|
| *A. velutinum* | Control | 4 | 368 | 75.64 | 15.89 | 1.67 |
| | Frozen | 1 | 260.2 | 78.187 | 20. 25 | 1.53 |
| *A. subcordata* | Control | 1.2 | 501.5 | 84.06 | 15.16 | 1.72 |
| | Frozen | 2.6 | 307.9 | 102.09 | 27.30 | 1.49 |
| *F. orientalis* | Control | 2.9 | 326.3 | 74.286 | 17.86 | 1.59 |
| | Frozen | 0.9 | 248.1 | 88.336 | 22.71 | 1.64 |
| *C. betulus* | Control | 32 | 720.5 | 97.98 | 22.26 | 1.62 |
| | Frozen | 3.4 | 597.3 | 132.78 | 30.43 | 1.66 |

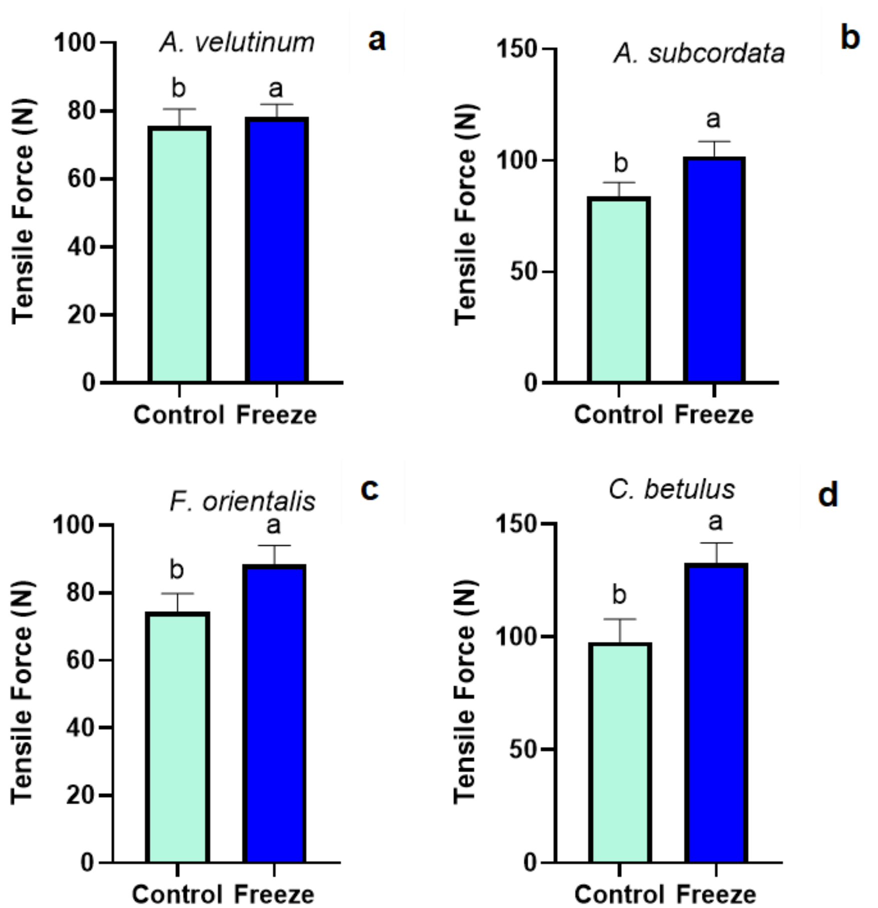



**Fig. 8.** Estimated marginal means (EMMs) of root tensile force (N) under control and freezing treatments in: (a) *A. velutinum*, (b) *A. subcordata*, (c) *F. orientalis*, and (d) *C. betulus*; Different letters indicate significant differences between treatments

The ANCOVA results demonstrated that both root diameter and freezing treatment significantly affected root tensile force in *A. velutinum* ($F = 5.17$, $p < 0.05$), *A. subcordata* ($F = 33.14$, $p < 0.001$), *F. orientalis* ($F = 7.68$, $p < 0.001$), and *C. betulus* ($F = 17.47$, $p < 0.001$). Overall, freezing treatment increased root tensile force across the studied species (Fig. 8).

Species had a significant effect on tensile force ($F = 6.67$, $p < 0.001$), indicating substantial interspecific variation. *Post hoc* comparisons revealed no significant differences among *F. orientalis*, *A. velutinum*, and *A. subcordata*. In contrast, *C. betulus* exhibited significantly higher tensile force than each of the other three species (Fig. 9).

