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MANEJO RADICULAR DE *QUILLAJA SAPONARIA* Y *ARISTOTELIA CHILENSIS* DURANTE LA VIVERIZACIÓN: IMPLICANCIAS SOBRE EL DESEMPEÑO AL DÉFICIT HÍDRICO

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POR

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RESUMEN

En un contexto de sequías prolongadas asociadas al cambio climático y de creciente demanda por la restauración de ecosistemas degradados, mejorar la calidad de las plantas se vuelve fundamental, particularmente en ecosistemas mediterráneos, donde un sistema radical bien desarrollado condiciona fuertemente el éxito del establecimiento. En este contexto, prácticas como la fertilización fosfórica y la poda química de raíces se han tradicionalmente asociadas con el desarrollo de la raíz. Sin embargo, la evidencia disponible se ha centrado mayoritariamente en atributos morfológicos y de supervivencia, existiendo escasa información sobre su efecto en el desempeño frente a eventos de déficit hídrico. El objetivo de esta tesis fue evaluar cómo la fertilización fosfórica y la poda química de raíces, aplicadas durante la viverización, modifican atributos morfológicos y nutricionales, incluida la arquitectura radical y condicionan el desempeño frente al déficit hídrico en dos especies nativas de importancia ecológica y económica en Chile, *Aristotelia chilensis* y *Quillaja saponaria*. Durante la viverización se aplicaron tratamientos de fósforo (0, 15, 60 y 120 mg L⁻¹) y poda química (WoCu: sin; WCu: con), evaluándose atributos morfofisiológicos, el potencial de crecimiento radical (PCR) y su desempeño en condiciones de déficit hídrico. Los resultados indicaron que el incremento de la fertilización fosfórica aumentó atributos de arquitectura radical, el estado nutricional y se asoció a un mejor desempeño del PCR y bajo déficit hídrico. En contraste, la poda química de raíces no generó beneficios consistentes frente al déficit hídrico, destacando la dependencia de la respuesta a las estrategias morfofisiológicas propias de cada especie.

ABSTRACT

In the context of prolonged droughts associated with climate change and the increasing demand for the restoration of degraded ecosystems, improving seedling quality has become essential, particularly in Mediterranean ecosystems, where a well-developed root system strongly determines establishment success. In this context, practices such as phosphorus fertilization and chemical root pruning have traditionally been associated with enhanced root development. However, available evidence has mainly focused on morphological attributes and survival, with limited information on their effects on plant performance under water deficit events. The objective of this thesis was to evaluate how phosphorus fertilization and chemical root pruning, applied during the nursery phase, modify morphological and nutritional attributes, including root architecture, and condition performance under water deficit in two native species of ecological and economic importance in Chile, *Aristotelia chilensis* and *Quillaja saponaria*. During the nursery phase, phosphorus levels (0, 15, 60, and 120 mg L⁻¹) and chemical root pruning treatments (WoCu: without; WCu: with) were applied. Morphophysiological traits, root growth potential (RGP), and performance under water deficit conditions were evaluated. The results indicated that increasing phosphorus fertilization enhanced root architectural traits and nutritional status and was associated with improved RGP performance and better responses under water deficit. In contrast, chemical root pruning did not generate consistent benefits under water deficit, highlighting the dependence of plant responses on the species-specific morphophysiological strategies.

INTRODUCCIÓN GENERAL

El calentamiento global, acelerado principalmente por la deforestación y el cambio de uso de la tierra, ha intensificado la ocurrencia de fenómenos extremos como sequías prolongadas e incendios forestales, comprometiendo la estabilidad de los ecosistemas (Vacek et al., 2020). Frente a este escenario, la restauración de bosques se reconoce como una estrategia esencial para mitigar los impactos de la crisis climática, reducir las concentraciones de CO₂ atmosférico y recuperar la funcionalidad ecológica de los territorios (Lewis et al., 2019). Con esta premisa, la Organización de las Naciones Unidas declaró el período 2021–2030 como la “Década de la Restauración de Ecosistemas”, con el objetivo de promover a escala global la recuperación de áreas degradadas (Aronson et al., 2020).

En Chile, los compromisos asumidos en el marco del Acuerdo de París 2015 (MMA, 2015) y de la Política Nacional Forestal 2015–2035 (CONAF, 2015) establecen metas ambiciosas de forestación, que incluyen la incorporación de 100.000 y 500.000 hectáreas, preferentemente con especies nativas, al 2030 y 2035, respectivamente. Posteriormente, en 2020, Chile actualizó su Contribución Determinada a Nivel Nacional (NDC), incrementando su compromiso a la forestación de 200.000 hectáreas al año 2030 (MMA, 2020). No obstante, el cumplimiento de estos objetivos enfrenta serios cuellos de botella asociados a la producción de plantas en vivero. Entre las principales limitantes se encuentran la escasa oferta de especies nativas actualmente producidas y la baja proporción de individuos que cumplen con atributos idóneos para programas de

restauración (Acevedo et al., 2021; Bannister et al., 2018). De mantenerse este escenario, las metas proyectadas se alcanzarían con décadas de retraso (Acevedo et al., 2021), lo que subraya la urgencia de mejorar las prácticas de manejo en vivero.

En este contexto, surge la necesidad de orientar la viverización hacia la producción de plantas con atributos que maximicen la probabilidad de un adecuado desempeño en campo, concepto generalmente conocido como calidad de planta. Este concepto ha evolucionado desde una visión centrada únicamente en el tamaño y la morfología de las plántulas (Duryea & Landis, 1984) hacia un enfoque más integral, que incorpora también atributos fisiológicos y su relación con el desempeño en terreno (Dumroese et al., 2016; Grossnickle, 2012).

Inicialmente, la discusión sobre calidad de planta se enmarcó en el concepto de “Aptitud para el Propósito” (*fitness for purpose*), el cual proponía que las plántulas debían producirse en función de los requerimientos específicos del sitio de plantación y no bajo estándares genéricos (Sutton, 1980). Sobre esta base conceptual, Rose et al. (1990) desarrollaron el Concepto de Plántula Objetivo (*Target Seedling Concept*), centrado principalmente en plántulas jóvenes propagadas desde semillas, y que estableció que cada se debía producir plantas con atributos morfológicos definidos según las condiciones del sitio y los objetivos operativos del proyecto.

Esta evolución conceptual derivó en el Concepto de Planta Objetivo (*Target Plant Concept*, TPC), un marco integrador aplicable a plantas producidas en vivero mediante distintas formas de propagación, que articula atributos morfológicos, fisiológicos y

funcionales asociados al desempeño en función de los objetivos del proyecto y de las condiciones del sitio de plantación (Dumroese et al., 2016). Bajo el enfoque del TPC, la calidad de una planta se define por el desempeño en campo, enfatizando que los atributos desarrollados en vivero deben traducirse en una mayor supervivencia y crecimiento inicial tras la plantación (Davis & Pinto, 2021; Dumroese et al., 2016; Haase & Davis, 2017).

En la región mediterránea de Chile, donde la disponibilidad hídrica es altamente estacional y se proyecta un aumento de la aridez con el cambio climático (Acevedo et al., 2020; Santibañez et al., 2014), atributos como la arquitectura del sistema radical desarrollada en vivero y su potencial de desarrollo en campo adquieren especial relevancia, ya que determinan la capacidad de exploración del suelo y la tolerancia a periodos de déficit hídrico (Sloan et al., 2020; Villar-Salvador et al., 2012).

En este marco, resulta clave identificar prácticas de manejo en vivero que fortalezcan los atributos asociados a la resistencia al estrés hídrico. Entre ellas, la fertilización fosfórica y la poda química de raíces se han reportado como determinantes en el desarrollo de plantas, particularmente por su influencia sobre el sistema radical. El fósforo (P) es un macronutriente esencial para la división celular, el metabolismo energético y el crecimiento vegetal, cumpliendo un rol clave durante las primeras etapas de viverización (Epstein & Bloom, 2004; Landis, 1989; Marschner, 2012; Razaq et al., 2017). Sin embargo, la baja disponibilidad de P en los suelos y la limitada capacidad de absorción de las plántulas hacen que su manejo sea un desafío (Hinsinger, 2001; Nkebiwe et al., 2016). Se ha reportado que solo entre el 10 y 25% del P aplicado vía fertilización es

absorbido por las plantas durante las primeras etapas de crecimiento (Ma et al., 2021). Esta situación se acentúa en sitios pobres, usualmente utilizados para programas de restauración, donde la baja disponibilidad edáfica de P y el escaso desarrollo radical inicial restringen aún más su adquisición (Van den Driessche, 1991), resaltando la importancia del manejo fosfórico en vivero como estrategia para generar reservas nutricionales y favorecer un adecuado desarrollo radical posterior.

Diversos estudios han demostrado que el P puede modificar la morfología y la arquitectura del sistema radical, aunque la magnitud y dirección de estas respuestas varían entre especies. Por ejemplo, en *Rosa multiflora*, el aumento de la disponibilidad de P incrementó la masa seca de raíces, el diámetro a la altura del cuello (DAC) y las concentraciones de P en raíces y hojas (Ma et al., 2021). De manera similar, en especies mediterráneas como *Quercus ilex*, la fertilización con P aumentó el tamaño foliar, el crecimiento radical y las concentraciones foliares de P (Sardans et al., 2006). En contraste, en *Fraxinus mandshurica*, una mayor disponibilidad de P promovió el crecimiento de la raíz primaria, pero redujo el número y la longitud de las raíces laterales (Wu et al., 2005). Asimismo, en *Pistacia lentiscus*, la aplicación de P aumentó el área foliar, el área foliar específica (specific leaf area; SLA) y las concentraciones nutricionales, aunque disminuyó atributos del sistema radical como la longitud y el área superficial de raíces (Trubat et al., 2012). Por otra parte, en especies forestales como *Pseudotsuga menziesii* y *Pinus ponderosa*, no se han observado respuestas significativas al incremento de la fertilización fosfórica (Fischer & Herbert, 1982; van den Driessche, 1980).

Además de sus efectos morfológicos, el P también influye sobre atributos fisiológicos. En este sentido, en *P. lentiscus*, la fertilización con P aumentó la conductancia hidráulica de la raíz (Trubat et al., 2012). Por otra parte, bajo condiciones de déficit hídrico, estudios en *Phoebe zhennan* han demostrado que la disponibilidad de P modula la respuesta fisiológica a la sequía, asociándose con mayores tasas fotosintéticas, mejor estado hídrico foliar y mayor eficiencia fotoquímica (F_v/F_m); sin embargo, bajo restricción hídrica, niveles elevados de P pueden afectar negativamente la asimilación de nitrógeno (N), evidenciando una interacción entre los metabolismos de P y N bajo limitación hídrica (Tariq et al., 2017, 2022). No obstante, en *Fagus sylvatica* y *Quercus petraea*, la fertilización con P aumentó las concentraciones foliares de este nutriente, pero bajo estrés hídrico se asoció con una disminución de la fotosíntesis neta y del rendimiento fotosintético (Vukmirović et al., 2024).

En conjunto, estos antecedentes indican que, si bien el P desempeña un papel relevante en el crecimiento aéreo y radical, así como en las estrategias de resistencia al estrés hídrico, sus efectos sobre los atributos morfofisiológicos y el desempeño de las plantas no son consistentes entre especies. Esta variabilidad refuerza la necesidad de profundizar su estudio en especies nativas, particularmente bajo escenarios de déficit hídrico. En este contexto, comprender el rol del P en el desarrollo radical en la viverización resulta clave desde la perspectiva del TPC, dado que estos atributos condicionan en gran medida la capacidad de las plantas para enfrentar el estrés post-trasplante y establecerse en ambientes con limitada disponibilidad hídrica.

En este contexto, además de la nutrición mineral, diversas prácticas de manejo en vivero han sido utilizadas para modificar intencionalmente la arquitectura y funcionalidad del sistema radical, con el fin de mejorar el desempeño. Entre ellas, la poda química de raíces, la cual consiste en la aplicación de soluciones que incluyen principalmente compuestos a base de cobre (Cu) en las paredes internas del contenedor. Si bien el Cu es un micronutriente esencial involucrado en el crecimiento y desarrollo de las plantas, en concentraciones elevadas induce la inhibición del crecimiento radical, la perturbación de los procesos mitóticos y alteraciones estructurales en las células epidérmicas y en las membranas celulares de la raíz (Arduini et al., 1995; Jiang et al., 2001; Montagnoli et al., 2022; Qin et al., 2015; Sheldon & Menzies, 2005). De este modo, cuando las raíces secundarias entran en contacto con superficies recubiertas con Cu, se produce una inhibición localizada de la actividad del meristemo apical, lo que conduce a la detención del crecimiento de la raíz (Marchioretto et al., 2020).

Esta práctica ha demostrado modificar la asignación de biomasa y la arquitectura del sistema radical en distintas especies. Por ejemplo, en *Populus nigra*, la poda química incrementó la biomasa radical total y promovió una mayor longitud y volumen de raíces en comparación con el control, resultando en una mayor asignación de biomasa al sistema radical (Montagnoli et al., 2022). De manera similar, en *Pinus palustris*, la aplicación de Cu alteró la morfología de las plántulas al aumentar el DAC y la biomasa de la raíz principal, aunque redujo el volumen total del sistema radical (Dumroese et al., 2013). En *Pinus halepensis*, la poda química no afectó significativamente la longitud del tallo durante la fase de vivero, pero incrementó el DAC y promovió un sistema radical más

fibroso y ramificado, caracterizado por un mayor número de raíces laterales, sin modificaciones en el área superficial, el volumen ni la longitud total de las raíces (Tsakalidimi & Ganatsas, 2006).

Desde el punto de vista fisiológico, la evidencia disponible es más limitada. Algunos autores han reportado modificaciones en la concentración de nutrientes, tales como una disminución del magnesio (Mg) en la parte aérea y del calcio (Ca) en las raíces, junto con un aumento del contenido de Cu en plantas sometidas a poda química (Tsakalidimi & Ganatsas, 2006). Además, el tratamiento con Cu influyó significativamente en el intercambio gaseoso en *P. palustris*; sin embargo, no se observaron evidencias de un efecto fisiológico positivo atribuible a la poda química (Dumroese et al., 2013).

A pesar de la escasa evidencia directa sobre los efectos fisiológicos de la poda química en plantas, diversos estudios sugieren que la mejora en la arquitectura radical asociada a esta práctica podría favorecer el establecimiento de estas en campo (Grossnickle & MacDonald, 2018; Ritchie & Dunlap, 1980). No obstante, los resultados sobre el desempeño posterior al trasplante son variables, ya que, mientras algunos autores no reportan efectos significativos sobre la supervivencia ni el crecimiento (Haywood et al., 2012; South et al., 2005), otros han observado respuestas positivas, como un mayor potencial de crecimiento radical (PCR) en *P. patula* (Aguilera-Rodríguez et al., 2021) y un mayor crecimiento en altura y volumen hasta cinco años después del trasplante en *P. palustris* (Haywood et al., 2012).

En general, la mayoría de los estudios sobre poda química se han centrado en atributos morfológicos, principalmente del sistema radical, y en sus consecuencias sobre el anclaje y la supervivencia. En contraste, la evidencia disponible sobre sus efectos fisiológicos y su relación con el desempeño en campo sigue siendo limitada, particularmente en especies de climas mediterráneos expuestas a condiciones recurrentes de déficit hídrico. En este sentido, evaluar cómo las modificaciones en la arquitectura radical inducidas por la poda química se traducen en mejoras funcionales, tales como una mayor capacidad de exploración del suelo y resistencia al déficit hídrico, resulta clave para comprender su contribución al desempeño en campo de plantas producidas bajo el enfoque del TPC.

En consecuencia, persiste un vacío de conocimiento respecto de cómo estas prácticas de manejo en vivero -la fertilización con P y la poda química de raíces- influyen en atributos clave asociados a la tolerancia al déficit hídrico en especies nativas, lo que limita la optimización de su producción tanto para fines productivos como para programas de restauración. En este contexto, dos especies nativas de importancia en la zona mediterránea de Chile son: *Quillaja saponaria* Mol., especie arbórea endémica que se encuentra entre las especies nativas más propagadas a nivel nacional, debido a su importancia ecológica y a su alto valor comercial asociado a la producción de saponinas (Espinoza et al., 2020; Magni et al., 2019; Pelah et al., 2002); y *Aristotelia chilensis* (Mol.) Stuntz, uno de los arbustos nativos más representativos del país, reconocido por su rápido establecimiento, su rol pionero en procesos sucesionales y el elevado contenido antioxidante de sus frutos (Benedetti, 2012; Yáñez et al., 2022). Ambas especies cumplen

un rol relevante en los procesos de restauración de ecosistemas degradados y, simultáneamente, poseen un valor productivo significativo, lo que incrementa el interés por su propagación y uso a escala nacional.

No obstante, pese a su relevancia ecológica y productiva, la producción en vivero de *Q. saponaria* y *A. chilensis* aún presenta limitaciones importantes en términos de cantidad y atributos de calidad de las plantas. De hecho, considerando únicamente las plantas producidas anualmente en Chile que cumplen con atributos de calidad para restauración, *Q. saponaria* corresponde a cerca del 16% y *A. chilensis* a apenas el 2% del total (modificado de Acevedo et al., 2021). Además, una proporción considerable de esta producción se realiza bajo criterios de manejo inadecuados o deficientes, principalmente mediante riego sistemático y con estrategias de fertilización poco definidas e incompletas, lo que se traduce en deficiencias nutricionales y un escaso desarrollo radical. Esta situación evidencia una brecha en el conocimiento y aplicación de prácticas de viverización orientadas a la producción de plantas nativas de alta calidad, particularmente en especies clave para programas de restauración y sistemas productivos.

En función de lo anterior, el primer capítulo de esta tesis aborda los efectos de la fertilización con P y la poda química de raíces sobre los atributos morfológicos y nutricionales, incluida la arquitectura radical, de *Q. saponaria* y *A. chilensis* durante la viverización. Considerando que el objetivo general de este trabajo fue analizar cómo estas prácticas de manejo influyen en el desempeño posterior de las plantas, particularmente frente al déficit hídrico, el segundo capítulo evaluó el efecto combinado de la fertilización

fosfórica y la poda química sobre el desarrollo radical y el desempeño fisiológico frente al déficit hídrico.

En conjunto, esta investigación busca generar evidencia científica y herramientas técnicas que contribuyan a la producción de plantas nativas de alta calidad, mejor adaptadas a condiciones de déficit hídrico, y que fortalezcan la producción de plantas para fines productivos y los programas de restauración ecológica en ambientes mediterráneos afectados por el cambio climático.

