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Article

Reliable Monitoring of Plantation Performance in Arid and Semiarid Lands Requires Both “In Vivo” and “Ex Vivo” Approaches.

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Abstract: Conventional monitoring relies on “in vivo” (non-destructive) measurements of aboveground organ traits (such as stems, branches, and leaves) to analyze plantation growth trends and assess the effectiveness of management practices like watering and fertilization. This study reveals the necessity of “ex vivo” (destructive) approaches, post-excavation, to ensure fully reliable monitoring of plantation performance, including belowground organ growth and development trends. We showcase the effectiveness of dendrometric analysis, which measures the annual ring width in the secondary xylem (wood) of the taproot and stem. Monitoring above- and belowground organ growth trends allow for the exploration of similarities and differences in how different tree organs respond to internal and external factors. Such monitoring is especially crucial for assessing plantation performance in challenging climates, such as those found in arid and semi-arid lands.

Keywords: arid lands; restoration; plantations; monitoring; tree performance

1. Introduction

Enhancing forest cover is crucial for enhancing carbon sequestration and mitigating the impact of climate change (Aronson et al., 2006; Clewell and Aronson, 2007). Natural forest regeneration is a recommended, cost-effective, and sustainable method for achieving this goal (Stanturf et al., 2020). However, it is essential to acknowledge that natural regeneration may not be feasible in regions with adverse climates hindering its success (Stanturf et al., 2014, 2015). For instance, in the arid and semiarid lands of Central Asia, environmental conditions hinder natural forest regeneration necessitating additional management measures such as irrigation and fertilization to facilitate the growth of planted forests.

A significant portion of Mongolia's territory faces a severe continental climate ranging from subarctic, to arid and semi-arid with 78% of the territory experiencing severe soil erosion and desertification (Country Programme of Mongolia, 2019; MNET 2010; Desertification Atlas of Mongolia, 2013). Climatic models predict a worsening of environmental conditions in these lands, leading to an expansion of desertification, loss of biodiversity, and a decline in residual forest cover (Yessekin et al., 2008; Hessel et al., 2016; Reyer et al., 2017; Stavi and Lal, 2015). In response to these challenges, the Government of Mongolia has initiated tree-planting projects, aiming to halt further land degradation and improve local livelihoods through economically viable agroforestry activities (Tsogtbaatar, 2013).

One tree-planting initiative is the Greenbelt project (Byambadorj et al. (2021 a,b), which commenced in 2009 and has contributed to afforestation in Mongolia's arid and semiarid land. These plantations (approximately 3000 ha) required the construction of water wells due to limited water

availability from rainfall. Consequently, thorough monitoring of the growth and developmental trend in these plantations was imperative to determine an optimal and sustainable amount of watering necessary to ensure the success of the planting initiative.

In a broad context, monitoring the growth and development of trees in a new plantation is a crucial step (McDonald et al., 2016) for at least three reasons. Firstly, it allows us to anticipate the success or failure of the plantation's objective (Menz et al., 2011; Abiyu et al., 2016; Guariguata and Evans, 2020), and, if necessary, make adaptive management adjustments (Le et al., 2012). Secondly, monitoring provides the opportunity to accumulate knowledge and expertise essential for scaling up or planning similar initiatives in the future (Kanowski et al., 2010; Derhé, 2016), and contributes to transparency and accountability (Benayas et al., 2009). Thirdly, monitoring is essential for generating comprehensive reports for investors, securing additional funds, and facilitating the exchange of information with similar initiatives (Guariguata and Evans, 2020).

Extensive literature exists on the various methods for measuring the biophysical parameters of growing trees. These methods can be broadly categorized into three overlapping groups: 1) Digital Tracking (DT), 2) Remote Sensing (RS), and 3) Drone Fly-Overs (DFO). RS utilizes LiDAR, while DFO employs lightweight uncrewed aerial vehicles. Both RS and DFO measure aboveground biomass, density, vegetation structure and composition, natural seedlings recruitment, tree architecture, flowering flushes, etc. DT involves field visits to the stand where morphological traits are hand-measured and recorded directly in a digital platform such as FARM-TRACE. Therefore, it is reasonable to consider DT as a "boots on the ground" monitoring method, whereas RS and DFO could be defined as "remote" monitoring methods, as both approaches collect imagery for later analysis using specific software to provide a Digital Terrain Model (DTM) and a Canopy Height Model (CHM) (Farajelahl et al., 2022).