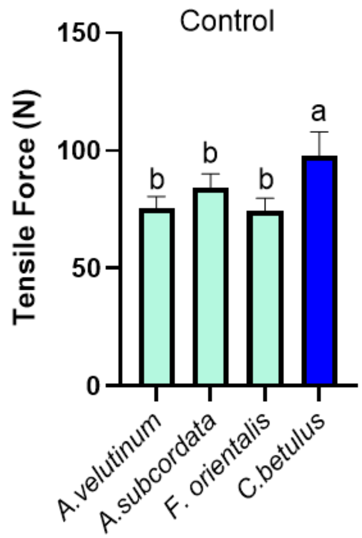



**Fig. 9.** *Post hoc* comparison of estimated marginal means of root tensile force (N) among the studied species under the control treatment; Different letters indicate significant differences between species

The relationship between root diameter and tensile force was significant across all species (F = 1045.452, $p < 0.001$), with tensile force increasing markedly as root diameter increased (Fig. 10).

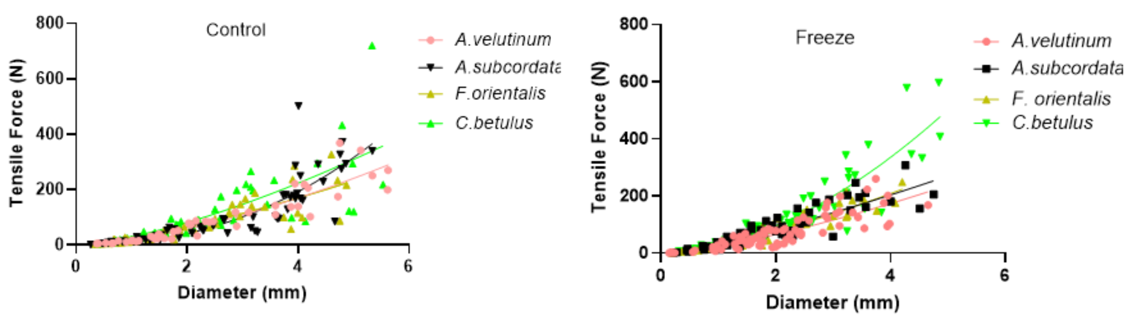



**Fig. 10.** General relationship between root tensile force (N) and root diameter (mm) across the studied species under both control and freezing treatments

## Tensile Strength

According to the statistical results, frozen roots generally exhibited higher coefficients of determination ($R^2$) than control roots, with $R^2$ values increasing by 12–57%. Among the studied species, *C. betulus* showed the highest estimated marginal mean tensile strength under freezing treatment (34.4 MPa), whereas *A. subcordata* exhibited the lowest value (22.5 MPa).

**Table 5.** Descriptive Statistics and Estimated Marginal Means (EMMs) of Root Tensile Strength (MPa) Under Control and Freezing Treatments

| Species | Treatment | Min | Max | EMMs | α | β |
|---|---|---|---|---|---|---|
| *A. velutinum* | Control | 7.28 | 37.42 | 18.30 | 19.34 | -.027 |
| | Frozen | 6.97 | 106.15 | 22.59 | 25.8 | -0.46 |
| *A. subcordata* | Control | 4.95 | 36.91 | 18.20 | 19.29 | -0.28 |
| | Frozen | 8.23 | 88.49 | 29.82 | 34.91 | -0.5 |
| *F. orientalis* | Control | 4.87 | 56.20 | 21.11 | 22.75 | -0.4 |
| | Frozen | 11.9 | 72.93 | 27.23 | 30.85 | -0.37 |
| *C. betulus* | Control | 6.14 | 43.07 | 25.43 | 28.37 | -0.37 |
| | Frozen | 9.33 | 83.51 | 34.48 | 38.76 | -0.33 |