HIPÓTESIS

El aumento de la fertilización fosfórica y la aplicación de poda química de raíces en *Q. saponaria* y *A. chilensis* producidas en vivero, modifican la arquitectura radicular en las plantas condicionando atributos fisiológicos que mejoran el desempeño frente al déficit hídrico.

OBJETIVO GENERAL

Evaluar el efecto del aumento de la fertilización fosfórica y la aplicación de poda química de raíces sobre atributos morfofisiológicos en vivero y su desempeño en *A. chilensis* y *Q. saponaria* frente al déficit hídrico.

OBJETIVOS ESPECÍFICOS

OE1. Evaluar el efecto de la concentración de fósforo aplicada y de la poda química de raíces en plantas de *Aristotelia chilensis* y *Quillaja saponaria* producidas en vivero, particularmente en sus atributos morfológicos, el estado nutricional y la arquitectura del sistema radical.

OE2. Analizar la relación entre las modificaciones morfofisiológicas y de la arquitectura radical previamente inducidas por la fertilización fosfórica y la poda química de raíces, y sus efectos sobre el potencial de crecimiento radical y la resistencia a la sequía en *Aristotelia chilensis* y *Quillaja saponaria*.

CAPÍTULO I: Phosphorus fertilization and chemical root pruning: Effects on root traits during the nursery stage in two Mediterranean species from Central Chile

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Abstract

The role of a plant root system in resource acquisition is relevant to confront drought events caused by climate change. Accordingly, nursery practices like phosphorous (P) fertilization and root pruning have been shown to modify root architecture, however, their combined benefits require further investigation in Mediterranean species. We evaluated the effect of applied P concentrations (0, 15, 60, and 120 mg L⁻¹ P) with or without chemical (copper) root pruning (WCu, WoCu, respectively) in *Aristotelia chilensis* and *Quillaja saponaria* on morpho-physiological and root architecture traits. Higher P concentration increased nutrient content in both species concurrent with higher growth. In *A. chilensis*, higher P concentrations only increased the length and volume of medium roots. In *Q. saponaria*, P additions increased root length and diameter and the length and volume of fine and medium roots. Root-to-shoot ratio declined with WCu in *A. chilensis* (23.1%) and *Q. saponaria* (15.7%). Unlike our hypothesis, fine root architecture remained unaffected with root pruning in *A. chilensis*, while fine root length and volume decreased with increasing P concentrations in *Q. saponaria*. Thus, P fertilization enhances root development more consistently than root pruning, highlighting the need for further testing under water deficit conditions to optimize nursery practices.

Keywords: fine roots; specific root length; root architecture; maqui; quillay.

Introduction

Mediterranean regions are considered highly vulnerable to climate change [1,2]. The Mediterranean region of south-central Chile is considered a biodiversity hotspot [3] and is being negatively impacted by the combination of prolonged drought, increasing temperatures, and decreasing amounts of precipitation [1,4–6]. Such disturbances constitute a threat to biodiversity by reducing an ecosystem's ability to acclimate to these new environmental conditions [7,8]. To improve adaptation of ecosystems to climate change, especially forest ecosystems, forest restoration has been considered a key tool by contributing to the reduction in greenhouse gases and by playing a role in mitigation [9]. However, the combination of inadequate seed supply [10], the low quantity and poor quality of available seedlings produced in nurseries, and low field survival [11,12] continue to limit the success of restoration in Mediterranean ecosystems [13].

In this regard, the Target Plant Concept seeks to define the adequate morpho-physiological plant attributes that favor increased survival and growth according to limiting environmental factors in the field [14–21]. Because of seasonal water scarcity in Mediterranean ecosystems, root architecture attributes are highly relevant, because, in addition to anchoring, the absorption of water and nutrients are fundamental for plant survival and growth after establishment [22]. For Mediterranean species, plant survival to drought events depends largely on the size of the root system and its ability to reach water stored in deeper soil horizons [23]. Nevertheless, initial root development in the field is mainly determined by the root architecture developed during the nursery stage [24–26]

concurrently with plant nutrient concentrations [23]. Nursery practices that maximize root attributes have, however, been scarcely documented.

Phosphorus (P) is an essential nutrient for plant development, being a fundamental component in nucleic acids, membrane phospholipids, and energy-dependent metabolic processes [27]. P has been traditionally described as a root growth stimulator [28], but few studies have documented such effect. In fertilization, additions of high amounts of P to *Fraxinus mandshurica* showed an increased primary root length, while P deficit promoted lateral root development [29]. In shrub species, an increase in applied P yielded higher total root length, surface area, and root dry mass in *Bauhinia faberi* [30], and root specific length in *Pistacia lentiscus* [31]. Furthermore, Mediterranean tree species such as *Quercus ilex*, P fertilization increased root biomass [32], which also correlated with field survival, as is shown in a study with *Acacia salicina* [33].

Along with P fertilization, chemical root pruning is also related to changes in root architecture. This practice is based on the application of compounds, such as copper (Cu), on the interior surfaces of the container, where the meristem of lateral roots ceases growth when coming into contact with the wall, thus promoting a more fibrous root system due to the loss of apical dominance [14,34,35]. This treatment avoids root spiraling inside the container [14,36,37] and is linked to an increase in the number of lateral roots [38], a higher number of growth points [39], and a more fibrous root system [40]. Such attributes could favor plant survival [23] because root growth after establishment increases water and nutrient uptake that promotes photosynthesis that supports root and shoot growth [41].

Other studies have shown, however, that Cu did not avoid spiral root growth and decreased root length and surface area in roots smaller than 5 mm diameter, thus decreasing total root length and surface area [42].

Although P fertilization and chemical root pruning influence root architecture, the response of the combination of these nursery practices in woody, Mediterranean species, and their possible effect when facing water deficit, is scarce. Accordingly, our hypothesis is that seedlings exposed to chemical root pruning in concert with P fertilization will develop a more fibrous root system having longer roots in smaller diameter classes (i.e., < 2 mm). Our aim is to evaluate the effect of applied P concentration and chemical root pruning in nursery-produced plants of *Aristotelia chilensis* and *Quillaja saponaria*, notably effects on morphological attributes, nutrient status, and root architecture. A pioneer native shrub from south-central Chile, *A. chilensis*, is desired for restoration purposes because its seeds germinate readily and abundantly [43], inducing high plant density when established in areas devoid of vegetation [44]. A Chilean endemic, *Q. saponaria* is one of the most produced native species in nurseries because its bark is a commercial source of saponin [47,48]. Found mainly in warm and dry climates, it is one of the most abundant species in the sclerophyllous forest of Mediterranean central Chile, because it also occupies cold and wet sites [49]. Despite their ecological and commercial importance, nursery production of these species has not focused on maximizing their root architecture to address water deficit after outplanting.

Materials and methods

Seedlings establishment in the nursery

We grew seedlings of *A. chilensis* and *Q. saponaria* in an outdoor nursery at the Centro Tecnológico de la Planta Forestal, Instituto Forestal (36.84° S; 73.13° W), Region of Biobío, Chile. During the nursery period (November 2022 – May 2023), the mean daily temperature was 16.8 °C. The maximum daily temperature (34.4 °C) was registered in February and the minimum (0 °C) in May. Seeds of both species were collected in El Morro (38.03°S; 72.68°W) and Quillón (36.74°S; 72.49°W), Region of Biobío, respectively. In late August 2022, seeds were soaked in water for 48 h, sown into germination beds filled with composted pine bark [86,87], and irrigated daily (Table S1). When seedlings developed their first set of true leaves (*A. chilensis*, end of October 2022; *Q. saponaria*, beginning of January 2023), we transplanted them individually into 32 trays per species (15 cm depth, 280 cm³ volume, 24 cavities; 768 seedlings) that had either been treated with a solution of 60 g L⁻¹ Cu₂(OH)₃Cl applied to the internal wall of the cavities (16 trays) for chemical root pruning treatment (WCu) or not (16 trays, WoCu). The trays were filled with composted pine bark as described above.

Fertilization

Phosphorous treatments began one month after transplanting. We applied 4 P concentrations: 0, 15, 60, and 120 mg L⁻¹, hereafter 0P, 15P, 60P, and 120P. Macro- and

micronutrients were applied at constant concentrations (mg L^{-1}): nitrogen (N, 400), potassium (K, 100), magnesium (Mg, 60), sulfur (S, 80), calcium (Ca, 80), iron (Fe, 10), manganese (Mn, 6), copper (Cu, 2), zinc (Zn, 6), molybdenum (Mo, 2), and boron (B, 2). Ammonium nitrate was the N source (proportion 1NO_3^- to 1NH_4^+). Available water was estimated using soil humidity sensors (ECH20 EC-5; Decagon, Pullman, WA USA); sensor values for volumetric water content ($\text{m}^3 \text{m}^{-3}$) were calibrated with the gravimetric mass to estimate the percentage of available water [88]. We applied phosphorous and the other nutrients as soluble fertilizer (fertigation) and alternated with irrigation events each time trays reached 50% of available water. Fertigation and irrigation were manually applied with a watering can until full container capacity was reached. We added macronutrients to every fertigation and micronutrients during every third fertigation. The N concentration of *A. chilensis* was reduced to 200 mg L^{-1} 96 days after transplanting.

Our experimental design for each species was a completely randomized factorial design; 4 P concentrations (0P, 15P, 60P, and 120P) x 2 chemical root pruning treatments (WoCu and WCu) x 4 replicates (trays) x 24 seedlings tray⁻¹; 768 seedlings total.

Morpho-physiological evaluations

Starting on day 14 after transplanting and continuing about every two weeks to the end of the nursery phase (12 and 9 measurements for *A. chilensis* and *Q. saponaria*, respectively) we selected 192 seedlings of each species (8 seedlings x 4 P concentrations x 2 root pruning

x 3 replicates) to determine stem length (SL, cm) and root collar diameter (RCD, mm). The measurements of SL and RCD were used to calculate relative growth rate (RGR) [89,90].

At the end of the nursery stage (June 2023; *A. chilensis*, 180 days; *Q. saponaria*, 140 days after transplanting) 64 seedlings of each species were randomly selected (2 seedlings x 4 P concentrations x 2 root pruning x 4 replicates) for root and shoot morphological assessment. Roots were separated from shoots, gently washed, dyed with a crystal violet solution (5 g L⁻¹), quantified with a high-resolution scanner (1200 DPI resolution, Epson Perfection 4490 Scanner®, Nagano, Japan), and analyzed with a root image analysis system (WinRhizo®, Regent Instrument Inc., Canada). The means for total root diameter, length, surface area, and volume were determined, as well as distribution of roots among diameter classes (fine roots < 1 mm, medium roots = 1-2 mm, and coarse roots > 2 mm) [24]. The area of fresh leaves (cm²) was measured using a leaf area meter (LI-3100, LI-COR Biosciences, Lincoln, Nebraska, USA). We dried shoots (i.e., stems and leaves) and roots at 60 °C for 72 h in a forced ventilation oven to determine dry mass per component. The root to shoot ratio (R:S) was calculated as the root dry mass x shoot dry mass⁻¹. We calculated specific root length (SRL, m g⁻¹) as root length x root dry mass⁻¹, and the root tissue density (RTD, g cm⁻³) as root dry mass x root volume⁻¹. The specific leaf area (SLA, cm² g⁻¹) was calculated as the foliar area x leaf dry mass⁻¹. The same suite of measurements with the same number of seedlings was used for *A. chilensis*, except that only 32 seedlings were used for root architecture (1 seedling x 4 P concentrations x 2 root pruning x 4 replicates).

We selected 3 foliar samples per treatment per species (4 P concentrations x 2 root pruning; 24 total) to obtain N, P, and K concentrations per species. We used the Kjeldahl digestion method and colorimetry for N [87]. For P and K, we prepared samples using dry calcination, and P concentration was determined using colorimetric dissolution in 1N hydrochloric, while K was determined by atomic emission spectrophotometry [87]. Nutrient concentrations and leaf dry mass were used to obtain nutrient content.

Data analysis

The SL, RCD, and RGR data during the nursery stage (180 days for *A. chilensis* and 140 for *Q. saponaria*) were modelled with the PROC NLIN procedure (SAS Institute Inc.) using Gauss-Newton method through a derivative-free algorithm. For SL and RCD, we adjusted a Weibull model for all treatments, with the exception for the SL in the zero P concentration of *Q. saponaria*, where an Asymptotic Regression model was adjusted. For RGR, we used a Gauss Peak Shape model for all treatments, except for RCD in *A. chilensis*, where a First-order Decay Kinetics model was adjusted.

The effect of P concentration and root pruning at the model level was evaluated using the additional sums of squares method [91]. The final morphological attributes, leaf area, SLA, root architecture (i.e., root diameter, length, volume, and surface area), SRL, RTD, biomass (leaves, stem, roots, and R:S), and nutrient concentration and content were assessed using two-way analysis of variance (ANOVA) for a completely randomized design, through PROC GLIMMIX (SAS Institute Inc. Cary, NC, USA) with a distribution selected

considering the Akaike Information Criterion (AIC). Statistical differences between means were performed with Tukey test (HSD) for multiple comparison with 95% confidence. All visualizations were made with graphing software (SigmaPlot 14.0, Systat Software Inc., San José, CA, USA).

Results

Morphological attributes

At the end of the nursery stage, P concentration was significant for root and leaf biomass, leaf area, and R:S (Table 1). The control (0P) had the lowest leaf area compared to all other P concentrations; the same trend was observed for leaf biomass. The highest P concentration (120P) yielded more root biomass than 0P (Table 2). In contrast, root pruning was significant only for root biomass and R:S; the control treatment (WoCu) yielded more root biomass than WCu, and the same trend was observed for R:S (Table 2). The interaction of P concentration and root pruning was significant for *A. chilensis* stem biomass (Table 1); for 0P, stem biomass was the same regardless of root pruning treatment, but at 120P, the WCu treatment had more stem biomass than the WoCu treatment (Table 2).

Table 1 *P*-values of morphological attributes, nutrient concentrations and content, and root architecture in *Aristotelia chilensis* and *Quillaja saponaria* in relation to applied phosphorous concentration (P), chemical root pruning (RP), and their interaction. R:S: root to shoot ratio; SRL: specific root length; RTD: root tissue density. Significant values at $p < 0.05$ in bold.

Variable	<i>Aristotelia chilensis</i>			<i>Quillaja saponaria</i>			
	Phosphorous (P)	Root Pruning (RP)	P x RP	Phosphorous (P)	Root Pruning (RP)	P x RP	
Morphological	Leaf area	< 0.0001	0.2788	0.9136	< 0.0001	0.0057	0.4347
	Leaf biomass	< 0.0001	0.1892	0.8667	< 0.0001	0.0029	0.3596
	Stem biomass	< 0.0001	0.0045	0.0006	< 0.0001	0.0004	0.4996
	Root biomass	0.0404	0.0086	0.3849	< 0.0001	0.1606	0.5951
	R:S	< 0.0001	0.0041	0.8804	0.0522	0.0228	0.8139
Nutritional	N concentration	< 0.0001	0.0006	0.1396	< 0.0001	0.0001	0.0131
	P concentration	< 0.0001	0.0124	0.1630	0.0016	0.0012	0.0074
	K concentration	< 0.0001	0.1950	0.8049	0.0043	0.9467	0.1298
	N content	< 0.0001	0.1172	0.8296	< 0.0001	0.3275	0.8368
	P content	< 0.0001	0.5542	0.9462	< 0.0001	0.2370	0.7441
	K content	0.0552	0.8510	0.7362	< 0.0001	0.0249	0.4022
Root architecture	Root length	0.2742	0.2417	0.4801	< 0.0001	0.1532	0.1353
	Root diameter	0.1095	0.1468	0.5625	< 0.0001	0.2709	0.3179
	Root volume	0.0785	0.0364	0.4313	< 0.0001	0.8007	0.0099
	Root surface area	0.1601	0.0616	0.4043	< 0.0001	0.4938	0.0140
	SRL	0.4362	0.1305	0.5325	0.0002	0.0350	0.0862
	RTD	0.1140	0.8988	0.3191	0.1142	0.0001	0.0263

For *Q. saponaria*, P concentration was significant for all morphological traits except R:S (Table 1). In general, the highest concentrations of P (120 and 60) had higher amounts of leaf area and leaf, stem, and root biomass than 60P, which had greater values for these variables than 0P. Root pruning was significant for leaf area, leaf and stem biomass, and R:S; leaf area and leaf and stem biomass were higher with copper pruning (WCu) but R:S

was lower compared to WoCu (Table 2). No interaction of P concentration and root pruning was observed.

Table 2. Mean values (\pm standard deviation; n=8) of leaf area (cm²) and leaf, stem, and root biomass (g) in *Aristotelia chilensis* and *Quillaja saponaria* in relation to applied phosphorous concentration (0, 15, 60, and 120 mg L⁻¹ P), chemical root pruning (with, WCu and without, WoCu), and its interaction. Different letters refer to significant differences between treatments at $p \leq 0.05$ according to Tukey; ns = non-significant.