An important difference between these monitoring methods consists in the type of information they provide. RS and DFO monitor a stand at a community level (Ruiz-Jaén and Aide, 2006; Monie et al., 2013), whereas DT monitors the growth and development of individual trees within a stand (Mahamoudou and Arakwiye, 2020; Reyter et al., 2020). DT monitoring is particularly relevant at the initial stage of forest landscape restoration (FLR) implementation to establish the compatibility between plant species and the environmental factors specific to the selected site.

We have been monitoring the performance of the Greenbelt Project plantations since 2009 through a DT approach based on the yearly "in vivo" (non-destructive) measurement of various morphological parameters of aboveground organs (stem, branches, and leaves). In particular, these studies have focused on *Populus sibirica* and *Ulmus pumila* and the data are summarized in several published papers that show the growth and development trends followed by these trees from 2009 to 2019. This monitoring approach has established that the higher levels of irrigation (4 and 8 Lh⁻¹) ensure the best growth and development trends (even in the absence of fertilization) (Byambadorj et al., 2021a,b; Nyam-Osor and al., 2021; Montagnoli et al., 2022). Regarding belowground organs, unfortunately, it has been impossible to use any "in vivo" monitoring approach due to the physical nature of the soil which limits the efficacy of the penetrating radar technology (Zhang et al., 2019). For this reason, in 2019, we utilized an "ex vivo" (destructive) approach (i.e., hand excavation) to measure the level of growth and development achieved by the root system of these trees after 11 years of growth. In particular, we measured various parameters such as root length, root diameter, and dry and fresh weight; for this purpose, the roots were categorized into taproots and lateral roots, divided into diameters classes and branching orders (Montagnoli et al., 2022).

The data collected in this study confirmed that *Populus sibirica* and *Ulmus pumila* trees subjected to the higher irrigation regime of 4 and 8 L h⁻¹, presented the highest values of all the morphological traits measured. Interestingly, the root-to-total-tree biomass ratio exceeded 40%. This ratio aligned with values derived from various allometric biomass equations found in the literature (He et al., 2018), indicating that at the time of excavation (2019), these trees exhibited a well-developed root system.

While the "ex vivo" approach provided valuable insights into root conformation, the data fell short of fully characterizing the growth trends in the root systems of *Ulmus pumila* over their 11-year

lifespan. To address this gap, the present study employed a dendrometric analysis of xylogenetic activity (Rathgeber et al., 2016) to characterize the wood in the upper portion of the taproot and the lower portion of the stem in excavated *Ulmus pumila* trees.

This approach rested on the assumption that the growth and development of the overall root system including the production of new lateral roots in a given year, are intricately linked to the width of the annual ring produced in the same year in the upper portion of the taproot (Kozłowski and Pallardy, 1997). Similarly, we hypothesized that the overall production of new branches and leaves aboveground is intricately linked to the width of the annual ring produced in the same year in the lower portion of the stem.

Furthermore, the dendrometric analysis of the wood organization in the upper portion of the taproot and the lower portion of the stem could illuminate the correlation established during the tree's lifespan between the transportation of raw sap and phloem sap produced by the leaves (Kozłowski and Pallardy, 1997).

The results obtained suggested the occurrence of similar growth and developmental trends in both above- and belowground organs in *Ulmus pumila* trees. In both cases, the trends feature an initial LAG phase, followed by an exponential LOG phase that decelerates without reaching the conventional STATIONARY phase observed in typical geometric growth trajectories.

Furthermore, this study revealed the occurrence of a difference between the trends obtained through the new dendrometric (i.e., "ex vivo") or traditional DT (i.e., "in vivo") approach. In this last case, the trends obtained by measuring stem height and diameter from 2009 to 2019 followed a typical geometric growth curve. The discussion in the paper delves into the difference between these trends emphasizing the importance of adopting both approaches for reliable monitoring of plantation performance.