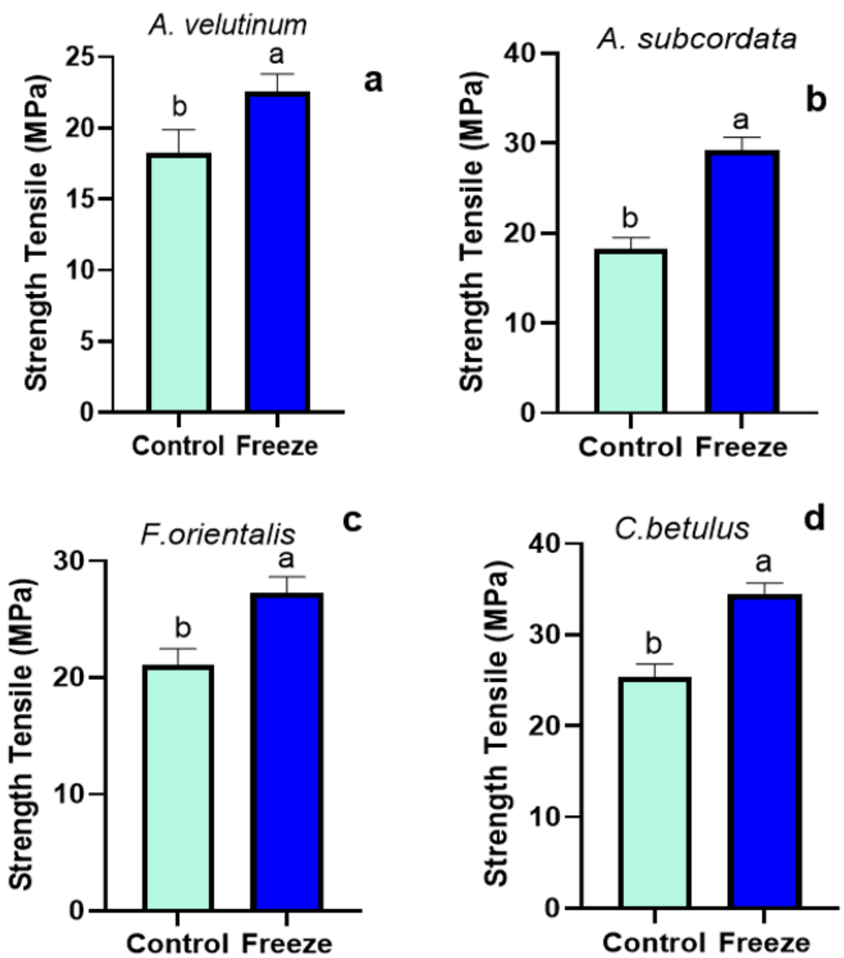



**Fig. 11.** Estimated marginal means (EMMs) of mean root tensile strength (MPa) under control and freezing treatments in: (a) *A. velutinum*, (b) *A. subcordata*, (c) *F. orientalis*, and (d) *C. betulus*; Different letters indicate significant differences between treatments.

The ANCOVA showed that freezing treatment significantly increased root tensile strength in *A. velutinum* ($F = 3.850$, $p < 0.05$), *A. subcordata* ($F = 41.243$, $p < 0.001$), *F. orientalis* ($F = 13.996$, $p < 0.001$), and *C. betulus* ($F = 26.20$, $p < 0.001$), relative to the control (Fig. 11).

In addition, a significant inverse relationship was observed between root diameter and tensile strength across all species ($F = 13.92$, $p < 0.001$), indicating that tensile strength declined according to a power-law relationship as root diameter increased (Fig. 12).

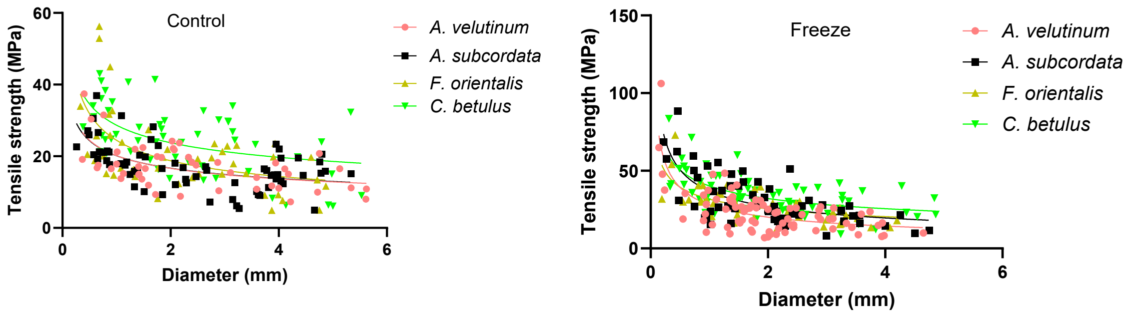



**Fig. 12.** General relationship between root tensile strength (MPa) and root diameter (mm) across the studied species under both the control and freeze treatment

Comparisons of estimated marginal mean tensile strength under control conditions demonstrated that *C. betulus* had significantly greater tensile strength than *F. orientalis*, *A. velutinum*, and *A. subcordata* ($F = 6.48$, $p < 0.001$) (Fig. 13).