		Biomass (g)					
		Leaf area (cm²)	Leaf	Stem		Root	
				WoCu	WCu	R:S	
<i>A. chilensis</i>		Phosphorous (P)					
	0P	383.15 \pm 102.21 ^b	4.03 \pm 0.45 ^b	5.63 \pm 0.61 ^d	5.05 \pm 0.53 ^d	3.48 \pm 1.87 ^b	0.36 \pm 0.08 ^a
	15P	759.53 \pm 217.65 ^a	7.66 \pm 1.52 ^a	12.3 \pm 0.10 ^c	16.00 \pm 2.62 ^{ab}	4.28 \pm 2.27 ^{ab}	0.20 \pm 0.05 ^b
	60P	781.03 \pm 218.31 ^a	7.75 \pm 1.28 ^a	14.62 \pm 1.28 ^{abc}	14.98 \pm 2.53 ^{abc}	3.85 \pm 2.05 ^{ab}	0.18 \pm 0.03 ^b
	120P	803.55 \pm 157.65 ^a	8.26 \pm 1.15 ^a	14.35 \pm 2.39 ^{bc}	18.08 \pm 1.81 ^a	4.40 \pm 2.32 ^a	0.19 \pm 0.04 ^b
		Root Pruning (RP)					
	WoCu	667.37 \pm 237.28 ^{ns}	7.07 \pm 1.98 ^{ns}	11.72 \pm 4.04 ^{ns}		4.33 \pm 2.25 ^a	0.26 \pm 0.10 ^a
	WCu	700.79 \pm 260.75 ^{ns}	6.78 \pm 2.13 ^{ns}	13.53 \pm 5.46 ^{ns}		3.67 \pm 1.95 ^b	0.20 \pm 0.08 ^b
<i>Q. saponaria</i>		Phosphorous (P)					
	0P	13.37 \pm 6.17 ^c	0.21 \pm 0.09 ^c	0.06 \pm 0.05 ^c		0.14 \pm 0.08 ^c	0.65 \pm 0.21 ^{ns}
	15P	44.55 \pm 19.47 ^b	0.72 \pm 0.36 ^b	0.25 \pm 0.15 ^b		0.69 \pm 0.44 ^b	0.75 \pm 0.15 ^{ns}
	60P	87.95 \pm 18.73 ^a	1.49 \pm 0.42 ^a	0.52 \pm 0.13 ^a		1.21 \pm 0.68 ^a	0.61 \pm 0.07 ^{ns}
	120P	109.69 \pm 44.19 ^a	1.81 \pm 0.92 ^a	0.71 \pm 0.43 ^a		1.31 \pm 0.76 ^a	0.56 \pm 0.07 ^{ns}
		Root Pruning (RP)					
	WoCu	55.84 \pm 39.26 ^b	0.88 \pm 0.66 ^b	0.30 \pm 0.23 ^b		0.77 \pm 0.56 ^{ns}	0.70 \pm 0.14 ^a
	WCu	71.94 \pm 50.34 ^a	1.23 \pm 0.94 ^a	0.47 \pm 0.41 ^a		0.90 \pm 0.63 ^{ns}	0.59 \pm 0.14 ^b

In *A. chilensis*, the RGR of the 120P concentration revealed maximum growth occurred sooner (43 days after transplanting) than that for 60P, 15P, and 0P (45, 59, and 61 days, respectively) (Figure 1B). The 0P-WoCu and the 0P-WCu treatments promoted the least amount of stem length during the entire growth season compared to all the other treatment combinations (Figure 1A). For RCD growth, only the 60P-WoCu and 120P-WoCu treatments shared similar growth rates throughout the nursery stage (Figure 1C). Whereas the rate of stem length growth increased during the nursery stage, the rate of RCD was greatest early in the nursery stage and then declined (Figure 1D), regardless of treatment.

In *Q. saponaria*, the 120P, 60P, and 15P concentrations (regardless of root pruning) reached maximum stem length RGR at day 50, twenty days sooner than the control (0P) (Figure 1F). The RCD reflected effects on P concentrations but not root pruning treatment (Figure 1G). The highest P concentrations (120 and 60) generated the most RCD throughout the growing period, and these rates achieved their maximum growth rate 69 days after transplanting, faster than the 71 days for 15P and 87 days for 0P (Figure 1H). Also, 0P and 15P concentrations, irrespective of root pruning treatment, and the 60P-WoCu and 120P-WoCu treatments shared the highest stem length dynamic (Figure 1E). The 120P-WoCu and 60P-WoCu treatments showed the maximum stem length, while the lowest stem length was observed in the 0P-WoCu and 0P-WCu treatments.

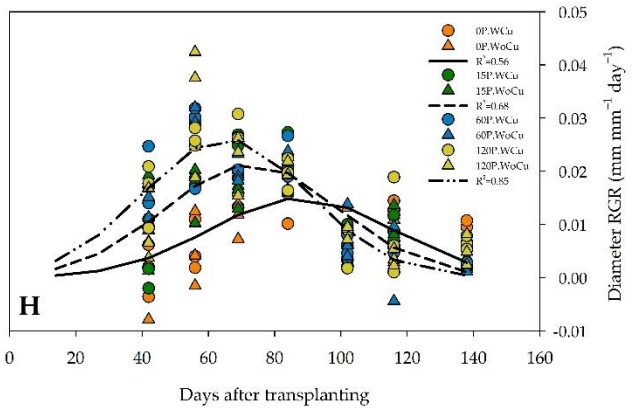
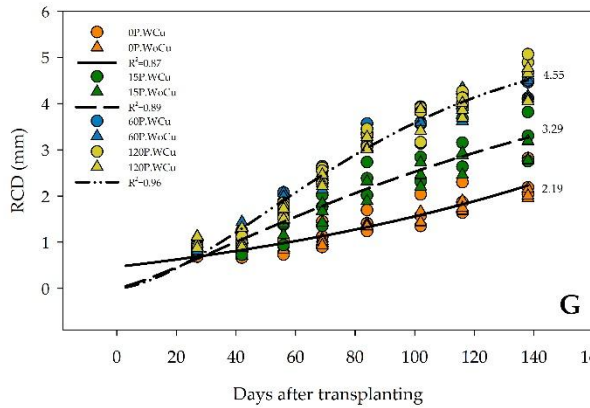
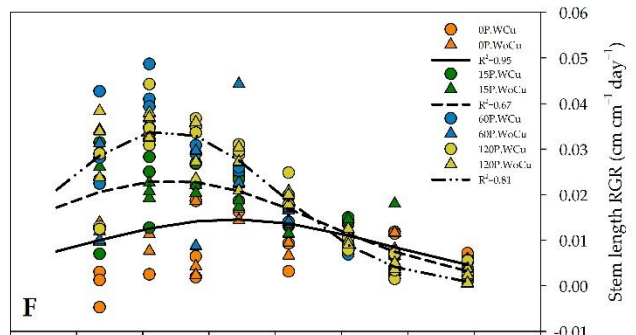
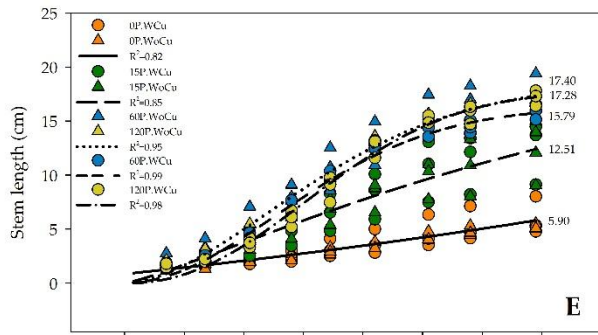
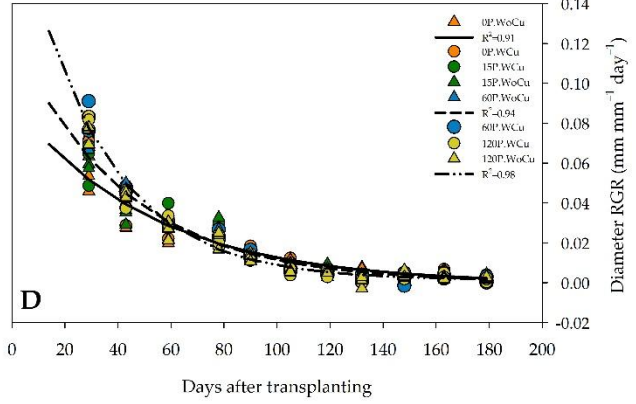
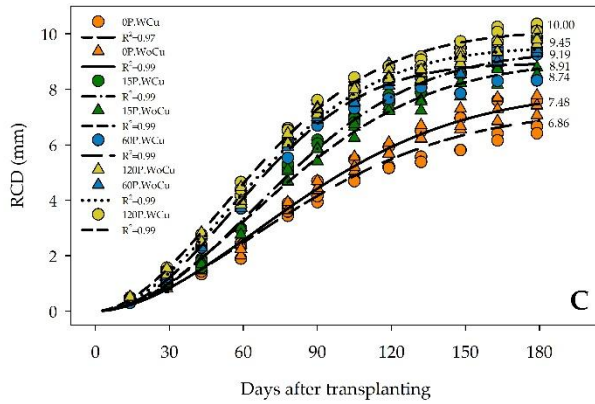
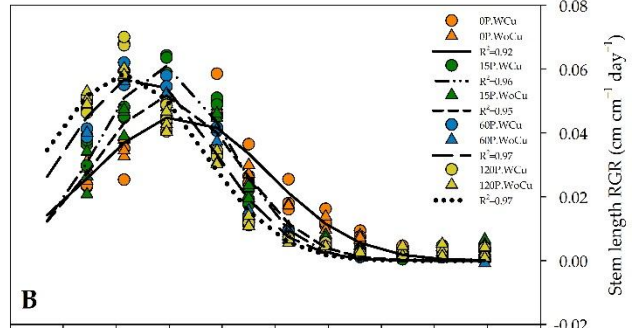
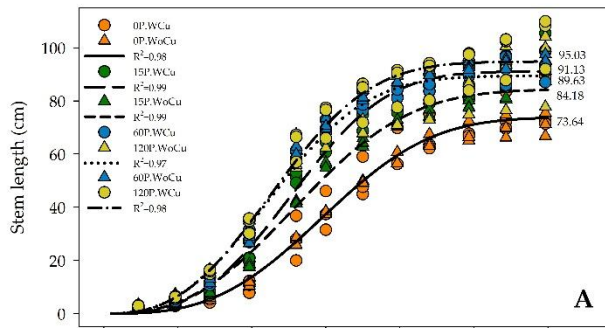


Figure 1. Growth dynamics of stem length and root collar diameter (RCD) for *Aristotelia chilensis* (A, C) and *Quillaja saponaria* (E, G), in relation to applied phosphorous concentration (0, 15, 60, and 120 mg L⁻¹ P), chemical root pruning (with, WCu and without, WoCu), and its interaction (n=24 for each treatment). Figures on the right illustrate the relative growth rate (RGR) of stem height and RCD for *A. chilensis* (B, D) and *Q. saponaria* (F, H). Colored symbols indicate the mean, and lines indicate modeled response for treatments.

Nutrient concentration and content analysis

In *A. chilensis*, the level of applied P concentration was significant for plant N, P, and K concentrations and N and P content (Table 1). The control (0P) had the lowest N concentration and content compared to all other applied P concentration treatments. Not surprisingly, sequential increases in applied P concentration increased plant P concentration and content. In contrast, sequential increases in applied P concentrations decreased K concentrations (Table 3). The root pruning treatment was significant for plant N and P concentrations (Table 1). The higher N concentration was observed in the WCu treatment compared to the WoCu treatment; the opposite behavior was observed for plant P concentration (Table 3). Also, no significant interaction between applied P concentration and root pruning was observed for plant nutrient concentration and content.

Table 3. Mean values (\pm standards deviation; n=3 for each treatment) of nitrogen (N), phosphorous (P) and potassium (K) nutritional concentration and content *Aristotelia chilensis* and *Quillaja saponaria* in relation to applied phosphorous concentration (0, 15, 60, and 120 mg L⁻¹ P), chemical root pruning (with, WCu and without, WoCu), and its interaction. Different letters refer to significant differences among treatments at $p \leq 0.05$ according to Tukey; ns = non-significant.

Nutrient concentration (mg g ⁻¹)					Nutrient content (mg)			
<i>A. chilensis</i>	N	P	K		N	P	K	
Phosphorous (P)								
0P	12.15 \pm 1.76 ^b	0.67 \pm 0.12 ^d	12.10 \pm 2.30 ^a		48.17 \pm 4.49 ^b	2.63 \pm 0.22 ^c	47.69 \pm 4.34 ^{ns}	
15P	16.47 \pm 2.76 ^a	1.28 \pm 0.15 ^c	8.28 \pm 0.90 ^b		125.22 \pm 23.96 ^a	9.81 \pm 1.80 ^b	63.77 \pm 14.95 ^{ns}	
60P	16.58 \pm 1.01 ^a	1.77 \pm 0.12 ^b	6.60 \pm 0.50 ^c		133.75 \pm 17.85 ^a	14.29 \pm 2.39 ^a	53.29 \pm 7.81 ^{ns}	
120P	17.60 \pm 1.87 ^a	2.08 \pm 0.23 ^a	7.43 \pm 0.80 ^{bc}		136.97 \pm 14.32 ^a	16.52 \pm 2.09 ^a	58.89 \pm 6.43 ^{ns}	
Root pruning (RP)								
WoCu	14.47 \pm 2.65 ^b	1.51 \pm 0.57 ^a	8.24 \pm 1.97 ^{ns}		105.56 \pm 39.07 ^{ns}	10.62 \pm 5.66 ^{ns}	55.87 \pm 12.91 ^{ns}	
WCu	16.78 \pm 2.44 ^a	1.39 \pm 0.58 ^b	8.97 \pm 2.95 ^{ns}		116.50 \pm 42.79 ^{ns}	11.01 \pm 5.95 ^{ns}	55.95 \pm 8.31 ^{ns}	
<i>Q. saponaria</i>								
	WoCu	WCu	WoCu	WCu				
Phosphorous (P)								
0P	34.43 \pm 4.89 ^a	27.33 \pm 1.99 ^b	2.10 \pm 0.26 ^{ab}	2.06 \pm 0.21 ^{ab}	9.38 \pm 1.21 ^b	7.09 \pm 1.82 ^c	0.49 \pm 0.16 ^c	2.23 \pm 0.82 ^c
15P	20.67 \pm 2.57 ^c	18.27 \pm 0.42 ^{cd}	1.83 \pm 0.25 ^{bc}	1.60 \pm 0.17 ^c	12.20 \pm 1.25 ^a	15.94 \pm 3.88 ^b	1.41 \pm 0.40 ^b	10.06 \pm 2.61 ^b
60P	15.90 \pm 0.69 ^d	16.20 \pm 0.70 ^d	1.80 \pm 0.17 ^{bc}	1.77 \pm 0.06 ^{bc}	10.75 \pm 1.19 ^{ab}	24.64 \pm 3.92 ^a	2.73 \pm 0.35 ^a	9.38 \pm 1.81 ^b
120P	21.07 \pm 0.70 ^c	15.47 \pm 0.49 ^d	2.43 \pm 0.15 ^a	1.70 \pm 0.17 ^{bc}	11.12 \pm 1.04 ^{ab}	34.15 \pm 8.84 ^a	3.80 \pm 0.76 ^a	12.20 \pm 6.83 ^a
Root pruning (RP)								
WoCu	23.02 \pm 7.59 ^{ns}		2.04 \pm 0.32 ^{ns}		10.93 \pm 1.87 ^{ns}	19.13 \pm 9.78 ^{ns}	2.00 \pm 1.28 ^{ns}	10.97 \pm 6.50 ^b
WCu	19.32 \pm 5.04 ^{ns}		1.78 \pm 0.23 ^{ns}		10.79 \pm 1.09 ^{ns}	21.78 \pm 13.10 ^{ns}	2.22 \pm 1.48 ^{ns}	13.97 \pm 9.45 ^a

For *Q. saponaria*, applied P concentration was significant for K concentration, and N, P, and K contents (Table 1). Potassium foliar concentration was highest in the 15P concentration treatment and lowest in the 0P control treatment, independent of root pruning treatment. As a general trend, N, P and K contents were higher in the 120P concentration and lowest in the 0P (Table 3). Root pruning was not significant for nutrient concentrations or contents. The applied P concentration x root pruning treatment interaction was significant for plant N and P concentrations (Table 1). The highest N concentrations were observed in the 0P-WoCu followed by the 0P-WCu treatments, while the 60P-WoCu, 60P-WCu, and the 120P-WCu treatment displayed the lowest N concentrations (Table 3). Regarding plant P concentration, higher values were observed in the 15P-WoCu, while the lowest values were found in the 15P-WCu treatment (Table 3). As a general trend, the presence of copper pruning resulted in lower plant P concentrations than that for seedlings grown without copper pruning.

Root architecture traits

For *A. chilensis*, P concentration was not significant for any of the root architecture traits (Table 1), whereas root pruning was significant for root volume. The WoCu treatment increased root volume 33% (10.15 cm^3) compared to the WCu treatment (7.83 cm^3).

For *Q. saponaria*, the P concentration was significant for root length, root diameter, and SRL (Table 1). Thus, root length and diameter were higher in any treatments with $P > 0$ compared to the control (0P) (Figure 2A and 2C, respectively).

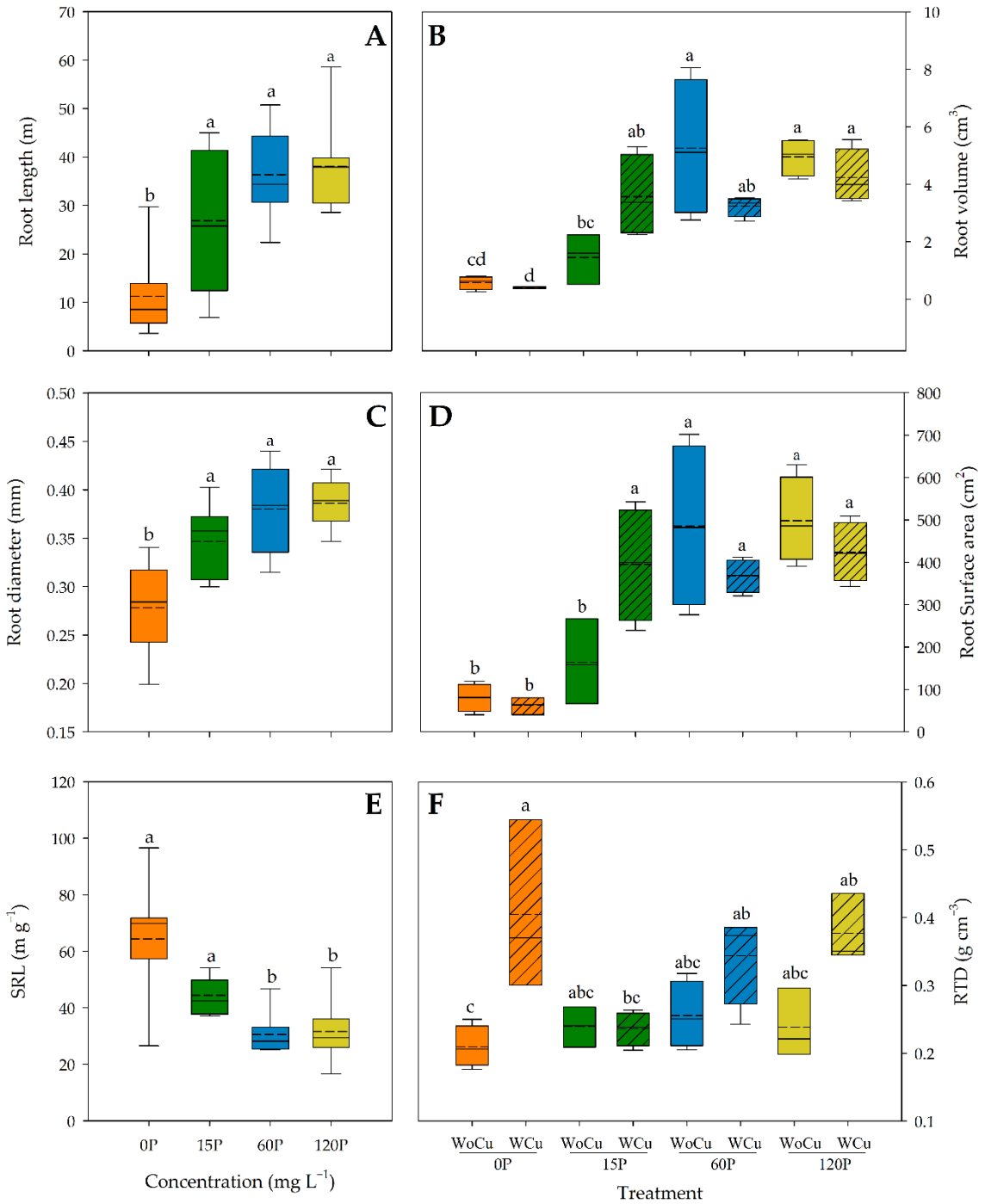


Figure 2. Root length (A), volume (B), diameter (C), surface area (D), specific root length (SRL) (E), and root tissue density (RTD) (F) of *Quillaja saponaria* in relation to applied phosphorous concentration (0, 15, 60, and 120 mg L⁻¹ P), chemical root pruning (without, WoCu and with, WCu), and its interaction (n= 8 for each treatment). Bottom and top boundaries of the boxes represent the 25th and 75th percentiles, respectively. The solid dashed on the center of each box represents the mean value and the solid line represents the median value. Different letters indicate significant different among treatments at p < 0.05 according to Tukey.