2. Materials and Methods

2.1. The Experimental Site

The experimental site is in Lun soum, Tuv province, Mongolia (47°52'15.43"N, 105°10'46.4"E) at an elevation of 1,130 m a.s.l. The site extends for 0.2 ha, and it is described as the Middle Khalkha dry steppe (Ulziykhutag 1989) dominated by xerophytic and mesoxerophytic graminoids (e.g., *Stipa sareptana* subsp. *krylovii* (Roshev.), *Cleistogenes squarrosa* (Trin.), *Agropyron cristatum* (L.) Gaertn, *Artemisia frigida* (Willd.), *Artemisia adamsii* (Besser), *Carex duriuscula* C.A.Mey., *Leymus chinensis* (Trin.)) (Lavrenko et al. 1991). Soil type is classified as Loamic Kastanozems (IUSS Working Group WRB, 2015), with topsoil characterized by a hardness of 4.5 kg cm⁻², while that of the subsoil was 1.7 kg cm⁻².

2.2. Climatic Characteristics of the Experimental Site

According to the National Agency for Meteorology and Environmental Monitoring of Mongolia (NAMEM, 2019), the annual average temperature is 0.6 ± 0.45 °C with a summer (May–September) average temperature of 16.29 ± 0.41 °C. Annual mean precipitation during the whole experiment (2000–2019) was 196 mm, with a maximum value between June and August that accounted for 80–90% of the total annual rainfall. The mean annual potential evapotranspiration is 752.12 ± 30.68 mm (Cao et al. 2018). The warmest month is July (16 °C) whereas the coldest month is January (–22 °C).

2.3. Plant Material and Management: Watering and Fertilization

Two-year-old saplings of *U. pumila* (grown from seeds) were transplanted in May 2009, in 60–70 cm-deep holes with a diameter of 50–60 cm. Immediately after planting, a sufficient level of watering was supplied to individual trees by compensating non-leakage (CNL) button drippers placed 10 cm from the stem of each sapling. After sapling acclimatization, an irrigation regime of 8 L h⁻¹=1.0 mm m⁻² was applied: The 5-hour duration of watering was done twice a week for the entire vegetative season (from the beginning of May to the end of August) and was repeated every year from 2009 to 2019.

Each plot measured 20 × 10 m, and the seedlings were planted in rows with a north-south orientation to ensure maximum light availability during the whole day (Johnson and Brandle, 2009). The distance between rows was 2.5 m and distance between trees was 2.5 m.

2.4. Monitoring by “In Vivo” Approach

The methods used for trait measurement “in vivo,” are described extensively in Byambadorj et al. (2021 a,b). In particular, the height and RCD of the stem of elm was measured by ruler and calliper at 30-day intervals during the entire vegetative season during the period 2009–2019. The seedling recruitment (i.e., seedling survival) rate was calculated as the number of living plants divided by the number of plants originally planted.

2.5. Monitoring by “Ex Vivo” Approach

Tree excavation is described extensively in Montagnoli et al. (2022). In summary, six trees were randomly selected and hand-excavated to recover the intact root system. Before excavation, a single screw was driven into the bark of each tree at the root-stem interface to delineate the north. Two screws were partially drilled into the stump about 20 cm apart with their heads adjusted to indicate the horizontal level of the soil. Two more screws, perpendicular to the first two, were installed similarly. Excavation of the trees was done to a depth of ~0.8–1m and the portions of roots extending more than 1m from the trunk were left in the soil. From six excavated trees an aliquot of disks was cut from the lower portion of the stem (10 cm above the soil surface) and from the upper portion of the taproot (10 cm below the soil surface) to be used for dendrometric studies. In particular, the disks were sanded with a series of increasingly fine grit sandpaper (ranging from 60 to 600-grit). The sanded sections were later scanned at 750 dpi sensitivity and images were analysed by a plug-in (ObjectJ) of ImageJ software to measure the annual ring thickness and the xylem diameter. The thickness of each annual ring was obtained as the mean of two measurements obtained by starting from the centre of the section and moving toward the cork in two opposite directions. This procedure was adopted to avoid the effect of the eccentricity of the section which could have a considerable effect on the thickness measurement.