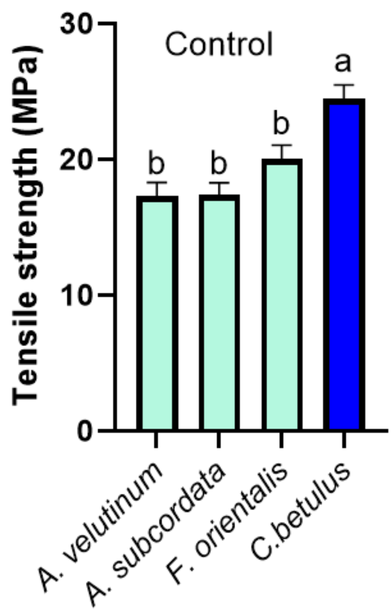



**Fig. 13.** *Post hoc* comparison of estimated marginal means of root tensile strength (MPa) among the studied species under the control treatment; Different letters indicate significant differences between species.

## Young's Modulus

The descriptive statistics presented in Table 6 indicate considerable variation in Young's modulus among species and between control and freezing treatments.

**Table 6.** Descriptive Statistics and Estimated Marginal Means (EMMs) of Young's Modulus (MPa) Under Control and Freezing Treatments

| Species | Treatment | Min | Max | EMMs | α | β |
|---|---|---|---|---|---|---|
| *A. velutinum* | Control | 91.88 | 594.85 | 295.597 | 299.63 | -0.21 |
| | Frozen | 115.75 | 1550 | 390.29 | 439.33 | -0.47 |
| *A. subcordata* | Control | 73.17 | 692.62 | 303.87 | 312.64 | -0.26 |
| | Frozen | 236.38 | 1014.73 | 503.87 | 543. 71 | -0.23 |
| *F. orientalis* | Control | 99.22 | 1270.65 | 353.39 | 337.33 | -0.27 |
| | Frozen | 239.90 | 1100 | 540.01 | 599.64 | -0.32 |
| *C. betulus* | Control | 163.84 | 865.75 | 433.41 | 458.51 | -0.23 |
| | Frozen | 257.12 | 978.44 | 518.29 | 549.05 | -0.18 |

The ANCOVA results showed that root diameter significantly affected Young's modulus ($F = 10.806$, $p < 0.001$). Freezing treatment also significantly influenced Young's modulus in *A. velutinum* ($F = 5.634$, $p < 0.05$), *A. subcordata* ($F = 56. 47$, $p < 0.001$), *F. orientalis* ($F = 32.011$, $p < 0.001$), and *C. betulus* ($F = 11.661$, $p < 0.001$) (Fig. 14).

A significant negative power-law relationship was observed between root diameter and Young's modulus in all studied species, indicating that Young's modulus decreased according to a power-law function with increasing root diameter under both control and freezing conditions (Fig. 15).