On the contrary, the 60P and 120P concentration had more SRL than their 0P and 15P counterparts (Figure 2E). Root pruning only affected the SRL (Table 1), with higher values in the WoCu treatment ($47.22 \pm 23.80 \text{ m g}^{-1}$) compared to WCu ($36.66 \pm 15.52 \text{ m g}^{-1}$). The interaction of P concentration and root pruning treatments was significant for root volume, root surface area, and RTD (Table 1). The lowest root volume was observed in the 0P concentration, followed by the 15P-WoCu treatment, while the 120P concentration showed the highest root volume (Figure 2B). Similarly, the 0P and the 15P-WoCu treatments had the lowest root surface area, compared to all the other treatments (Figure 2D). For RTD, the 0P-WoCu and 15P-WCu treatments had significantly lower tissue density, while the highest RTD was observed in the 0P-WCu treatment (Figure 2F).

Table 4. *P*-values of root architecture in *Aristotelia chilensis* and *Quillaja saponaria* in relation to the applied phosphorus concentration (P), chemical root pruning (RP) and its interaction (P x RP) in the distribution of fine, medium and coarse roots. Significant differences at $p < 0.05$ in bold.

	Source of variation	Length			Volume		
		fine	medium	coarse	fine	medium	coarse
<i>A. chilensis</i>	Phosphorous (P)	0.2792	0.0021	0.2954	0.0675	0.0038	0.0004
	Root Pruning (RP)	0.2472	0.0380	0.0807	0.0485	0.0382	0.9086
	P x RP	0.4802	0.5809	0.4547	0.5076	0.4450	0.8502
<i>Q. saponaria</i>	Phosphorous (P)	0.0003	0.0008	0.0146	0.0001	0.0008	0.0035
	Root Pruning (RP)	0.0322	0.0264	0.1082	0.0199	0.0428	0.0677
	P x RP	0.0011	0.0778	0.0899	0.0516	0.0379	0.0343

For *A. chilensis* root traits by size class, P concentration was significant for length of medium-sized roots, and on the volume of medium- and coarse-sized roots (Table 4). When P was applied (i.e., 120P, 60P, or 15P), medium-sized roots were longer than those in the control (Figure 3B). The same trend was observed for the volume of medium- and coarse-sized roots (Figure 3F and 3H, respectively). Root pruning was significant for length of medium-sized roots, and volume of fine- and medium-sized roots (Table 4), where shorter lengths and lower volumes were observed in the WCu treatment (Figure 3C, 3E, and 3G, respectively). Also, no interaction between P concentration and root pruning was observed.

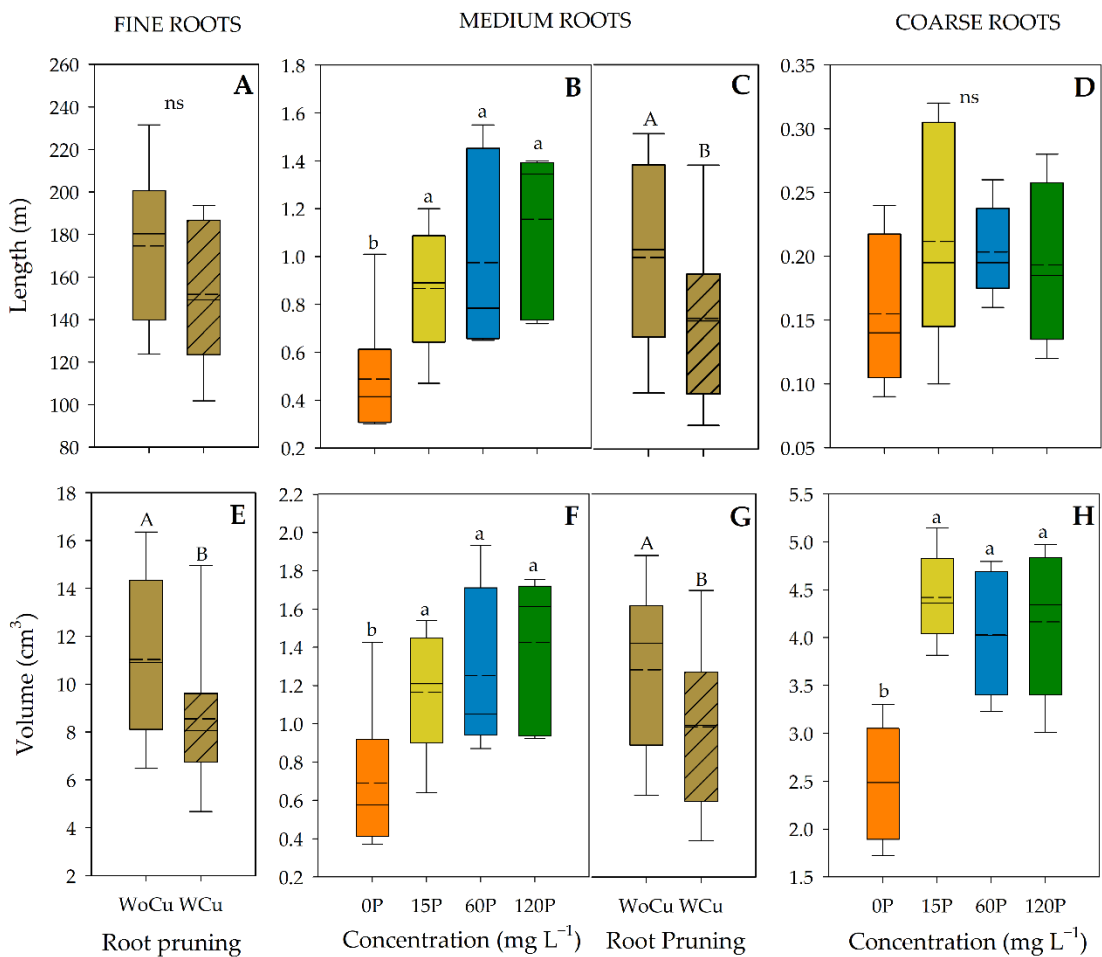


Figure 3. Root length (A-D) and volume (E-H) of *Aristotelia chilensis* in relation to applied phosphorous concentration (0, 15, 60, and 120 mg L⁻¹ P), chemical root pruning (without, WoCu and with, WCu) according to the diameter distribution (fine, medium, and coarse roots) (n= 4 for each treatment). Bottom and top boundaries of the boxes represent the 25th and 75th percentiles, respectively. The dashed line in the center of each box represents the mean value and the solid line represents the median value. Different letters indicate significant difference between treatments at $p \leq 0.05$ according to Tukey; ns = non-significant.

For *Q. saponaria*, P concentration was significant for length of medium- and coarse-size roots, and on volume of fine-size roots (Table 4). The 120P concentration showed higher length compared to 15P and 0P in medium-size roots (Figure 4B). The 120P and 15P concentrations had the highest length in coarse-size roots, meanwhile control (0P) showed the lowest (Figure 4D). All P concentrations (120P, 60P, or 15P) had higher root volume than 0P concentration (Figure 4E). Root pruning was significant for the length of medium-sized roots, and volume of fine-sized roots (Table 4), where shorter lengths and lower volumes were observed in the WCu treatment (Figure 4C and 6F, respectively). The interaction of P concentration and root pruning was significant for the length of fine-sized roots and volumes of medium- and coarse-sized roots (Table 4). The lowest length in fine-size roots was observed in 0P-WoCu treatment and highest in 60P-WoCu (Figure 4A). For medium-sized roots, 0P-WoCu treatment yielded significantly lower volume, while the highest was observed in 120P-WoCu (Figure 4G). Similarly, the 120P-WoCu treatment had the highest volume in the coarse-size roots and 60P-WCu showed the lowest (Figure 4H).

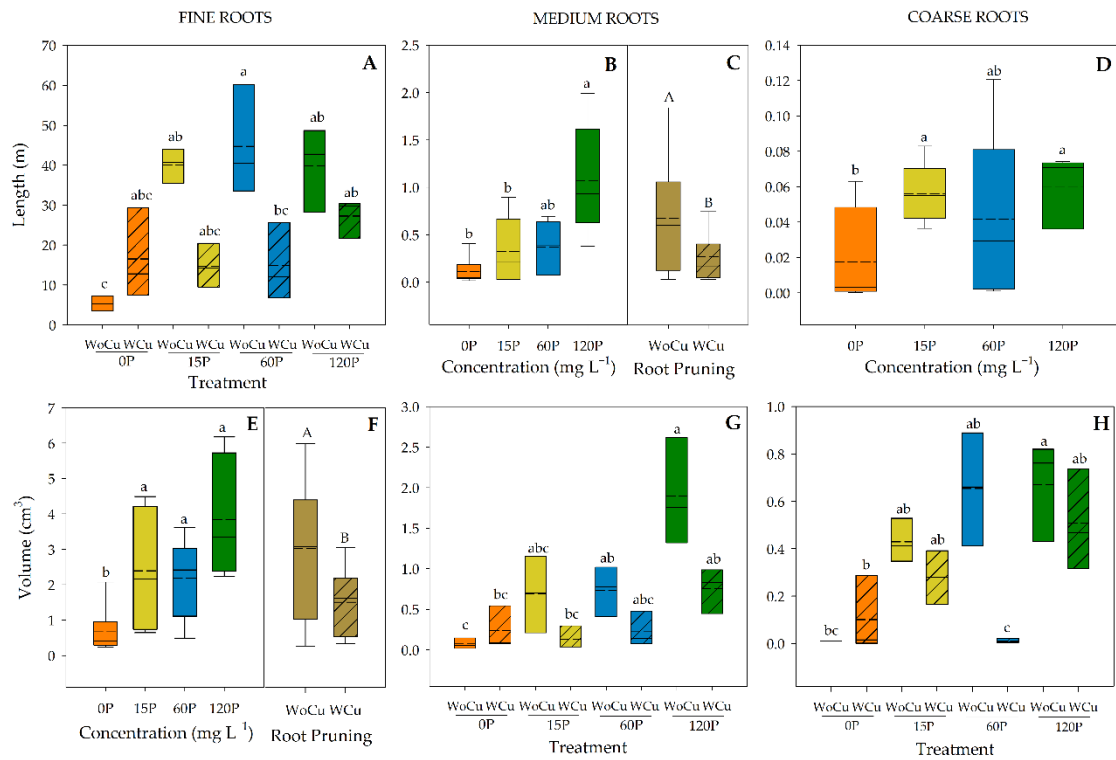


Figure 4. Root length (A-D) and volume (E-H) of *Quillaja saponaria* in relation to applied phosphorous concentration (0, 15, 60, and 120 mg L⁻¹ P), chemical root pruning (without, WCu and without, WoCu), and its interaction according to the diameter distribution (fine, medium, and coarse roots) (n= 8 for each treatment). Bottom and top boundaries of the boxes represent the 25th and 75th percentiles, respectively. The dashed line in the center of each box represents the mean value and the solid line represents the median value. Different letters indicate significant difference between treatments at $p \leq 0.05$ according to Tukey.

Discussion

In *A. chilensis*, we observed that increasing P concentration applied during nursery production significantly increased RGR, resulting in greater plant growth and size. Increase in growth is well correlated with the increase in N and P content observed in the applied P concentrations, and this effect has been previously observed in *Acer mono* and *Quercus ilex* [50,51]. In our study, applied P concentration increased leaf area (47%) and leaf biomass (51%) more than root biomass (21%) relative to the 0P control treatment, thus causing a decrease in the R:S. Similar results were observed in *Araucaria angustifolia* cultivated with concentrations greater than 118 mg L⁻¹ P [52]. The decrease in the R:S could induce an imbalance between water absorption and transpiration leading to water stress and decreased survival after field establishment [53,54]. Despite that, increasing P concentration also promoted higher root biomass, which agrees with other research [30,32,33]. This was, however, not related to changes in the architecture of fine roots in *A. chilensis*, which are mainly responsible for water and nutrient absorption [30,55–57]. Contrary to our expectations, the increase in medium- and coarse-sized root length and volume observed with increases in applied P concentration were mostly responsible for the observed increase in total root biomass.

The chemical (i.e., copper) root pruning decreased *A. chilensis* root biomass, which agrees with several reports [40,42,58]. The objective of chemical root pruning is to limit the growth of lateral roots and stimulate growth of higher order-roots [34,35], thus developing a fibrous root system with higher growth of shorter and thinner roots [40,42].

Some studies have shown no differences in root architecture [14,38]. However, contrary to our hypothesis, root pruning did not increase the volume or length of fine roots as expected, and significantly decreased the length and volume of medium roots, thus explaining the overall lower root biomass in this treatment. This also led to a decline in R:S which, as previously discussed, could negatively impact plant performance in drought stress conditions.

Similar to the results observed in *A. chilensis*, increasing the rate of applied P to *Q. saponaria* increased stem length, diameter, biomass, and leaf area. Contrary to *A. chilensis*, P concentration had, however, no effect on the R:S. Higher applied P concentration also enhanced plant N, P, and K content, which is associated with increased growth and above- and below-ground biomass. Similar results were observed in *Eucalyptus grandis*, where plants exhibited higher shoot growth and promoted accumulation of N, P, and K content with increased applied P concentration [59]. Development of more leaf area and shoot biomass with increasing applied P has also been reported in *Swietenia macrophylla* [60]. Although the effects of P have been commonly researched by assessing the effects of its deficiency, contrasting results have been observed in leaf area, shoot biomass, and R:S in different species [31,51,61,62], indicating that the effects of P on plant growth and biomass distribution are highly species-specific. This agrees with our distinct results observed between *A. chilensis* and *Q. saponaria*.

Although the applied P concentration interacted with root pruning, we noted that N foliar concentrations decreased as the rate of applied P increased. Similar results have been

recorded for *E. grandis*; the effect was described as antagonism between phosphate and nitrate (NO_3^-) [63]. Similar results were observed in *Phoebe zhennan* [64]. However, lower N concentrations caused by increased rate of applied P in our study had no implications on growth and biomass accumulation, probably due to higher N content in P fertilized plants.

Contrary to our results for *A. chilensis*, P concentration and chemical root pruning had major effects on *Q. saponaria* root architecture. The literature shows that P deficiency induces a decrease in root diameter and an increase in root length toward expanding the area of soil explored, thus increasing root surface area (RSA) [65–69] in trees and shrubs [29,31]. In *Q. saponaria* we observed, however, that P deficiency in the 0P concentration decreased root diameter and length, in contrast to previous studies. The lower root diameter and root biomass led to an increase in SRL in the 0P control, which is commonly observed in P deficient soils linked to higher P uptake efficiency [70,71] and increasing soil exploration volume per unit of carbon invested in root length [72–75]. Besides root diameter, a meta-analysis revealed that higher SRL correlated with decreased RTD [76]. In our study, however, this relationship depended on the application of chemical root pruning and level of applied P. In our 0P concentration, the expected low RTD in concert with high SRL was noted in the WoCu treatment, but in WCu, RTD and SRL were both high. Although there was no difference in root diameter under P-deficient conditions, the application of Cu may promote development of denser roots that might increase plant tolerance to drought conditions [76] and nutrient acquisition in poorly nourished soils after outplanting [73]. This combination of high SRL and RTD related to chemical root pruning was absent when P was applied (i.e., 15P, 60P, and 120P). Additionally, the application of

Cu induced a decrease in length and volume of fine roots and showed a tendency to decrease the volume of medium and coarse roots, which can negatively affect nutrient and water absorption in resource limited conditions.

The application of P induced a lower SRL in *Q. saponaria*, which agrees with results from several species [50,68,77]. Because a higher SRL has been linked to resistance to water stress [76,78,79] by increasing the efficiency in resource acquisition, these results could imply that P could induce lower drought resistance after establishment. However, in *Q. saponaria*, the lower SRL of P-fertilized plants was also linked to higher RSA, root diameter, root length, volume, RTD, and biomass, traits that are usually present in species subjected to water deficit or present in habitats with lower rainfall [61,80]. All these traits should confer high resource acquisition due to increased root-soil interface. The high nutrient content induced by P fertilization in *Q. saponaria* could imply a high resource investment in denser roots with longer lifespans, as it has been described in roots with higher RTD [73,78,81,82]. Phosphorous fertilization also had a high impact on root architecture at different diameter distributions. More importantly, increasing P concentration induced the development of longer fine roots with increased volume, implying a larger volume of soil exploited per unit of biomass invested [76]. Fine roots play a significant role in soil exploration for water and nutrient acquisition and can account for up to 80% of total root length [83,84]. Thus, minute changes in fine root architecture could result in great changes in plant performance after field establishment. Similarly, not accounting the effect of chemical root pruning, higher P concentration also increased volume and length of medium- and coarse-sized roots. This could also have a beneficial

effect in resource-limiting conditions, because, beyond anchorage, coarse roots serve as carbohydrate reserve and absorption of water from deeper soil horizons [83,85].