2.6. Statistical Analysis

Statistical analysis used the SAS software package, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). One-way analysis of variance (ANOVA) with Duncan’s multiple range test (DMRT) was used for multiple comparisons among the 2019 data. Permanent plots were considered independent replicates. During the 10-year monitoring period, the morphological traits (height and RCD) in stems of trees within each plot were measured and the data treated as a mean.

3. Results and Discussion

The widely accepted definition of growth and development in plant biology suggests that while plant growth involves organ dimension increase over time, plant development refers to structural changes. These processes are closely related and should be studied together as part of a single “integrative development” event (Dambreville et al., 2015), influenced by both genetic properties and environmental factors (Kholmanskiy, 2015). Therefore, optimal and coordinated growth and development of both above and belowground organs are crucial for plant health. Consequently, monitoring plantation performance should involve evaluating the simultaneous growth and developmental trends of all tree organs, considering the anatomical and physiological differences between aboveground and belowground organs and their distinct living environments (i.e., air and soil).

Regrettably, current plantation monitoring, whether at the level of single trees or communities, relies solely on “in vivo” measurements of aboveground tree organs (Padua et al., 2017). It is surprising that this limitation exists, considering the critical role played by belowground organs in the life of higher plants (Ryan et al., 2016). However, it is not difficult to understand this limitation,

given that the opaque nature of soil still hinders the “in vivo” study of belowground organs, such as the root system.

Since 2009, our laboratories have been involved in monitoring the performance of plantations established in arid and semiarid sites of Mongolia under the Greenbelt project (Byambadorj et al., 2021a,b; Nyam-Osor et al., 2021; Montagnoli et al., 2022). Until 2019, we monitored these plantations through a DT approach by measuring “in vivo” year after year the morphological traits of aboveground (stem, branches, and leaves) organs. These studies succeeded in establishing that the irrigation regime of 8 L h⁻¹ was the management measure that better ensured an optimal growth and development trend of *Ulmus pumila* trees even in the absence of fertilization (Byambadorj et al., 2021a,b; Nyam-Osor et al., 2021; Montagnoli et al., 2022). Unfortunately, until 2019 no monitoring had been possible to investigate the growth and development trends of belowground organs of these trees.

For this purpose, in 2019 we adopted an “ex vivo” (i.e., after excavation) monitoring approach and excavated six *Ulmus pumila* trees to analyze the architectural conformation of their root system present at the time of tree excavation (Montagnoli et al., 2022). These “ex vivo” studies succeeded in providing a clear picture of the health condition of the root systems at the time of tree excavation but were useless in reconstructing the growth and developmental trends followed by the root systems in the period 2009-2019.

The study presented here shows for the first time how it is possible to obtain an indication of the growth and development trend followed by the root system of a tree using a dendrometric approach. This approach is obviously to be considered as an “ex vivo” monitoring approach and is based on the analysis of annual ring width over the years. In this regard, Figure 1 A shows the aspect of a wood section of the upper portion of a taproot of *Ulmus pumila* where a considerable variation can be observed in the width of the annual rings took place in the years 2009-2019.