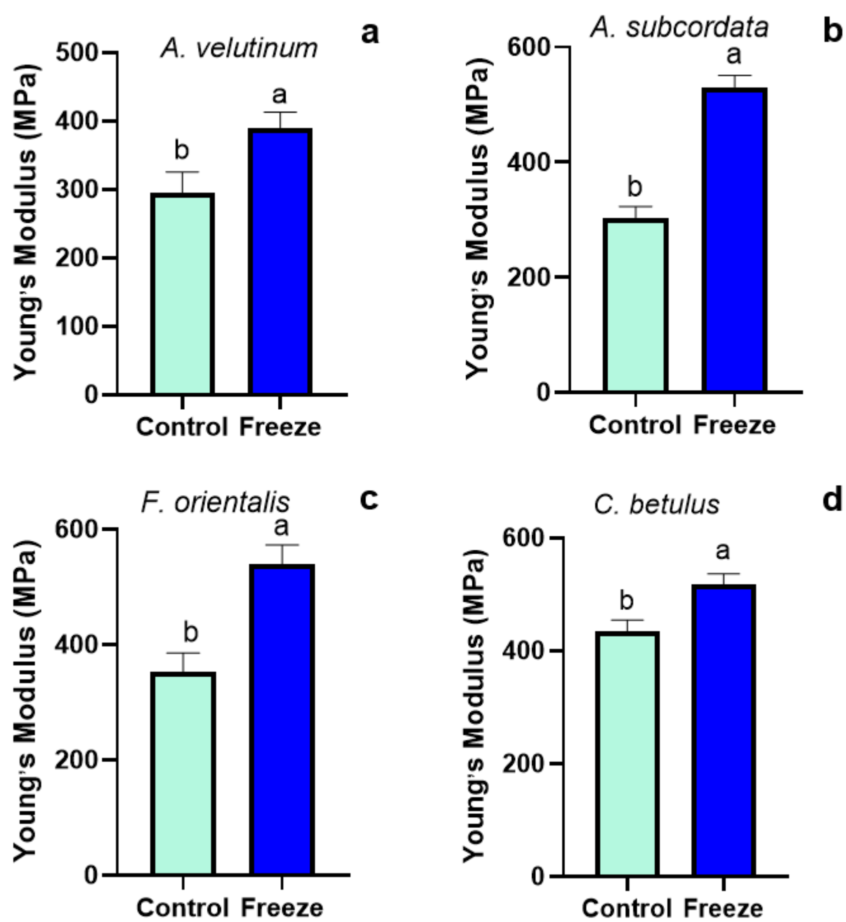



**Fig. 14.** Estimated marginal means (EMMs) of mean Young's modulus (MPa) under control and freezing treatments in: (a) *A. velutinum*, (b) *A. subcordata*, (c) *F. orientalis*, and (d) *C. betulus*; Different letters indicate significant differences between treatments.

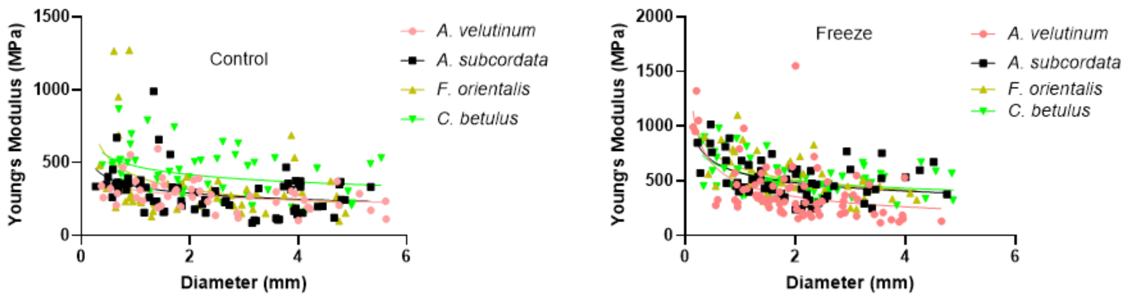



**Fig. 15.** Relationship between Young's modulus (MPa) and root diameter (mm) in the studied species under both the control and freeze treatment

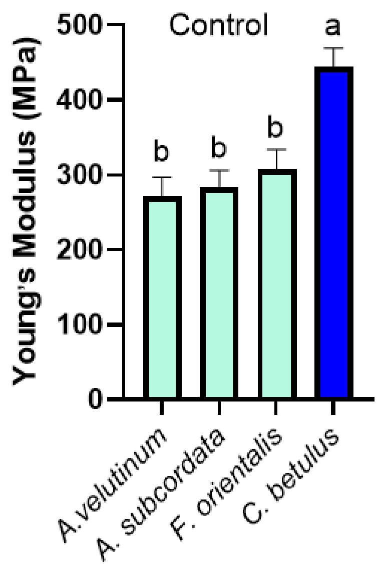



**Fig. 16.** *Post hoc* comparison of estimated marginal means of Young's modulus (MPa) among the studied species under the control treatment; Different letters indicate significant differences between species.

Among the studied species, *C. betulus* exhibited significantly greater Young's modulus values than *F. orientalis*, *A. velutinum*, and *A. subcordata* (Fig. 16).

## DISCUSSION

### Effect of Loading Rate on Root Mechanical Properties

Loading rate is a critical experimental variable in tensile testing because it influences the mechanical response of viscoelastic materials. Consequently, the relationship between loading rate and root tensile properties has received increasing attention (Zhang *et al.* 2012; Melese *et al.* 2021; Zhang *et al*. 2021), although the reported findings remain inconsistent across species and experimental conditions. In the present study, increasing the loading rate from 10 to 400 mm min$^{-1}$ did not significantly affect tensile force or tensile strength in *Fagus orientalis*, *Carpinus betulus*, or *Acer velutinum* (Figs. 2 and 4). This result agrees with Zhang *et al.* (2021), who observed no significant changes in root tensile strength over the same loading-rate range. Likewise, Wu *et al.* (2021) reported that increasing the loading rate from 16 to 200 mm min$^{-1}$ did not significantly alter the tensile strength of *Chrysopogon zizanioides* roots. Although the tested loading-rate ranges differed among studies, these findings collectively suggest that, when factors, such as root moisture content and sample geometry, are carefully controlled, root tensile properties remain relatively stable across a broad range of loading rates.

In contrast, *A. subcordata* exhibited a significant increase in both tensile force and tensile strength at the higher loading rate. Similar rate-dependent strengthening has been reported by Cofie and Koolen (2001), who also observed greater rupture strength under rapid loading conditions. These contrasting responses among species are likely associated with differences in root anatomical organization, tissue density, and the presence of microstructural defects that influence stress distribution during tensile loading (Zhang *et al.* 2012; Freschet *et al.* 2021). Such interspecific variation suggests that root mechanical responses to loading rate are governed not only by the intrinsic viscoelastic properties of root tissues but also by species-specific anatomical characteristics. Consequently, integrating anatomical analyses with biomechanical testing may provide a more comprehensive understanding of root mechanical behavior.