Although *A. chilensis* and *Q. saponaria* had effects due to P concentrations and RP, the rapid shoot and root growth of *A. chilensis* was unexpected. This growth response resulted in the roots of this species densely occupying the entire container substrate by the end of the nursery period (Figure S1), and that might have limited the accurate root development response for the effects of P and RP. While for *Q. saponaria* at conclusion of the nursery phase, root plugs were intact, but roots were not densely packed, allowing the determination of differences in response to applied treatments (Figure S2).

Conclusions

Our study shows that the efficacy of nursery treatments, namely applied P concentration and use of chemical (i.e., copper) root pruning, both of which aim to modify root architecture traits, are species specific. We observed that *A. chilensis* fully colonized the container substrate during nursery production, which could have influenced the lack of response in root pruning treatments. This shows the need to account for the growth dynamics of Mediterranean species to select suitable containers and/or fertility regimes for optimal development in the nursery. Contrary to our hypothesis, P concentration did not affect the development of fine roots and only had minor effects on length and volume of medium roots. Regarding *Q. saponaria* and in agreement with our hypothesis, P concentration induced the expected changes in root architecture, especially in fine- and medium-sized roots. Considering whole root architecture, P concentration induced changes that could relate to higher plant performance under water limited conditions and had an improvement in plant nutritional status, which has been extensively correlated to better plant performance in Mediterranean climates. However, the implications of these changes on plant performance require further evaluation. In disagreement with our hypothesis, chemical root pruning prompted changes that could negatively impact plant performance, mostly in fine- and medium-sized roots. Considering that root pruning during nursery production is costly and time-consuming, our results do not present a clear benefit of its application toward improving root systems to increase drought avoidance.

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CAPÍTULO II: Phosphorus fertilization and chemical root pruning during nursery production shape root growth and drought response of two Mediterranean woody species

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Abstract

Recent work demonstrated that P fertilization and chemical root pruning (RP) during nursery production of *Aristotelia chilensis* and *Quillaja saponaria*, two important tree species in the mediterranean region of Chile, affected root architecture and nutritional status. Here we report on how the nursery-induced modifications through P fertilization and RP influence post-transplanting root growth potential (RGP) and drought resistance. RGP was quantified through the number, length, and biomass of newly formed roots, while leaf water potential (Ψ_L) and stomatal conductance were monitored during a controlled drought experiment. Higher P fertilization enhanced CO₂ assimilation, water-use efficiency, and RGP in both species. It also promoted species-specific drought strategies: *A. chilensis* displayed a more isohydric response, tightly regulating water loss, whereas *Q. saponaria* maintained gas exchange under declining Ψ_L , reflecting anisohydric behavior. In contrast, RP reduced RGP and impaired drought performance by limiting new root development and restricting gas exchange. These results highlight the positive role of P fertilization in producing drought-resilient plants and question the suitability of RP for mediterranean woody species intended for restoration. Understanding how nursery practices shape morpho-physiological and root traits is essential to optimize plant quality and post-planting survival under increasingly frequent drought conditions.

Keywords: Target Plant Concept, plant performance, root growth potential, drought resistance, stomatal regulation, isohydric-anisohydric behavior.

Introduction

Forest ecosystems worldwide are under growing pressure from the interacting effects of climate change, land-use change, intensified wildfire regimes, and biological invasions (Bussotti & Pollastrini, 2020; Cawson et al., 2018; Cueto et al., 2025; Underwood et al., 2018). Among these, the rising frequency and duration of droughts has emerged as a critical constraint, impairing natural regeneration and threatening long-term ecosystem sustainability (Acevedo et al., 2024a; Erickson and Halford, 2020; Giorgi, 2006; Peñuelas & Sardans, 2021; Santibáñez et al., 2025). Given these challenges, planting seedlings for ecological restoration has become a key strategy, yet its success largely depends on high-quality plants capable of establishing under drought prone conditions (Haase & Davis, 2017; Stanturf et al., 2014). Currently, the limited availability of seedling material with suitable morphological and physiological traits remains one of the main bottlenecks for restoration programs in mediterranean-type ecosystems (Acevedo et al., 2021; Bannister et al., 2018).

The suite of morphological and physiological traits needed by planted seedlings to ensure survival and growth can be defined through the Target Plant Concept (Dumroese et al., 2016). Target plants in mediterranean ecosystems specifically require, among other traits, development of a robust root system for post-planting survival, as increased soil exploration, particularly to deeper soil substrates, allows plants to access water reserves during drought periods (Dhief et al., 2011; García-Pérez et al., 2021; Padilla et al., 2007). Root growth potential (RGP), defined as a seedling's capacity to produce new active roots

for water and nutrient uptake (Grossnickle, 2005; Ritchie & Dunlap, 1980), is one way to quantify this target attribute, which has been positively associated with field performance (Grossnickle & Ivetić, 2022), in gymnosperms (L'Hirondelle et al., 2007; Ritchie, 1985) and mediterranean broadleaved species (Puértolas et al., 2024), including *Nothofagus glauca* (Acevedo et al., 2024b). Because of the importance of RGP, improving our understanding of the nursery practices that enhance root development and subsequent post-planting establishment is critical.

Among nursery practices that affect root development, phosphorus (P) fertilization increases seedling root length, branching, biomass, and nutrient use efficiency (Salim et al., 2023). Moreover, P fertilization can increase leaf relative water content, net photosynthesis, and photochemical efficiency, thereby improving drought resistance (Tariq et al., 2017). Thus, the benefit of P fertilization during nursery production to these morphological and physiological attributes is enhanced plant performance during field establishment (Vukmirović et al., 2024). Similar to P fertilization, chemical root pruning (RP) of container seedlings in nurseries also promotes early and controlled root branching, yielding a more fibrous root system with reduced deformations such as root spiraling (Montagnoli et al., 2022; Gilman and Beeson, 1995; Sword et al., 2009; Wenny et al., 1988). Root pruning aims to promote development of more horizontal roots to improve seedling stability after outplanting (Grossnickle & Ivetić, 2022) and most available evidence on its effects on post-planting performance comes from conifer species. For example, in *Pinus palustris*, RP increased biomass of first- and second-order roots without affecting survival, and these effects persisted up to eight years after establishment (Sung

et al., 2019; Sword et al., 2009, 2011). The influence of RP on plant performance after field establishment, particularly under drought conditions, remains poorly documented.

We recently reported that P fertilization during nursery production increased root length and volume, as well as leaf nitrogen (N), P, and potassium (K) contents—attributes associated with improved plant performance under drought stress (Grossnickle & MacDonald, 2018; Vukmirović et al., 2024)—in *Aristotelia chilensis* and *Quillaja saponaria* seedlings, two woody species from Mediterranean Chile (Calderón-Ureña et al., 2025). In our study, we also found that chemical RP reduced root biomass, root-to-shoot ratio (R:S), and leaf N and P concentration—attributes linked to lower survival and growth after field establishment (Grossnickle, 2012; Villar-Salvador et al., 2012). Such changes in root architecture and biomass allocation between below- and above-ground organs may translate into adjustments in plant physiological behavior during water deficit, particularly in the dynamics of stomatal closure regulating leaf water potential (Ψ_L), thereby influencing the balance between carbon assimilation and water loss (Bartlett et al., 2012; Grossnickle & Ivetić, 2022).

In mediterranean ecosystems, species with contrasting drought-response strategies often co-occur, ranging from conservative species that tightly regulate transpiration to acquisitive (i.e., opportunistic) species that maintain gas exchange even as Ψ_L declines (Martínez-Vilalta et al., 2014; Nardini et al., 2014; Vilagrosa et al., 2010). Yet these aboveground strategies represent only one axis of drought acclimation, as species also differ substantially in the structure and function of their root systems.

Considering below-ground organs, a conceptual framework known as the root economic spectrum (RES) classifies species along a continuum from conservative to acquisitive strategies based on root architectural traits (McCormack et al., 2012; Prieto et al., 2015; Roumet et al., 2016; Weemstra et al., 2016). Conservative species are characterized by the development of short and thick roots reflected in lower SRL, with greater carbon investment per unit of root tissue. In contrast, acquisitive species exhibit higher SRL, investing less carbon per unit root length while achieving greater resource uptake capacity under limiting conditions.

Interestingly, in our previous study (Calderón-Ureña et al., 2025), we noted that while both species generally responded similarly to increases in P fertilization, there was a distinct difference in the specific root length (SRL, m g^{-1}). For instance, *A. chilensis* had lower SRL ($40.6 \pm 1.29 \text{ m g}^{-1}$) than *Q. saponaria* ($61.3 \pm 13.52 \text{ m g}^{-1}$), suggesting a more conservative root strategy in the former and a more acquisitive strategy in the latter. Moreover, *A. chilensis* had higher root length and volume than *Q. saponaria*, key traits often linked to drought resistance (McCormack et al., 2012). Even so, in woody species, including *Q. saponaria*, the acquisitive strategy has also been associated with increased drought resistance (Ovalle et al., 2015; Tan et al., 2023), where greater root depth and more fine-root development are key traits enhancing water acquisition during soil drying (Padilla & Pugnaire, 2007; Tron et al., 2015). However, it remains unclear how nursery-induced variation in root growth and architecture translates into physiological drought responses after transplanting, particularly in mediterranean woody species with contrasting root strategies.

Given these considerations, our objective was to analyze the relationship between morpho-physiological and root-architectural modifications previously induced by P fertilization and chemical RP, and their effects on RGP and drought resistance in *A. chilensis* and *Q. saponaria*. We hypothesize that the changes in morpho-physiological attributes induced by P fertilization during nursery production will enhance drought resistance during water deficit, whereas the opposite will occur in plants subjected to RP. Additionally, we expected that the acquisitive root traits exhibited by *Q. saponaria* would confer greater drought resistance compared to the more conservative *A. chilensis*. Together, these results should improve our understanding about how nursery practices modulate physiological behavior among species used for restoration in drought-prone environments.

Materials and methods

Nursery stage

Seedling Production

We grew *A. chilensis* and *Q. saponaria* seedlings in the Instituto Forestal (INFOR; Forest Institute) Centro Tecnológico de la Planta Forestal (CTPF; Technology Center for Forest Plants) outdoor nursery located in the Biobío region, Chile (36.84° S; 73.13° W) between November 2022 and June 2023. We used 64 trays each with 24 cavities (280 cm³).

Experimental design by species consisted of a completely randomized factorial design with two factors: chemical root pruning (RP) and P fertilization. For the RP treatment, 32 trays were submerged in a $60 \text{ g L}^{-1} \text{ Cu}_2(\text{OH})_3\text{Cl}$ solution following Acevedo et al. (2021) (with RP: WCu), while remaining trays were not treated (without RP: WoCu). Trays were filled with a substrate of *Pinus radiata* bark compost (Acevedo et al., 2024; González et al., 2020). For the P treatment, seedlings received 0, 15, 60, or 120 mg L^{-1} (hereafter 0P, 15P, 60P, or 120P, respectively). Remaining macro and micronutrients were applied at a constant rate as soluble fertilizer (fertigation) alternated with irrigation events each time trays reached 50% of available water (described below; Calderón-Ureña et al. 2025). Experimental unit was constituted by a tray, replicated 4 times.

During nursery production we assessed morphological attributes (stem length; root collar diameter, RCD; leaf, stem, and root biomass), nutrient content and concentration (N, P, and K), and root architecture traits (diameter; total length and volume; length and volume of fine, medium and coarse roots). Methodology and results of these evaluations are detailed in Calderón-Ureña et al. (2025).

Gas exchange evaluation

At the end of nursery production (June 2023), 48 seedlings per species (6 seedlings x 4 P fertilization x 2 RP treatments) were randomly selected across the experimental units for gas exchange evaluation. We used a portable infrared gas analyzer (WALZ GFS-3000, equipped with the LED-Array/PAM fluorescence module, GFS-3000FL; Effeltrich,

Germany) to generate a species-specific light-response curve to identify the light saturation point. A fully expanded leaf from the upper third of each seedling was used to measure net CO₂ assimilation (A_n; μmol CO₂ m⁻² s⁻¹), stomatal conductance (g_s; mmol H₂O m⁻² s⁻¹), and transpiration rate (E; mmol H₂O m⁻² s⁻¹). Intrinsic water use efficiency (iWUE; μmol CO₂ mol⁻¹ H₂O) was calculated as A_n/g_s. For measurements, CO₂ concentration in the leaf cuvette was set to 400 ppm, while leaf temperature and photosynthetically active radiation (PAR) were maintained at 15 ± 1 °C and 1,000 μmol photons m⁻² s⁻¹, respective

Plant Performance

Growing Conditions

In August 2023, we randomly selected 128 plants per species (16 plants × 4 P fertilization × 2 RP) for the RGP experiment and 192 plants per species (24 plants × 4 P fertilization × 2 RP) for the water-restriction experiment. For the RGP evaluation, all white (non-suberized) roots were removed prior to transplanting. For both experiments, plants were transplanted into large containers (5.7 L; 60 cm length, 5 cm width) filled with the same *P. radiata* bark compost described above. Plants were placed outdoors, irrigated initially to container capacity, and subsequently irrigated once per week to container capacity for 100 days (period selected to allow sufficient expression of new root growth under non-limiting conditions). Volumetric water content (VWC) was monitored following Alvarez-

Maldini et al. (2020). During this period, mean air temperature was 11.0 °C, maximum temperature 21.0 °C, maximum PAR 1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and mean relative humidity 75%.

RGP

After 100 days, plants were removed from containers and their root systems were carefully washed to assess RGP traits. We quantified the number of white (non-suberized) new roots and measured the average length of the three longest new roots (cm; this average hereafter simply “longest new root”), following Acevedo et al. (2021). Biomass of new roots (g) was determined after drying 24 hrs at 105 °C in a forced ventilation oven.

Water Restriction

After 100 days, *A. chilensis* and *Q. saponaria* plants were placed under greenhouse conditions and randomly divided into two irrigation treatments: well-watered plants (WW), which were subsequently maintained with the same once-per-week irrigation to container capacity; and a water restriction (WR) treatment. For WR, plants were irrigated to container capacity on day 101, and then irrigation was fully stopped to allow a sustained decrease in substrate water content. Substrate volumetric water content (VWC) was monitored with moisture sensors (ECH2O EC-5, Decagon, Pullman, WA, USA) that were calibrated with gravimetric mass to estimate the percentage of available water (Dumroese et al., 2015). Each time available water in the WR treatment decreased by 10 percentage points, we measured leaf water potential (Ψ_L) and stomatal conductance (gs). We

measured WW plants every other time that we measured WR plants. Once available water reached approximately 10%, we continued taking measurements at similar time intervals even though available water no longer decreased, until no leaves remained suitable for measurement. Consequently, Ψ_L and g_s were measured 11 and 10 times along the experiment in *A. chilensis* and *Q. saponaria*, respectively. On measurement days, pre-dawn (Ψ_{pd} ; MPa; between 05:00 and 07:00 hrs) and midday minimum (Ψ_{min} ; MPa; between 13:00 and 14:00 hrs) water potentials were assessed on 3 randomly selected leaves using a pressure chamber (Scholander 1505D-EXP, PMS Instruments, Albany, OR, USA). Stomatal conductance (g_s ; $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) was measured with a steady state porometer (model SC-1, Meter Group, Pullman, WA, USA). Thus, on WR sampling days we measured 24 plants per species (3 plants \times 4 P fertilization \times 2 RP) whereas we measured 48 on days when we measured WR and WW treatments (3 plants \times 4 P fertilization \times 2 RP \times 2 irrigation treatments). During the water-restriction experiment, mean air temperature was 19.2 °C, maximum temperature of 32 °C, maximum PAR of 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and mean relative humidity of 72%.

Data analysis

Gas exchange parameters (i.e., A_n , g_s , E and iWUE), and RGP data (number, longest, and biomass of new roots) were analyzed using a two-way ANOVA with the PROC GLIMMIX procedure (SAS Institute Inc., Cary, NC, USA) taking into consideration the distribution selection based on the lower Akaike information criteria (AIC). Significant

differences between means were analyzed with the Tukey (HSD) test for multiple comparisons at 95% confidence.

Hydroscape areas, which describe the range of Ψ_{pd} and Ψ_{min} values compatible with gas exchange, were calculated following Meinzer et al. (2016) with the modifications described by Johnson et al. (2018). The lower boundary of the hydroscape was defined using a convex hull constructed from the average Ψ_{pd} and Ψ_{min} values for each measurement time. Unlike previous implementations, we did not extrapolate these limits to the point where $\Psi_{pd} = \Psi_{min}$ (i.e., their intersection on the 1:1 line), because this equality was never observed in our dataset. The relationship between gs and Ψ_{min} was modeled using the PROC NLIN procedure (SAS Institute Inc.) using the Gauss-Newton method through an algorithm without derivatives and a logistic model was adjusted. The effects of P fertilization and RP were assessed using the squared sum method (Bergerud, 1996). The water potential at the loss of 12%, 50%, and 90% of gs (P_{gs12} , P_{gs50} , and P_{gs90} , respectively) was calculated according to Li et al. (2019) using the fitplc package (R statistical software, version 4.4.2).

Independently for *A. chilensis* and *Q. saponaria*, we performed a relative importance analysis for the new root number variable (RGP) and the P_{gs50} variable (WR phase). To identify which nursery traits measured in our previous study (Calderón-Ureña et al., 2025) best explained variation in these two response variables generated in the present experiment, we used a Random Forest model following Acevedo et al. (2024). The Random Forest algorithm assessed predictors with the highest relative contribution for

explaining new root number and P_{gs50} , quantified as mean square error. All predictor variables (nursery traits) and response variables (new root number and P_{gs50}) were normalized by standardizing values between zero and one. The analyses were performed using the Random Forest library (Liaw and Wiener, 2002) and VIP library (Greenwell and Boehmke, 2020) (R software; R Core Team, 2023). All visualizations were made with graphing software (SigmaPlot 14.0, Systat Software Inc., San José, CA, USA).

Results

Gas exchange

In *A. chilensis*, the interactions between P fertilization and RP were not significant for any gas exchange parameter. P fertilization was significant for An, E, and iWUE. The lowest values for An and iWUE were observed in the 0P treatment. The lowest E was observed in the 0P and 60P treatments followed by the 15P and 120P treatments. RP was significant for An and E, with WoCu yielding increases of 13.5% and 9.2%, respectively, compared with WCu (Table 1).