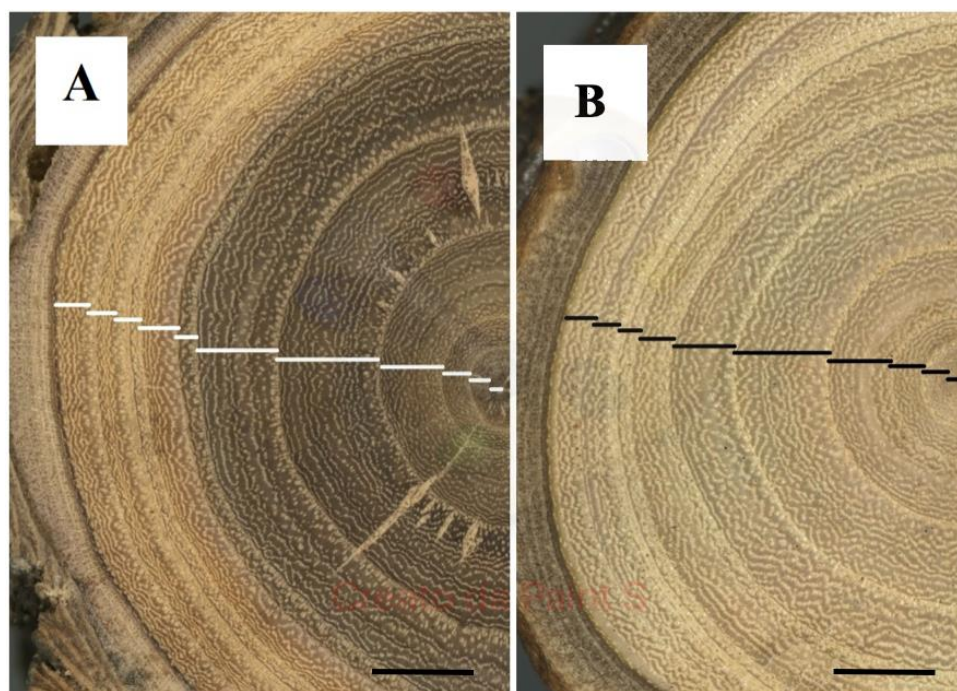


Figure 1. Annual rings in the wood of the stem and root of *Ulmus pumila*. The wood sections of root (A) and stem (B) have been sanded and analyzed by a scanner at 700 PSI sensitivity. The images have been analyzed by using Object J, which is a plugin of Image J software. The width (in microns) of each annual ring (white or black lines) has been measured. The section was sanded by hand with increasing sandpaper grit up to P600. The black lines at the bottom-right side of the two panels represent 4 mm of wood.

When we plotted each annual ring width over the years (Figure 2A), we observed a curve with the highest value in 2014. When we plotted the cumulative sum of values of the annual rings year after year, then we obtained a curve of “cumulative root biomass” (Figure 2B). We assumed that each annual ring present in the upper portion of a taproot could represent an indicator of the overall biomass that the plant yearly invested in the growth and development of the root system. In particular, this curve showed an initial increase followed by a continuous exponential phase of width increase. Moreover, the standard deviation reported in both panels suggested clearly that the same pattern characterized the variation of annual ring width in the woody sections of all the excavated trees.

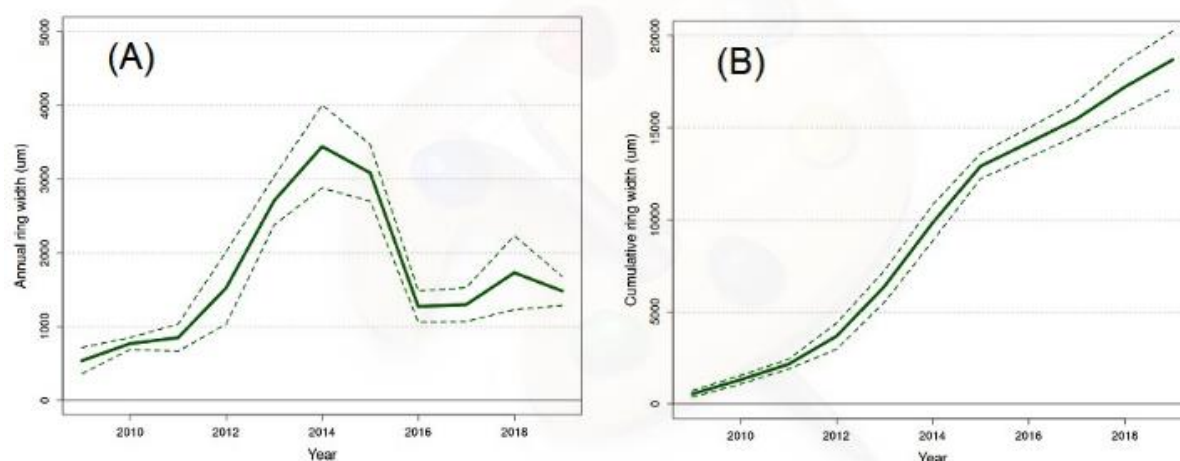


Figure 2. Kinetics of growth and development measured using the annual ring width of *Ulmus pumila* taproot sections = Panel 2A shows the pattern formed by plotting the width of each annual ring measured for the period 2009-2019. Panel 2 B represents the pattern formed by plotting the cumulative diameter increments obtained in a wood section of a root when all widths (measured in microns) of each annual ring are progressively added. The dotted lines represent +/- the standard error.