A similar species-dependent response was observed for Young's modulus. Increasing loading rate significantly increased Young's modulus in *A. subcordata* and *A. velutinum*, whereas no significant change was detected in *F. orientalis*. In contrast, *C. betulus* exhibited the opposite pattern, with significantly higher Young's modulus at the lower loading rate. These contrasting responses indicate that elastic stiffness is regulated by species-specific structural characteristics and that different tissues may respond differently to loading velocity. Such variability further supports the view that root biomechanics cannot be adequately described using generalized mechanical parameters.

From a practical perspective, loading rates applied during laboratory testing may represent different deformation rates experienced by roots during natural slope failures, ranging from slow soil creep to rapid shallow landslides (Castelli *et al.* 2009). Therefore, understanding loading-rate sensitivity is important for interpreting laboratory-derived biomechanical data within realistic geomorphological contexts.

Overall, the present results indicate that elasticity-related parameters appear more sensitive to loading rate than failure-related parameters. The greater sensitivity of Young's modulus to changing loading rate than tensile force and tensile strength may be related to the viscoelastic and heterogeneous nature of root tissues. Because Young's modulus is derived from the elastic portion of the stress–strain curve, faster loading reduces the time available for viscous deformation, therefore resulting in greater apparent stiffness and a response closer to the unrelaxed modulus (Bidhendi and Geitmann 2019). Loading rate can also alter the development of the elastic phase and the stress–strain relationship of roots (Chen et al. 2014), while rate-dependent viscoelastic behavior has been reported in root tissues (Nwakuba *et al.* 2023). Moreover, the distinct mechanical properties of root tissues, particularly the cortex and stele, contribute to their nonlinear mechanical response (Meijer *et al.* 2018; Meijer *et al.* 2024). Thus, loading-rate effects may be more evident in the elastic response measured by Young's modulus than in ultimate failure parameters such as tensile force and tensile strength.

Future studies incorporating detailed anatomical investigations of root tissues are recommended to further describe the mechanisms underlying these rate-dependent responses

## Effect of Freezing Preservation on Root Mechanical Properties

The second objective of this study was to determine whether freezing, a widely used preservation method in root biomechanical studies, alters the measured mechanical properties of roots. The results clearly demonstrated that storing roots at −20 °C for one month significantly increased tensile force, tensile strength, and Young's modulus compared with fresh samples. These findings indicate that freezing does not simply preserve root tissues but systematically modifies their mechanical behavior, suggesting that measurements obtained from frozen samples may not accurately represent the *in vivo* mechanical properties of roots.

The increase in root mechanical properties following freezing may reflect the combined effects of freeze-induced dehydration, water redistribution, and structural alterations within root tissues. During extracellular freezing, ice formation drives water from living cells toward extracellular spaces, resulting in cellular dehydration and changes in the mechanical state of the cell-wall matrix (Stegner *et al*. 2022). During thawing, part of this water may remain redistributed or be lost, reducing tissue moisture content and increasing apparent tissue stiffness. Because water acts as a plasticizer within plant cell walls, its loss can increase the rigidity of cell-wall components and enhance stress transfer within the tissue, potentially resulting in greater apparent tensile resistance. Similar moisture-dependent increases in root tensile strength have been reported earlier by Hales *et al.* (2013) and Yang *et al*. (2016), highlighting the importance of tissue water content in root biomechanical behavior. Freezing and thawing may also induce ice-crystal formation and membrane or intercellular structural alterations, which can further modify tissue stiffness and post-thaw mechanical behavior. The magnitude of these effects may vary among species because freeze-induced dehydration is influenced by anatomical characteristics such as cell size, cell-wall properties, and intercellular spaces (Stegner *et al.* 2022). Thus, differences in root anatomy and tissue properties may partly explain the species-specific responses observed in the present study. However, because root moisture content and anatomical changes were not directly measured in the current study, these mechanisms should be considered plausible explanations rather than experimentally confirmed causes.

Freezing also strengthened the relationships between root diameter and the measured mechanical properties, producing more consistent regression models than those obtained from fresh samples. Although this improvement might initially suggest reduced experimental variability, it should not be interpreted as evidence of improved measurement accuracy. Rather, the increased consistency likely reflects the homogenizing effects of dehydration and structural modification, which reduce natural biological variability among root samples. Consequently, stronger statistical relationships after freezing probably represent experimental artefacts rather than true physiological patterns.