For *Q. saponaria*, no interaction between P fertilization and RP was observed for any gas exchange parameter. P fertilization was significant for An and iWUE (Table 1), with higher values in 60P and 120P compared to 0P. The RP treatment was not significant for any gas exchange parameter.

Table 1. Mean values (\pm standard error) of gas exchange parameters and p-values for *Aristotelia chilensis* and *Quillaja saponaria* in relation to phosphorus (P) fertilization treatments (0, 15, 60, and 120 mg L⁻¹), chemical root pruning treatments (RP; without, WoCu; with, WCu), and their interaction. Net photosynthesis (An), stomatal conductance (gs), transpiration (E), and intrinsic water use efficiency (iWUE). Different letters, according to Tukey (HSD), differ significantly ($p \leq 0.05$).

	<i>A. chilensis</i>				<i>Q. saponaria</i>			
	An ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	gs ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	iWUE ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)	An ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	gs ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	iWUE ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)
Phosphorus (P)								
0	3.68 \pm 0.87 ^b	63.37 \pm 19.20	0.55 \pm 0.24 ^b	7.34 \pm 2.54 ^b	8.68 \pm 1.14 ^b	179.74 \pm 48.79	1.45 \pm 0.37	6.23 \pm 1.15 ^b
15	6.81 \pm 1.28 ^a	86.48 \pm 22.76	0.76 \pm 0.18 ^a	9.35 \pm 2.00 ^a	10.63 \pm 1.70 ^{ab}	173.56 \pm 42.82	1.43 \pm 0.34	7.88 \pm 2.19 ^{ab}
60	5.68 \pm 1.95 ^a	69.39 \pm 26.31	0.58 \pm 0.20 ^b	9.95 \pm 2.75 ^a	10.74 \pm 2.21 ^a	164.74 \pm 57.54	1.39 \pm 0.46	8.07 \pm 1.52 ^a
120	6.24 \pm 0.10 ^a	68.44 \pm 12.51	0.61 \pm 0.10 ^{ab}	10.36 \pm 1.05 ^a	11.57 \pm 2.50 ^a	172.60 \pm 28.63	1.37 \pm 0.25	8.40 \pm 0.84 ^a
Root Pruning (RP)								
WoCu	6.01 \pm 1.64 ^a	74.72 \pm 21.01	0.65 \pm 0.18 ^a	9.34 \pm 1.97	9.94 \pm 2.33	175.08 \pm 51.38	1.43 \pm 0.42	7.26 \pm 1.54
WCu	5.20 \pm 1.82 ^b	69.12 \pm 23.03	0.59 \pm 0.22 ^b	9.16 \pm 2.84	10.86 \pm 1.96	170.24 \pm 37.39	1.39 \pm 0.29	8.03 \pm 1.78
Source of variation								
P	< 0.0001	0.0572	0.0004	0.0009	0.0050	0.7795	0.9483	0.0034
RP	0.0279	0.2552	0.0333	0.9820	0.0723	0.8910	0.9697	0.0953
P x RP	0.0983	0.0753	0.1505	0.6799	0.2547	0.1872	0.1337	0.1684

Root Growth Potential (RGP)

In *A. chilensis*, the interaction of P fertilization and RP treatments was not significant for new root number or longest new root. P fertilization was significant for both variables.

The highest number of new roots was recorded in the 15P treatment, while the lowest was observed in the 0P treatment. In contrast, the longest new roots were observed in the 0P treatment, whereas the 60P treatment exhibited the shortest maximum new root length. For new root biomass, P fertilization was significant; the 0P value was lower than all the P concentrations > 0 , which were not statistically different (Table 2). Regarding the RP treatment, WCu plants had a lower number of new roots and less root biomass than WoCu plants (Table 2).

For all RGP traits in *Q. saponaria*, the interaction between P fertilization and RP was not significant, but P fertilization was significant. Treatments with $P > 0$ had higher number and more biomass of new roots than the 0P treatment. The longest new roots were observed in 60P and 120P, while the shortest lengths were observed in 15P and 0P treatments. The RP treatment was not significant for any RGP parameter (Table 2).

Table 2. Mean values (\pm SE) and p -values of the number, length (cm), and dry weight (g) of new roots in the root growth potential experiment for *Aristotelia chilensis* and *Quillaja saponaria* in relation in relation to phosphorus (P) fertilization treatments (0, 15, 60, and 120 mg L⁻¹), chemical root pruning treatments (RP; without, WoCu; with, WCu), and their interaction. Different letters, according to Tukey (HSD), differ significantly ($p \leq 0.05$).

	<i>A. chilensis</i>			<i>Q. saponaria</i>		
	New roots (N°)	Length (cm)	Biomass (g)	New roots (N°)	Length (cm)	Biomass (g)
Phosphorus (P)						
0	708 \pm 69 ^b	44.5 \pm 1.1 ^a	4.29 \pm 0.22 ^b	53 \pm 70 ^b	43.3 \pm 2.2 ^b	0.61 \pm 0.09 ^b
15	928 \pm 88 ^a	42.5 \pm 1.1 ^{ab}	6.99 \pm 0.42 ^a	122 \pm 18 ^a	46.8 \pm 1.6 ^b	2.06 \pm 0.29 ^a
60	783 \pm 68 ^{ab}	40.2 \pm 1.5 ^b	6.82 \pm 0.47 ^a	126 \pm 17 ^a	55.2 \pm 1.5 ^a	2.63 \pm 0.30 ^a
120	815 \pm 69 ^{ab}	44.0 \pm 1.0 ^{ab}	7.60 \pm 0.42 ^a	114 \pm 12 ^a	58.4 \pm 2.3 ^a	3.28 \pm 0.45 ^a
Root Pruning (RP)						
WoCu	1006 \pm 34 ^a	43.91 \pm 0.78	7.21 \pm 0.36 ^a	102 \pm 13	52.5 \pm 2.0	2.30 \pm 0.31
WCu	606 \pm 30 ^b	41.72 \pm 0.94	5.65 \pm 0.32 ^b	105 \pm 10	49.4 \pm 1.6	1.99 \pm 0.28
Source of variation						
P	0.0043	0.0406	<0.0001	0.0002	<0.0001	<0.0001
RP	<0.0001	0.0567	<0.0001	0.3599	0.1725	0.1676
P x RP	0.8983	0.4275	0.6567	0.6279	0.4936	0.7659

Water restriction

For both species, the relationship between Ψ_{pd} and Ψ_{min} was represented with hydroscares for different P fertilization and RP treatments. In *A. chilensis*, the hydroscape area (HA) values decreased with increasing P fertilization up to 60P (i.e., 3.23, 2.61, and 1.90 MPa² for 0P, 15P, and 60P, respectively) before increasing slightly to 2.14 MPa² in the 120P

treatment (Fig. 1). The HA for WCu (2.82 MPa²) was smaller than that of WoCu (3.45 MPa²) (Fig. S1).

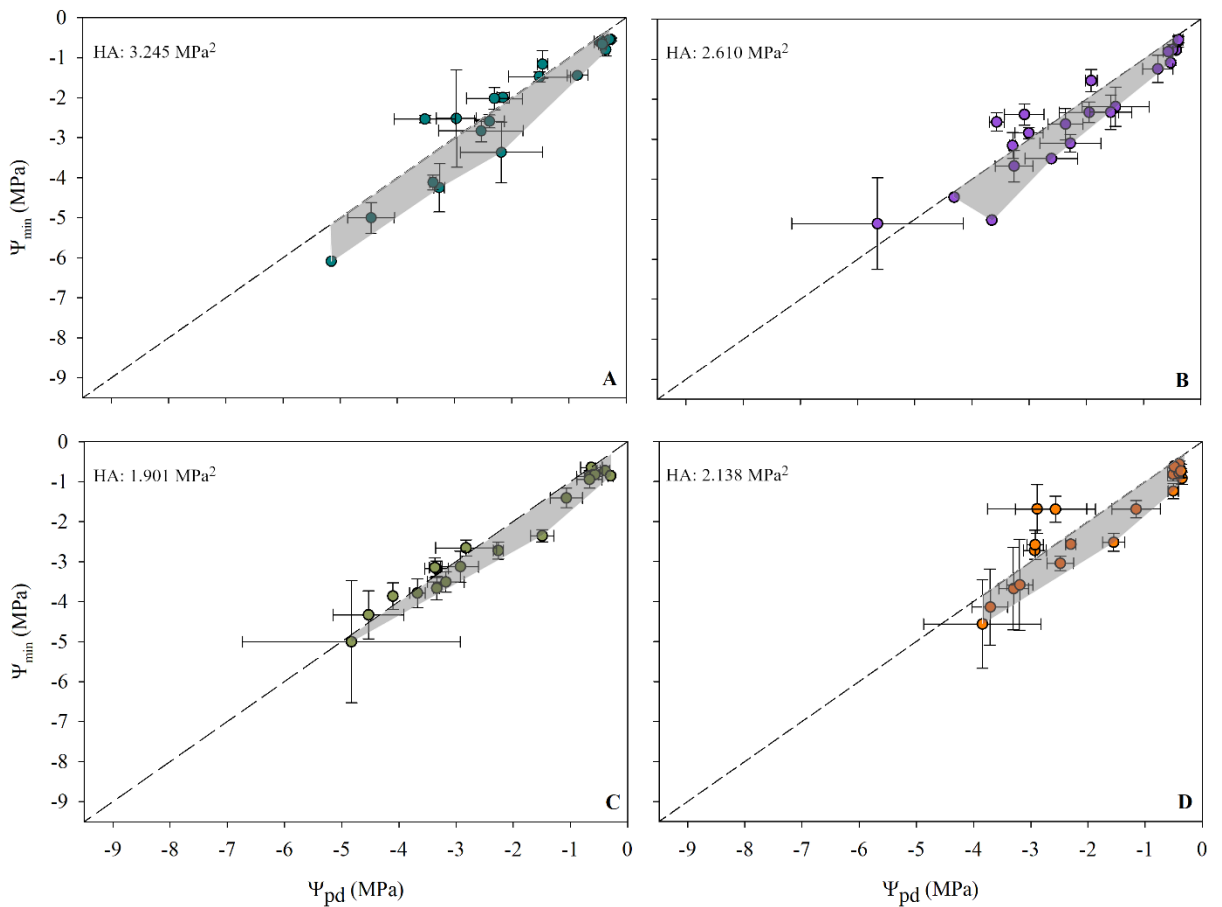


Figure 1. Relationship between predawn water potential (Ψ_{pd}) and minimum water potential (Ψ_{min}) during the water restriction experiment in *Aristotelia chilensis* in relation to the application of phosphorus (P) fertilization treatments (0, **A**; 15, **B**; 60, **C**; and 120,

D; mg L^{-1}). Symbols represent mean Ψ_{pd} and Ψ_{min} (\pm SE) for each measurement time, and the shaded gray area indicates the hydroscape area (HA). The gray dashed line represents the 1:1 relationship between Ψ_{pd} and Ψ_{min} .

In *Q. saponaria*, the HA showed a tendency to decrease with increasing P (i.e., 5.41, 3.19, 3.69, and 2.97 MPa^2 for 0P, 15P, 60P, and 120P, respectively) (Fig. S2). Regarding RP, the WCu treatment produced higher HA (6.62 MPa^2) than WoCu (4.64 MPa^2) (Fig. 2).

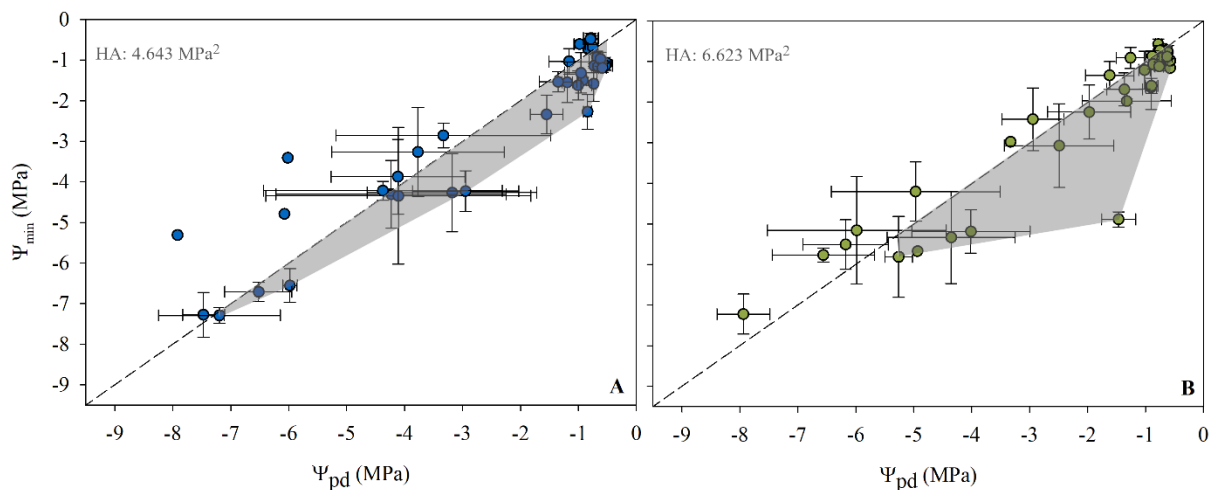


Figure 2. Relationship between predawn water potential (Ψ_{pd}) and minimum water potential (Ψ_{min}) during the water restriction experiment in *Quillaja saponaria* in relation to root chemical pruning treatments (RP; without: WoCu, **A**; with: WCu, **B**). Symbols mean Ψ_{pd} and Ψ_{min} (\pm SE) error for each measurement time, and the shaded gray area

indicates the hydroscape area (HA). The gray dashed line represents the 1:1 relationship between Ψ_{pd} and Ψ_{min} .

With water restriction, a nonlinear relationship between Ψ_{min} and gs was observed for both species (Fig. 3). In *A. chilensis*, P fertilization significantly affected the Ψ_{min} vs. gs relationship, with the 120P treatment exhibiting the lowest maximum gs (476.1 mmol H₂O m⁻² s⁻¹) compared to 0P (675.0 mmol H₂O m⁻² s⁻¹), 15P (698.0 mmol H₂O m⁻² s⁻¹), and 60P (691.6 mmol H₂O m⁻² s⁻¹) (Fig. 3a). The RP treatment was not significant for the Ψ_{min} vs. gs relationship.

For *Q. saponaria*, P fertilization was not significant for the Ψ_{min} vs. gs relationship. In contrast, model comparison indicated that RP was significant with the WCu treatment showing a lower steepness parameter ($b_2 = 0.501$ MPa⁻¹) compared to WoCu ($b_2 = 0.671$ MPa⁻¹) (Fig. 3b).

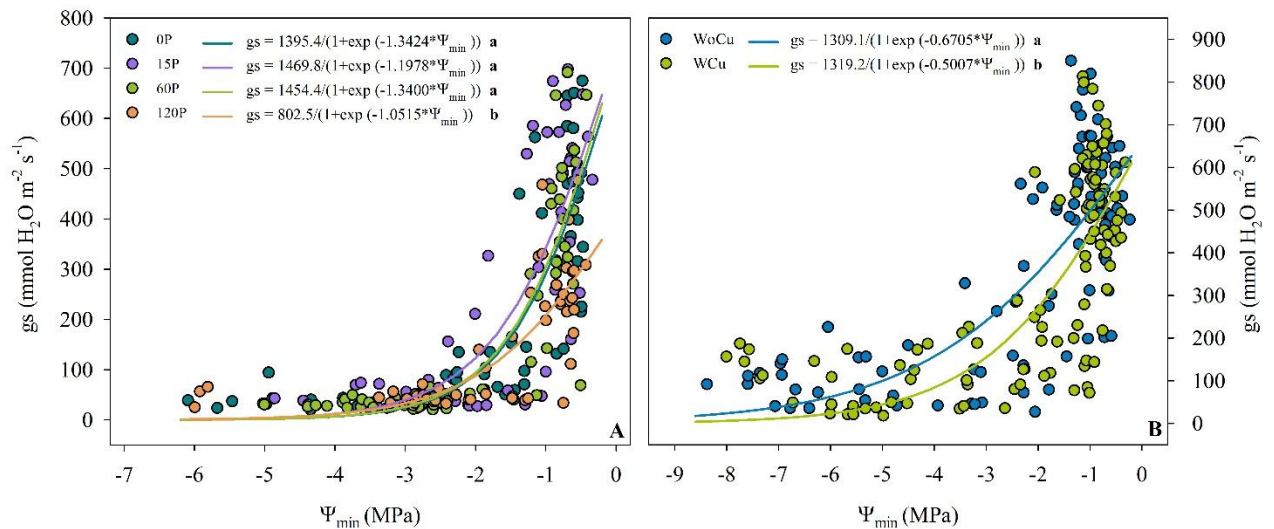


Figure 3. Relationship between minimum water potential (Ψ_{\min}) and stomatal conductance (gs) during the water restriction experiment in *Aristotelia chilensis* in relation to the application of phosphorus (P) fertilization treatments (0, 15, 60, and 120 mg L⁻¹) (A) and *Quillaja saponaria* in relation to chemical root pruning treatments (RP; without, WoCu; with, WCu) (B). Symbols represent measured data, and lines correspond to the modeled data for each treatment. Different letters indicate significant differences between models.

In *A. chilensis*, P_{gs12} and P_{gs50} values increased with P fertilization (Table 3). P_{gs12} shifted from -0.35 MPa in the 0P treatment to -0.07 MPa in the 120P treatment. Similarly, P_{gs90} moved toward less negative values as P fertilization increased. Regarding root pruning, P_{gs12} values were comparable between treatments, whereas P_{gs50} and P_{gs90} were slightly more negative in WCu plants (Table 3).

In *Q. saponaria*, the patterns of water potential at loss of stomatal conductance were less consistent than in *A. chilensis*. Plants in the 0P and 60P treatments exhibited similar P_{gs12} , P_{gs50} , and P_{gs90} values at higher Ψ_F , whereas plants in the 15P and 120P treatments exhibited similar values at lower Ψ_F (Table 3). With respect to root pruning, *Q. saponaria* showed the same pattern observed in *A. chilensis* (Table 3).