In plant biology, the literature predicts the occurrence of a theoretical and empirical geometrical pattern of growth where an initial slow increase (LAG phase), is followed by an exponential increase (LOG phase), that ends with an equilibrium (STATIONARY phase) (Deng et al., 2012). According to this theoretical definition of a growth trend, the data collected by our dendrometric approach on one hand confirm the possibility to reconstruct the growth and development trend of the root system, but on the other hand, suggest that our data do not follow a geometrical trend. The initial LAG phase (2009-2012) seems to be followed by a continuous LOG phase (lasting at least until the time of root excavation).

Furthermore, our dendrometric approach reveals consistent trends in taproot and stem (Figure 1 A, B) suggesting that *Ulmus pumila* in the Greenbelt Project plantations adheres to the “pipe model theory”. This theory as proposed by Shinozaki in 2017 posits a robust correlation between plant development and the connectivity between belowground (roots) and aboveground (leaves) organs. Moreover, the absence of discernible differences in the trends in our trees indicates both ontogenetic and environmental factors, such as variation in soil nutrient concentration, have not affected the allocation of biomass between above- or belowground organs as shown by other authors (Helmisari et al., 2002; Poorter et al., 2011; Mathew et al., 2016; Jevsenak and Levanic, 2018; Holland et al., 2019; Soong et al., 2020). Regarding environmental factors, a meticulous analysis of meteorological data (air relative humidity, air temperature, precipitation, soil water content, soil temperature, wind strength) recorded from 2009 to 2019 did not reveal any variability that could have influenced the biomass production and allocation of our trees (data not shown).

The difference emerging from the present study between the trend described by this novel “ex vivo” dendrometric approach, with the trend obtainable by the traditional plotting of the variation

of morphological traits (stem height and RCD) collected through DT monitoring (Byambadorj et al., 2021a,b; Nyam-Osor et al., 2021) is surprising. This difference is reported in Figure 3 wherein the same graph we have reported the trends obtained through “in vivo” (stem height and RCD) and “ex vivo” approaches (annual ring width measured in the taproot wood). In this figure, it is possible to observe that both height and RCD trends can be characterized as geometric trends (LAG, LOG, and STATIONARY) contrary to the annual ring trends that lack the STATIONARY phase.