Direct comparison with previous studies is limited because the influence of freezing preservation on root mechanical behavior has rarely been investigated. Nevertheless, extensive research on plant freezing physiology demonstrates that freeze-induced membrane disruption, cellular dehydration, and irreversible structural alterations are common consequences of freezing stress (Steponkus 1984; Steponkus *et al.* 1993; Thomashow 1999). These physiological responses provide a plausible mechanistic explanation for the systematic increase in mechanical properties observed in the present study.

From an applied perspective, these findings have important methodological implications for soil bioengineering research. Root mechanical properties are widely used to parameterize root reinforcement models; therefore, systematic overestimation of tensile force, tensile strength, and Young's modulus may propagate into predictions of vegetation-induced soil reinforcement. Although freezing offers a practical and inexpensive solution for preserving large numbers of samples when immediate testing is not feasible (Freschet *et al.* 2021), its use should be carefully considered because it may introduce systematic bias into biomechanical datasets. When long-term storage is unavoidable, preservation methods that better maintain the native structure and moisture status of root tissues, such as freeze-drying, may provide a more appropriate alternative, although their availability is often limited by laboratory facilities.

Overall, the present findings demonstrate that freezing preservation is not a neutral storage procedure but a methodological factor capable of systematically altering measured root mechanical properties. Consequently, the use of frozen samples may lead to overestimation of vegetation-induced soil reinforcement unless these effects are explicitly considered during data interpretation and biomechanical modelling.

## Diameter Dependence and Species-specific Mechanical Behavior

In agreement with numerous previous studies, root diameter exerted a strong influence on all measured mechanical properties. Tensile force increased with root diameter, following a positive power-law relationship, reflecting the greater load-bearing capacity associated with larger cross-sectional areas (Bischetti *et al.* 2005; Genet *et al.* 2005; Abdi 2018). In contrast, tensile strength generally declined as root diameter increased, confirming the inverse scaling relationship widely reported in the literature (Capilleri *et al.* 2016; Patil *et al.* 2023) greater absolute loads, they are mechanically less efficient on a unit-area basis than finer roots.

The negative relationship between tensile strength and root diameter is primarily explained by age-related and anatomical changes that occur during root development. As roots increase in diameter, secondary growth modifies tissue composition through progressive lignification, thickening of secondary cell walls, and changes in the relative proportions of xylem, fibres, and parenchyma (Oladi *et al.* 2025). These structural changes alter the way tensile stresses are distributed within the root and generally reduce specific tensile strength. Previous studies have shown that tensile strength is negatively correlated with lignin content and the lignin-to-cellulose ratio, while positively associated with cellulose and hemicellulose concentrations (Ye *et al.* 2017). Because lignin content generally increases with root diameter (Zhang *et al.* 2019), thicker roots are expected to exhibit lower tensile strength despite their greater absolute resistance to failure.

Root anatomical organization further contributes to this scaling behavior. Xylem architecture, the distribution of radial parenchyma, fibre morphology, and the arrangement of supporting tissues all influence the mechanical performance of woody roots (Reiterer *et al.* 2002; Bari *et al.* 2015). Thinner roots generally possess longer fibres and relatively thinner cell walls, characteristics that contribute to higher tensile strength (Oladi *et al.* 2025). Conversely, thicker roots contain a larger proportion of supporting tissues and exhibit changes in fibre architecture that reduce mechanical efficiency per unit cross-sectional area (Norris 2005). These observations indicate that root biomechanics cannot be explained by diameter alone but must also consider the internal organization and composition of root tissues.

Species identity also significantly influenced root mechanical behavior. *Carpinus betulus* exhibited significantly greater estimated marginal mean tensile force than the other three species, whereas *Fagus orientalis*, *Acer velutinum*, and *Alnus subcordata* did not differ significantly from one another. Similar interspecific variation has been widely documented and is commonly attributed to differences in genetic background, anatomical structure, wood density, and ecological adaptation (De Baets *et al.* 2008; Ji *et al.* 2012; Schwarz *et al.* 2013; Vergani *et al.* 2012, 2016). In addition, environmental factors, such as soil moisture, soil compaction, and nutrient availability, can modify root development and tissue properties, further contributing to variation in mechanical characteristics among species and study sites (Loades *et al.* 2013; Zhou *et al.* 2022).

A similar pattern of interspecific variation was observed for Young's modulus. *Carpinus betulus* exhibited the highest elastic stiffness, followed by *Fagus orientalis*, *Alnus subcordata*, and *Acer velutinum*. These differences most likely reflect species-specific variation in tissue composition, xylem structure, and mechanical adaptation to environmental conditions. Together, these findings indicate that the mechanical behavior of tree roots is governed by the combined effects of root diameter, anatomical organization, and species identity, rather than by any single morphological characteristic.