Table 3. Leaf water potential at 12% (P_{gs12}), 50% (P_{gs50}), and 90% (P_{gs90}) loss of stomatal conductance (mean, CI in brackets) during the water restriction experiment in *Aristotelia chilensis* and *Quillaja saponaria* in relation to phosphorus (P) fertilization treatments (0, 15, 60, and 120 mg L⁻¹) and chemical root pruning (without, WoCu and with, WCu).

	<i>A. chilensis</i>			<i>Q. saponaria</i>		
	P_{gs12} (MPa)	P_{gs50} (MPa)	P_{gs90} (MPa)	P_{gs12} (MPa)	P_{gs50} (MPa)	P_{gs90} (MPa)
Phosphorus (P)						
0P	-0.35 [na ; 0.56]	-1.02 [0.85 ; 1.20]	-2.17 [1.63 ; 2.72]	-0.12 [na ; na]	-1.16 [0.88 ; 1.50]	-5.75 [4.01 ; na]
15P	-0.29 [na ; 0.52]	-1.02 [0.80 ; 1.22]	-2.44 [1.73 ; 2.85]	-0.34 [na ; 0.56]	-1.63 [1.34 ; 1.94]	-4.97 [3.49 ; 7.33]
60P	-0.22 [na ; na]	-0.78 [0.63 ; 0.88]	-1.92 [1.27 ; 2.41]	-0.09 [na ; na]	-1.26 [0.89 ; 1.66]	-7.96 [5.65 ; na]
120P	-0.07 [na ; na]	-0.52 [na ; 0.62]	-2.12 [1.79 ; 2.68]	-0.46 [na ; 0.86]	-1.85 [1.42 ; 2.25]	-4.99 [2.51 ; 6.94]
Root Pruning (RP)						
WoCu	-0.18 [na ; 0.27]	-0.83 [0.70 ; 0.95]	-2.41 [2.00 ; 2.77]	-0.14 [na ; 0.244]	-1.33 [1.10 ; 1.59]	-6.68 [4.95 ; na]
WCu	-0.15 [na ; na]	-0.70 [1.60 ; 0.80]	-2.09 [1.68 ; 2.41]	-0.10 [na ; na]	-1.00 [0.82 ; 1.18]	-5.22 [4.14 ; 6.50]

Relative importance analysis

Our analysis revealed that the main predictors of the number of new *A. chilensis* roots initiated during the RGP experiment were, by percentage, root pruning (17.9), fine root volume (9.2), total root volume (8.6), coarse root volume (7.3), N content (5.2), root biomass (5.1) and RCD (4.8) (Fig. 4a). In *Q. saponaria*, the main predictors of new roots were root volume (8.2), root diameter (7.2), RCD (6.6), iWUE (6.3) stem length (5.9), medium root volume (5.7), and N content (5.7) (Fig. 4b).

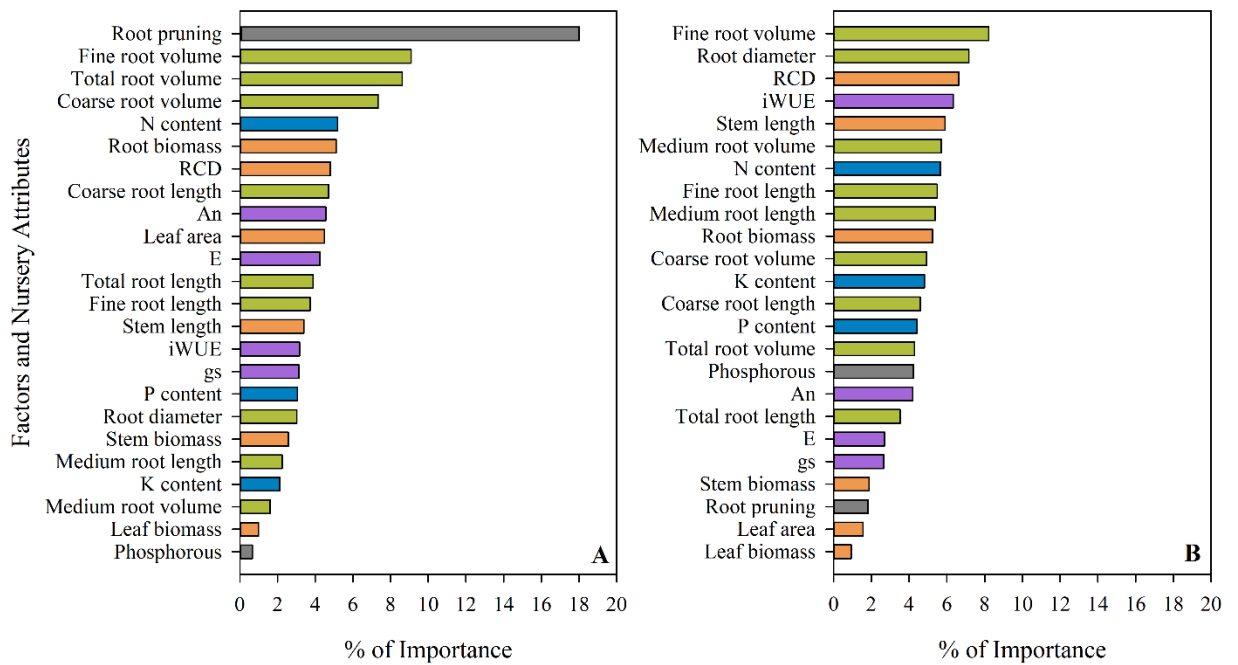


Figure 4. Relative importance analysis (%) of the factors and attributes evaluated during the nursery stage on the number of new roots in *Aristotelia chilensis* (A) and *Quillaja saponaria* (B). Bar color represents factor categories: Gray = treatments; orange = morphological; green = root architecture; purple = gas exchange; blue = nutritional. RCD: root collar diameter, An: net CO₂ assimilation rate, E: transpiration rate, gs: stomatal conductance, iWUE: intrinsic water use efficiency.

When considering the influence of nursery morpho-physiological traits on the dynamics of stomatal closure, the factors or attributes that best explained P_{gs50} in *A. chilensis* were (by percentage) root pruning (12.9), RCD (9.0), net photosynthesis (An; 8.4), P

fertilization (8.3), and N, P, and K contents (~8.1) (Fig. 5a). In *Q. saponaria*, the factors were root pruning (13.9), coarse root length (11.0), total root volume (10.0), coarse root volume (9.6), fine root volume (9.2), iWUE (9.1), and root diameter (8.2) (Fig. 5b). Overall, in *Q. saponaria*, variables related to root architecture ranked high in importance whereas these same variables ranked low for *A. chilensis*.

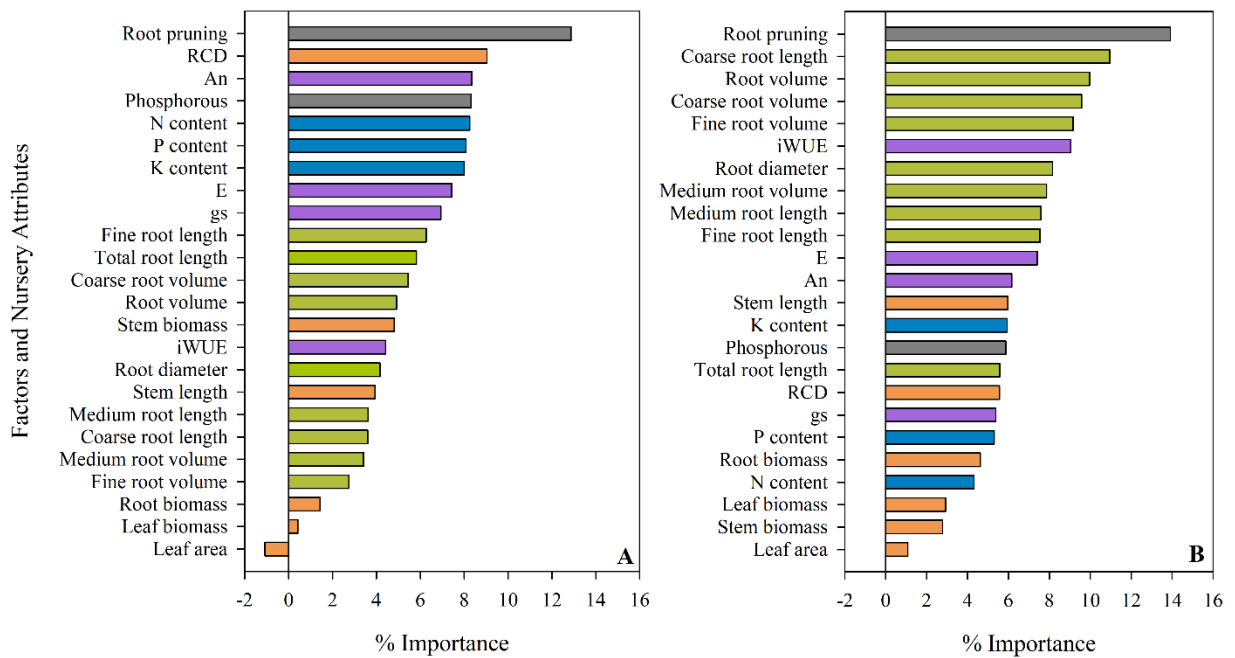


Figure 5. Relative importance analysis (%) of the factors and attributes evaluated during the nursery stage on the P_{gs50} during the water restriction experiment in *Aristotelia chilensis* (A) and *Quillaja saponaria* (B). Bar color represents factor categories: Gray = treatments; orange = morphological; green = root architecture; purple = gas exchange; blue = nutritional. RCD: root collar diameter, An: net CO₂ assimilation rate, E: transpiration rate, gs: stomatal conductance, iWUE: intrinsic water use efficiency.

Discussion

In line with the Target Plant Concept, our results show that P fertilization can be strategically used to produce seedlings with root and physiological traits better matched to water-limited environments, whereas chemical root pruning may compromise these attributes.

In our previous study, P fertilization improved seedling quality in both *A. chilensis* and *Q. saponaria* by increasing leaf and stem biomass, N and P content, and modifying roots, with *A. chilensis* showing greater length and volume of medium roots and *Q. saponaria* exhibiting increases in fine and medium root traits (Calderón-Ureña et al., 2025). These increases in shoot size, nutrient content, and root development are consistent with well-established evidence that larger, nutrient-rich seedlings have potential for better survival and field performance (Grossnickle & MacDonald, 2018; Villar-Salvador et al., 2012, 2026; Vukmirović et al., 2024).

In our current study, P fertilization enhanced key morpho-physiological attributes after both species were transplanted; photosynthetic capacity, water-transport efficiency, and root growth were increased, which collectively translated into improved performance under water stress. In contrast, RP reduced root growth, photosynthesis rates, and water-use efficiency, resulting in lower resistance to water stress.

In *A. chilensis* and *Q. saponaria*, P fertilization significantly increased An and iWUE, and in both species these improvements were driven by higher CO₂ assimilation rather than changes in g_s, which remained largely unaffected. This response aligns with

findings in *Phoebe zhennan*, where P fertilization enhanced photosynthesis and water-use efficiency through increases in leaf N (Tariq et al., 2017). Consistent with our previous results, P additions increased N and P content in both species (Calderón-Ureña et al., 2025), likely supporting the elevated An and iWUE observed here. Because P plays a central role in ATP synthesis and RuBP regeneration, its deficiency can limit photosynthetic capacity (Grant et al., 2001), as reflected in the 0P treatment where seedlings showed reduced biomass and growth during nursery production. These results agree with reports from mediterranean ecosystems showing that greater nutrient availability enhances water-use efficiency primarily through increased CO₂ assimilation (El-Madany et al., 2021).

Phosphorus fertilization markedly improved root development in both species, as shown in the RGP experiment where P-treated seedlings produced a greater number, length, and biomass of new roots. These responses are consistent with the increases in medium and coarse root length and volume observed at the end of nursery production (Calderón-Ureña et al., 2025). Interpreted within the root economic spectrum (RES) framework, this shift toward greater root elongation reflects a more acquisitive strategy (Roumet et al., 2016; Weemstra et al., 2016), facilitating enhanced soil exploration and nutrient uptake. Consequently, seedlings subjected to higher P fertilization exhibited increased N and P content (Calderón-Ureña et al., 2025), whereas 0P plants consistently showed reduced root development and physiological performance, indicating strong nutrient limitation during early growth. Such changes in root architecture are well known to improve plant performance under water scarcity, as a more developed root system

enhances both nutrient and water absorption (Holz et al., 2024; Villar-Salvador et al., 2012, 2026; Zhou et al., 2020).

Under water-restricted conditions, the two species exhibited contrasting stomatal responses to P fertilization. In *A. chilensis*, P fertilization promoted a more conservative stomatal behavior: P_{gs50} and P_{gs90} increased with higher P supply, indicating that stomatal closure occurred at less negative Ψ_L , consistent with a more isohydric strategy (Martínez-Vilalta & Garcia-Forner, 2017). This pattern was supported by the hydroscape area, which decreased progressively with increasing P, reflecting a narrower operational range in Ψ_L for maintaining stomatal opening. These responses likely stem from the stimulation of root growth associated with P fertilization, also evidenced by the greater number and biomass of new roots in the RGP experiment, which enhances water and nutrient uptake and helps maintain higher Ψ_L during drought (Grossnickle & Ivetić, 2022; Grossnickle & MacDonald, 2018). This may imply a trade-off between higher growth and a tighter regulation in the Ψ_L drop, favoring the protection of the hydraulic system over maximization of gas exchange during drought (Hochberg et al., 2018; Klein, 2014; Martínez-Vilalta et al., 2014). Such a trade-off, frequently documented in mediterranean species, supports the notion that improve growth potential is often associated with strict control of stomatal closure to minimize the risk of drought induced cavitation (Bartlett et al., 2012; Tardieu & Simonneau, 1998).

In contrast, *Q. saponaria* showed little variation in P_{gs50} and P_{gs90} across P fertilization levels, with overlapping confidence intervals among treatments.

Interpretation of P_{gs90} should be cautious, as the Ψ_{min} - g_s curve did not reach full stomatal closure in this species. However, a marked difference emerged between the 0P and 120P treatments: P_{gs50} was lower in 120P, indicating that stomatal closure occurred at more negative Ψ_L and reflecting a more anisohydric strategy. This behavior implies that P-fertilized maintain transpiration at lower Ψ_L , maximizing water uptake capacity. This response, which prioritizes carbon gain while accepting greater hydraulic risk, is commonly reported in drought-tolerant mediterranean species (Martínez-Vilalta et al., 2014; Vilagrosa et al., 2010).

Importantly, these results highlight that aboveground drought-response strategies are not necessarily mirrored by belowground traits. While P fertilization promoted more acquisitive root development in both species, its effects on stomatal regulation diverged, underscoring a partial decoupling between root economic strategies and aboveground water-use regulation (Kramer-Walter et al., 2016; Weemstra et al., 2016). This emphasizes the need to jointly consider root architecture and physiological traits when interpreting drought responses and predicting field performance, particularly in mediterranean environments where species with contrasting water-use strategies, such as those observed here, commonly coexist (Martínez-Vilalta et al., 2014).

With respect to chemical RP, in *A. chilensis*, copper application induced unfavorable morpho-physiological responses and reduced root growth. WCu seedlings showed significant declines in An and iWUE, along with fewer number and lower biomass of new roots in the RGP. These effects were likely driven by reduced volume of fine and

coarse roots observed at the end of nursery production (Calderón-Ureña et al., 2025), which likely limited nutrient and water uptake and contributed to poorer performance during water stress (Hodge et al., 2009; Sun et al., 2024). During water restriction, WCu plants exhibited higher P_{gs50} and P_{gs90} values and a smaller hydroscape area (Fig. S1), indicating earlier stomatal closure and a more isohydric response (Hochberg et al., 2018). Such patterns align with established effects of excess copper, oxidative stress and inhibition of root elongation (Yruela, 2009), and suggest that reduced formation of new roots constrained water absorption, triggering faster declines in g_s with decreasing Ψ_L .

In *Q. saponaria*, RP produced a different pattern. Despite reductions in the length and volume of fine and medium roots at the end of nursery production (Calderón-Ureña et al., 2025), copper application did not significantly affect gas exchange or RGP parameters. During water restriction, WCu seedlings showed higher P_{gs12} , P_{gs50} , and P_{gs90} values than WoCu plants, a pattern also reflected in the Ψ_{min} vs. g_s curves and in larger hydroscape areas. These results were, however, driven mainly by lower Ψ_{min} during early drought (i.e., higher Ψ_{pd}), rather than by a fundamentally looser stomatal regulation. Therefore, this does not translate into a less stringent stomatal regulation compared to the WoCu control plants.

When considered alongside the nursery results—where chemical RP reduced root length and volume across all diameter classes—and the modest RGP performance, our findings suggest that this practice may limit early drought resistance under the conditions evaluated here. Nonetheless, these implications should not be generalized. Substantial

evidence shows that chemical RP can be effective in nursery systems where root plug stability, root containment, or shoot–root balance are primary objectives (Krasowski, 2003; Montagnoli et al., 2022; South et al., 2005). Accordingly, the performance of chemically pruned seedlings depends strongly on species traits, copper formulation and dose, and the target planting environment. In drought-prone planting sites, maintaining an intact root system may instead favor a larger proportion of active roots capable of sustaining water uptake as Ψ_L declines (Grossnickle, 2005).

The relative importance analysis revealed that, in *A. chilensis*, chemical RP and the volume of the different root diameter classes as the most influential variables for the number of new roots. For P_{gs50} , RP, RCD, and An ranked the highest in importance. These results indicate that performance during water deficit is driven by the interaction between morphological and physiological traits, with RP emerging as one of the main factors shaping responses in both performance traits. The RP effect, however, appears to induce changes associated with reduced performance during drought. For example, during nursery production, RP decreased the length and volume of fine and medium roots (Calderón-Ureña et al., 2025), likely limiting water and nutrient uptake and constraining stomatal regulation and the water use efficiency. Consequently, RP may increase plant vulnerability during water deficit.

In *Q. saponaria*, the variables most strongly influencing the number of new roots and P_{gs50} were predominantly root-related traits, in contrast to *A. chilensis*, where physiological traits were more influential. This difference underscores the importance of

assessing root architecture traits, given their central role in determining exploration capacity, water uptake, and overall drought performance. These findings also highlight that the traits best predicting plant performance are species-specific and reflect the species' adaptive strategies. In *A. chilensis*, the predominance of physiological control and tighter stomatal regulation during drought is consistent with a more conservative root development pattern, as predicted by the RES framework. Conversely, *Q. saponaria* maintained stomatal conductance at lower Ψ_L , displaying a more acquisitive strategy aligned with the acquisitive root development described in the RES framework.