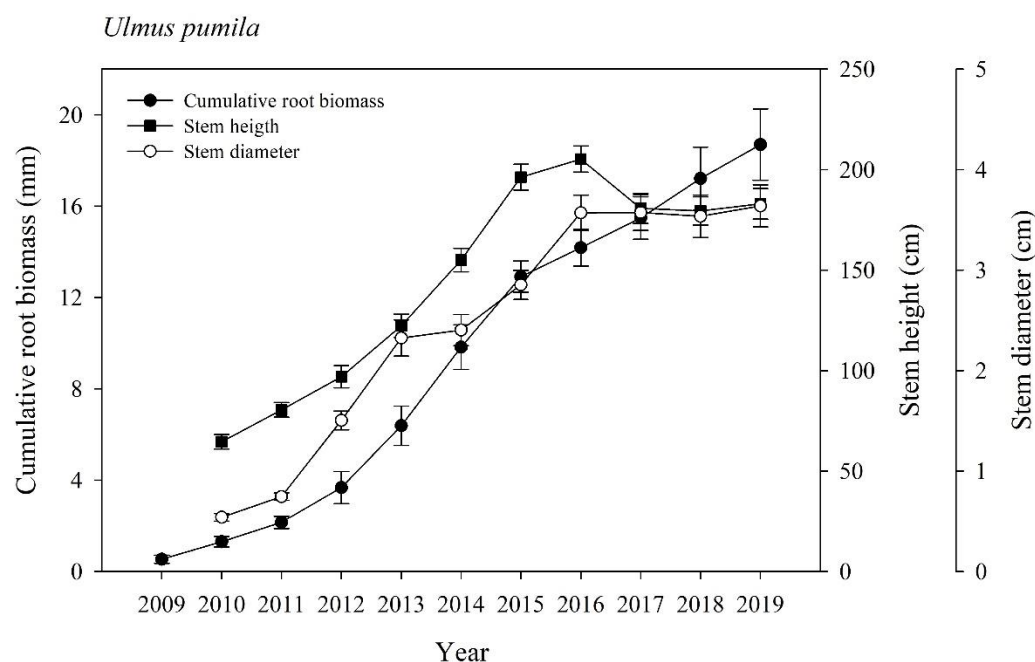


Figure 3. Growth and development trends in *Ulmus pumila*. The curves represent, respectively: the cumulative root biomass (closed circle) obtained by measuring the annual ring width in taproots; 2) the stem height (closed square) measured during the 10-year-period; 3) the stem diameter (RCD) (open circle).

An explanation for this difference could be that in the case of the arrest of stem height increase, the trees achieve a maturity stage (i.e., after the LOG phase) when biomass is used for the production and development of branches (branching plasticity) as reported by Yoshihira et al. (2020). Alternatively, it could be suggested that at a certain stage (tree maturity?), there is the onset of a “diminishing return” effect that arrests stem height increase. The diminishing return consists of a change in biomass allocation between organs that responds to the need of a plant to enable physiological adjustments (Shi et al., 2019). For example, this effect has been reported in natural forests where variations in the carbon allocation among organs are part of the plant’s strategy for responding to competition for light (Mensah et al., 2016).

The onset of a STATIONARY phase in the RCD trend could be the consequence of the onset of a bark peeling event. In fact, despite there being no data regarding bark shedding for *Ulmus pumila*, this event would hide the overall diameter increase of the stem that should take place as a consequence of the yearly production of new secondary xylem and phloem.

Our finding that a difference occurs when monitoring the growth and development trends of trees through an “in vivo” or “ex vivo” approach is similar to the lack of linearity that has been observed in other tree species when morphological traits (such as leaf area index) are compared to the biomass-related relative growth rate (Weraduwege et al., 2015). Moreover, a loss of correlation has been observed during the development of a leaf when comparing the relationship between specific traits such as dry or fresh leaf biomass, leaf thickness, and leaf area (Shi et al., 2020).

4. Conclusions

This study presents a novel monitoring approach allowing simultaneous comparison of growth and developmental trends in both aboveground and belowground organs of the same tree. Currently, radar scanning technology for “in vivo” monitoring of root system growth and development lacks reliability (Zhang et al., 2019), necessitating the use of the “ex vivo” monitoring approach, especially in harsh soil environments like arid and semiarid lands.

The combined use of “in vivo” and “ex vivo” monitoring approaches demonstrates the effectiveness of management measures under the Greenbelt Project in promoting optimal growth and development performance. In fact, it confirms that an irrigation regime of 8 Lh⁻¹ enables coordinated growth and development of belowground organs (Montagnoli et al., 2022), and avoids the need for measuring numerous morphological traits, preventing the collection of disconnected and unreliable trait measurements (Viani et al., 2018).

The observed differences in trends between “in vivo” and “ex vivo” monitoring approaches affirm the possibility of predicting aboveground organ trait variations from root trait variations, while reverse prediction is not feasible (Caldwell and O’Hara, 2017). The significance of monitoring root system growth and development trends supports previous conclusions on the critical nature of investigating the root system, given its high sensitivity to environmental and mechanical factors (Montagnoli et al., 2022; James et al., 2022).

Author Contributions: BN-O, SO-B, and DC conceived the research project. BN-O provided primary funding. SO-B, BN-O, AM, and DC developed the methodological approaches. FD performed the dendrometry data analysis. AM, GSS, JS, and DC provided important insights into the research process. DC and JS wrote the manuscript.

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Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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