Overall, the present results demonstrate that root diameter provides a robust predictor of mechanical behavior; however, anatomical structure and species identity substantially modify these relationships. Consequently, the use of generalized mechanical parameters across different species may oversimplify root biomechanics and reduce the reliability of vegetation-induced soil reinforcement models.

## Implications for Root Reinforcement Modelling and Soil Bioengineering

The considerable variability in root mechanical properties reported among previous studies has often been attributed to differences in species, site conditions, and environmental factors. The present findings demonstrate that methodological factors should also be considered an important source of variation. In addition to biological influences, such as species identity, root diameter, anatomical structure, and environmental conditions, differences in sample preservation, loading rate, and testing protocols can substantially affect the measured mechanical properties of roots. Consequently, direct comparisons among studies should be made with caution unless experimental methodologies are comparable.

Power-law relationships between root diameter and mechanical properties constitute the foundation of many root reinforcement models used in soil bioengineering and slope-stability analyses (Waldron 1977; Operstein and Frydman 2000; Fan and Su 2008). Although the present study confirmed the existence of these relationships, it also demonstrated that both their strength and model parameters are influenced by species identity and experimental methodology. In particular, freezing preservation systematically increased the measured mechanical properties and strengthened diameter–property relationships, indicating that model parameters derived from frozen samples may differ substantially from those obtained using fresh roots. Therefore, applying generalized coefficients across different species or combining datasets generated using different testing protocols may introduce additional uncertainty into root reinforcement predictions.

The observed species-specific responses further emphasize that root biomechanics cannot be adequately represented using universal mechanical parameters. Instead, species-specific parameterization should be preferred whenever possible, particularly in quantitative models that estimate vegetation-induced soil reinforcement. Incorporating anatomical characteristics together with biomechanical measurements may further improve model performance by providing a more mechanistic representation of root behavior under tensile loading.

From a practical perspective, the present findings have direct implications for laboratory testing protocols and engineering applications. The results indicate that the commonly used loading rate of 10 mm $min^{-1}$ provides representative measurements for most of the studied species, whereas freezing preservation systematically overestimates root tensile properties. Consequently, fresh samples should be preferred whenever feasible, and any preservation procedure should be clearly documented to facilitate meaningful comparisons among studies. Establishing standardized testing protocols—including sample collection, storage conditions, loading rate, and specimen preparation—will substantially improve the reproducibility, comparability, and reliability of biomechanical datasets.

Ultimately, improving the quality and consistency of laboratory-derived root mechanical data will enhance the parameterization of root reinforcement models and reduce uncertainty in predicting vegetation-induced soil stabilization. Such improvements will contribute to more reliable implementation of nature-based solutions for slope stabilization and support the development of more effective soil bioengineering strategies under a wide range of environmental conditions.

## CONCLUSIONS

1. Root biomechanical properties were influenced by species, root diameter, and, depending on species and property, loading rate and freezing treatment.
2. A consistent power-law relationship was observed between root diameter and mechanical properties. Tensile force increased with increasing root diameter, whereas tensile strength (per unit of cross-sectional area) generally decreased as root diameter increased.
3. Only *Alnus subcordata* exhibited significant increases in tensile force and tensile strength at the higher loading rate, whereas Young’s modulus also showed significant species-dependent responses.
4. Freezing preservation significantly increased tensile force, tensile strength, and Young's modulus. These increases most likely resulted from dehydration and structural changes induced by freezing rather than reflecting the true in vivo mechanical behavior of roots.
5. The use of frozen samples may systematically overestimate root mechanical performance and, consequently, root-induced soil reinforcement. Because root mechanical properties are key input parameters in root reinforcement models, their overestimation may lead to overestimation of root-induced soil cohesion and shear strength, and consequently the factor of safety of slope. Such bias may result in overly optimistic assessments of slope stability and increase the risk of unanticipated instabilities. Therefore, practically frozen samples should be used with caution in biomechanical studies, and alternative preservation methods should be further evaluated.
6. The results demonstrate that root mechanical properties should be treated as species-specific parameters. The use of standardized testing protocols together with species-specific mechanical data will improve the reliability of biomechanical datasets and the

predictive accuracy of root reinforcement models for soil bioengineering and slope stabilization applications.

7. A limitation of this study is that root moisture content and anatomical characteristics were not examined before and after freezing; therefore, the relative contributions of moisture loss and structural changes to the observed mechanical responses could not be distinguished.

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