Conclusions

Phosphorus fertilization during nursery production enhanced physiological performance in *A. chilensis* and *Q. saponaria*, increasing CO₂ assimilation and intrinsic water-use efficiency, and promoting greater root growth capacity after transplanting. These attributes positioned P-fertilized seedlings to more fully exploit their substrate and, upon water restriction, avoid the effects of drought better than non-P fertilized seedlings. We suggest that these P-fertilizer improvements to seedling quality may improve drought avoidance of outplanted seedlings experiencing the summer dry period typical of mediterranean climates. Although the two species exhibited contrasting drought-response strategies, *A. chilensis* expressing a more isohydric behavior and *Q. saponaria* a more anisohydric response, the same nursery P-fertilization practices consistently improved

morpho-physiological performance in both species, indicating a broadly applicable management approach despite species-specific functional differences.

Chemical root pruning, by contrast, tended to constrain drought performance under the conditions evaluated here. In both species, this practice was associated with more stringent stomatal regulation and reduced capacity to sustain gas exchange at lower Ψ_L , in parallel with reductions in RGP traits related to soil exploration and water uptake. Although the relative importance analysis identified RP as a key factor influencing performance metrics such as root growth potential and P_{gs50} , seedlings grown without this treatment developed morpho-physiological and behavioral traits more closely associated with drought resistance. Nonetheless, these findings should not be interpreted as a general rejection of RP. Its effectiveness is context-dependent, depending on species traits and nursery objectives. For drought-prone mediterranean planting environments, however, maintaining root system integrity appears more beneficial for early establishment and water acquisition during declining Ψ_L .

These findings reinforce the need to design nursery protocols based on functional targets linked to post-planting performance, thereby improving the effectiveness and resilience of restoration efforts under a changing climate.

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Supplementary Data

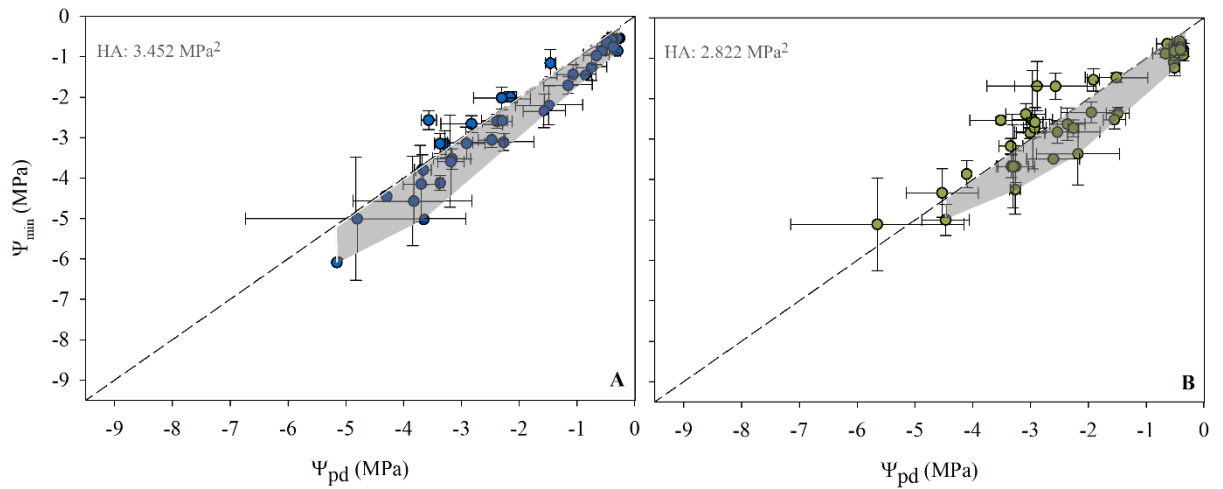


Figure S1. Relationship between predawn water potential (Ψ_{pd}) and minimum water potential (Ψ_{\min}) during the water restriction experiment in *Aristotelia chilensis* in relation to root chemical pruning treatments (without: WoCu, **A**; with: WCu, **B**). Symbols mean Ψ_{pd} and Ψ_{\min} (\pm SE) error for each measurement time, and the shaded gray area indicates the hydroscopical area (HA). The gray dashed line represents the 1:1 relationship between Ψ_{pd} and Ψ_{\min} .

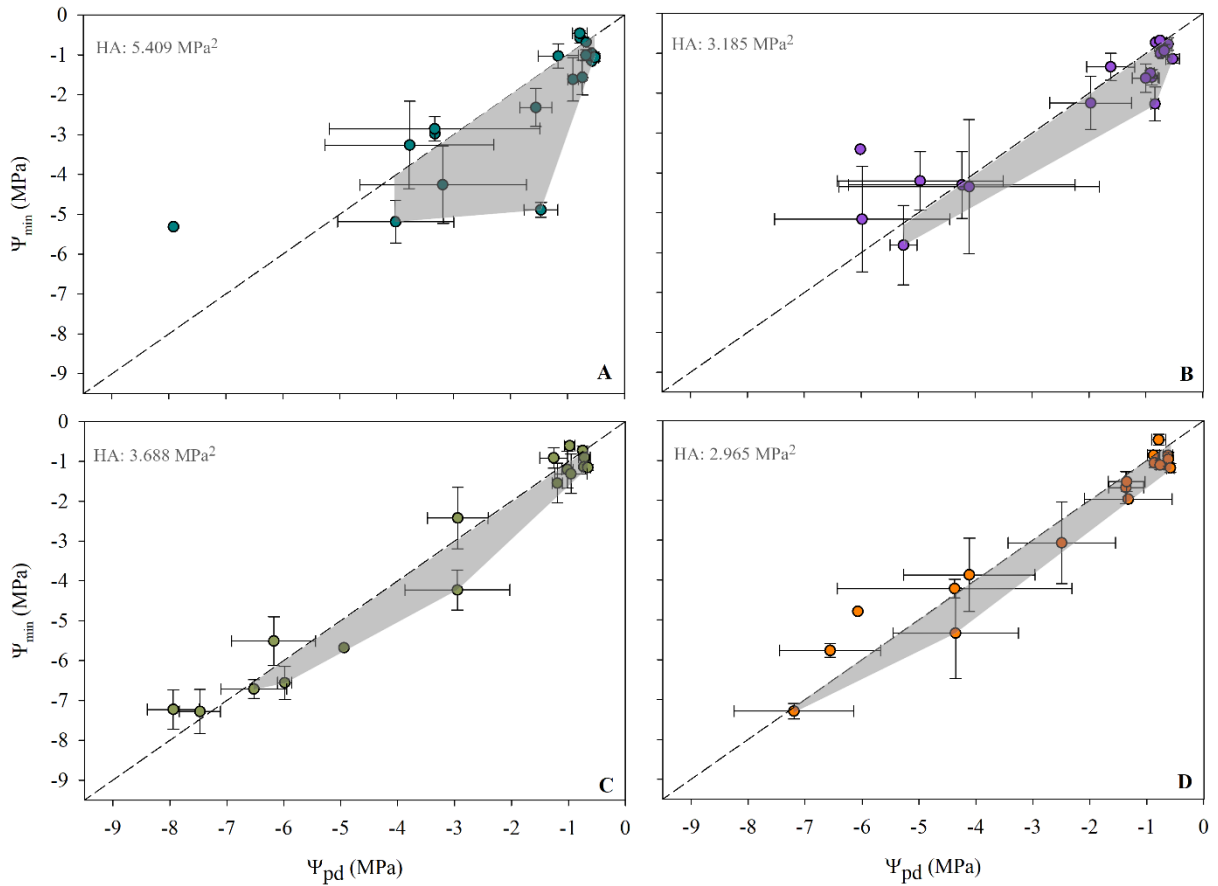


Figure S2. Relationship between predawn water potential (Ψ_{pd}) and minimum water potential (Ψ_{min}) during the water restriction experiment in *Quillaja saponaria* in relation to the application of phosphorus fertilization treatments (0P, **A**; 15P, **B**; 60P, **C**; and 120P, **D**). Symbols represent mean Ψ_{pd} and Ψ_{min} (\pm SE) for each measurement time, and the shaded gray area indicates the hydroscapse area (HA). The gray dashed line represents the 1:1 relationship between Ψ_{pd} and Ψ_{min} .

DISCUSIÓN GENERAL

Los ecosistemas mediterráneos de Chile se caracterizan por una marcada estacionalidad en la disponibilidad hídrica, con veranos prolongados y secos que se han intensificado durante las últimas décadas debido al cambio climático (Boisier et al., 2016; Garreaud et al., 2017). En este escenario, la producción de plantas de alta calidad, capaces de establecerse y sobrevivir bajo condiciones restrictivas, se vuelve un desafío central para la restauración (Padilla & Pugnaire, 2007; Valladares et al., 2007). Comprender cómo los rasgos morfológicos y fisiológicos interactúan para aumentar la resistencia al déficit hídrico resulta fundamental para optimizar tanto la viverización como el establecimiento en campo.

Una de las contribuciones más relevantes de esta investigación fue la inclusión explícita de la arquitectura radical como variable central de evaluación en vivero. Tradicionalmente, las mediciones se concentran en los atributos aéreos de la planta por ser más accesible, rápida y no destructiva, mientras que el sistema radical suele quedar relegado debido al esfuerzo técnico que implica su extracción, lavado y análisis (Poorter et al., 2012; Sutton, 1980). Sin embargo, las raíces constituyen la base estructural y fisiológica para la adquisición de agua y nutrientes; siendo determinantes en el establecimiento y la resistencia al déficit hídrico (Grossnickle, 2005, 2012). Por ello, comprender su distribución espacial, su biomasa y atributos como la longitud y el volumen por clases diamétricas resulta fundamental para evaluar adecuadamente los efectos de los

manejos aplicados y orientar la viverización hacia la producción de plantas de la más alta calidad.

Se evidenció que la fertilización fosfórica promovió aumentos progresivos a medida que se incrementó la concentración de P aplicada en los atributos morfológicos (longitud de tallo, DAC, área foliar y biomasa de hojas, tallo y raíz) y nutricionales (contenido de N, P y K) en *A. chilensis* y *Q. saponaria*, además de generar cambios significativos en la arquitectura radical, expresados en incrementos de longitud, diámetro, volumen y área superficial total en *Q. saponaria*, y en longitud y volumen de las diferentes clases diamétricas en ambas especies. Estos efectos son coherentes con el rol central del fósforo en procesos como la síntesis de ATP, el intercambio energético y la formación de biomasa, así como con su influencia en la división celular y la elongación de raíces (Lynch, 2011, 2019; Veneklaas et al., 2012). En particular, los cambios observados en la arquitectura radical sugieren una mayor expansión y complejidad del sistema radical en ambas especies, lo que potencialmente se traduce en una mayor capacidad de exploración del sustrato bajo condiciones de restricción hídrica, tal como se ha reportado en otras especies arbóreas.

Por el contrario, la aplicación de poda química de raíces generó respuestas opuestas a las esperadas. Si bien se observó un aumento del área foliar y de la biomasa aérea en *Q. saponaria*, esta práctica redujo la biomasa radical en *A. chilensis* y disminuyó la longitud y volumen en las raíces finas y medianas en ambas especies, modificando además la relación R:S. Aunque la poda química busca inducir sistemas radicales más

fibrosos y prevenir el enrollamiento de raíces (Ruehle, 1985; Wenny & Woollen, 1989), los resultados sugieren que los cambios inducidos durante la viverización no se tradujeron en una arquitectura funcionalmente ventajosa. En particular, la redistribución del crecimiento radical promovida por esta práctica no habría compensado la reducción en la capacidad exploratoria del sistema radical, considerando que un mayor grado de ramificación no necesariamente implica una exploración eficiente del volumen de suelo ni una mayor conductancia hidráulica a escala de planta (Lynch, 1995), y podría haber generado un desbalance entre la inversión aérea y radical.

En este contexto, la respuesta diferencial entre especies refleja contrastes en plasticidad y estrategias de asignación de biomasa. *Q. saponaria* mostró una mayor capacidad de compensación frente a la reducción del sistema radical, promoviendo el crecimiento aéreo, lo que es consistente con una estrategia más adquisitiva orientada a maximizar la captura de recursos (Reich, 2014; Wright et al., 2004). Por el contrario, *A. chilensis*, al reducir su biomasa radical sin inducir respuestas compensatorias en la parte aérea, evidencia una estrategia más conservadora, caracterizada por una menor plasticidad y una asignación más restrictiva de recursos (Poorter et al., 2012; Reich, 2014).

Bajo condiciones mediterráneas, donde la disponibilidad hídrica es marcadamente estacional, este tipo de desacople puede limitar la absorción de agua y nutrientes. No obstante, siguiendo TPC, estos efectos deben interpretarse siempre en función del desempeño en campo o evaluado bajo condiciones que representen el ambiente real de establecimiento (Duryea & Landis, 1984; Grossnickle, 2012). De este modo, la hipótesis

del primer capítulo se cumple parcialmente: mientras la fertilización fosfórica generó efectos positivos consistentes en atributos morfológicos y nutricionales, la poda química redujo atributos morfológicos clave, particularmente relacionados a su arquitectura radical, en ambas especies.

El segundo capítulo evaluó si las diferencias morfológicas y nutricionales generadas por la fertilización fosfórica y la poda química se traducían en un mejor desempeño bajo estrés, integrando el PCR y la respuesta al déficit hídrico. En esta fase, las mayores concentraciones de P incrementaron la An y la iWUE al final de la viverización, sin diferencias significativas en gs. Este efecto sugiere que la respuesta estuvo asociada a una mayor capacidad de asimilación de CO₂, más que a modificaciones en la apertura estomática (He & Dijkstra, 2014; Veneklaas et al., 2012). Este patrón indica una mayor capacidad fotosintética previa al estrés, lo que potencialmente podría contribuir a un mejor desempeño frente a eventos de déficit hídrico. Asimismo, luego del trasplante, el P incrementó de manera marcada los atributos de PCR en comparación con el tratamiento 0P en ambas especies (número, longitud y biomasa de raíces nuevas), rasgos que están en plena concordancia con la arquitectura radical generada durante la viverización y que están estrechamente vinculados al establecimiento y la supervivencia bajo estrés (Grossnickle, 2012; Villar-Salvador et al., 2012). Dado que el PCR refleja la capacidad de las plantas para producir raíces tras el trasplante, su incremento bajo fertilización fosfórica sugiere una mayor capacidad de acceso temprano al agua del suelo, un rasgo crítico para reducir el riesgo de fallas hidráulicas durante los primeros episodios de sequía.

Frente al déficit hídrico, las respuestas divergieron entre especies. *A. chilensis* mostró una estrategia conservadora o isohídrica, reduciendo la g_s a potenciales hídricos foliares (Ψ_f) relativamente altos para limitar la pérdida de agua (Martínez-Vilalta et al., 2014; Tardieu & Simonneau, 1998). En esta estrategia, las plantas utilizan los recursos lentamente, mantienen tasas fotosintéticas moderadas e invierten en tejidos de mayor longevidad, priorizando la supervivencia frente a episodios de sequía (Reich, 2014). En contraste, *Q. saponaria* mostró una estrategia más adquisitiva o anisohídrica, manteniendo la actividad fotosintética a Ψ_f más negativos, un patrón que puede asociarse a especies que exploran intensivamente el suelo mediante raíces finas y de rápido recambio, maximizando la captura de carbono cuando el agua está disponible (Comas et al., 2013; Reich, 2014). Estas diferencias sugieren que *Q. saponaria* puede sostener la asimilación de carbono bajo condiciones hídricas más restrictivas, mientras que *A. chilensis* prioriza la seguridad hídrica, alineándose con el gradiente seguridad–eficiencia descrita para especies leñosas en climas mediterráneos (Klein, 2014). En conjunto, estas estrategias permiten interpretar con mayor precisión la respuesta de ambas especies a la fertilización fosfórica frente al déficit hídrico y refuerzan la necesidad de ajustar los criterios de viverización a las estrategias de cada especie en programas de restauración mediterránea.

Desde una perspectiva ecológica, estas diferencias tienen implicancias directas para la restauración. *A. chilensis* podría presentar ventajas en sitios con restricción hídrica recurrente pero moderada, mientras que *Q. saponaria* podría desempeñarse mejor en ambientes con pulsos temporales de agua y con periodos de déficit hídrico más severo. En este contexto, la combinación de especies con estrategias contrastantes podría ser una

alternativa para aumentar la estabilidad y resiliencia funcional de los proyectos de restauración, considerando la severidad y frecuencia de la restricción hídrica.

Por su parte, la poda química limitó el desarrollo de nuevas raíces en el PCR, principalmente en *A. chilensis*, un efecto que ya se manifestaba durante la viverización mediante la reducción de atributos de la arquitectura radical. Esta menor capacidad de regeneración tras el trasplante sugiere una limitación en la exploración del suelo (Grossnickle, 2005), lo que podría haber condicionado la respuesta fisiológica ante condiciones restrictivas. Bajo déficit hídrico, este tratamiento mostró además un cierre estomático más estricto, reflejando un comportamiento más conservador o isohídrico (Martínez-Vilalta et al., 2014; Tardieu & Simonneau, 1998), consistente con la respuesta observada en vivero. Si bien esta estrategia no es intrínsecamente negativa, su combinación con una menor capacidad de regeneración y exploración radical podría restringir la recuperación fisiológica y el acceso al agua del suelo tras el establecimiento. En conjunto, estos resultados sugieren que la aplicación de poda química podría comprometer el desempeño de especies mediterráneas bajo condiciones de restricción hídrica severa y que, considerando además los mayores costos y requerimientos de manejo asociados, su uso debería ser evaluado críticamente en programas de viverización y restauración en ambientes con marcada estacionalidad hídrica.

En *Q. saponaria*, en cambio, la poda no afectó el PCR y, durante el déficit hídrico, presentó una respuesta fisiológica en general comparable a la de *A. chilensis* reflejado en la relación de la conductancia estomática (gs) y el potencial hídrico mínimo (Ψ_{\min}) y en el

P_{gs50}. Si bien la hydroscape generó resultados menos consistentes, la respuesta observada en el tratamiento WCu se asoció principalmente a valores de Ψ_{\min} particularmente bajos registrados en las etapas iniciales del déficit, en comparación con los potenciales hídricos a prealba (Ψ_{pd}). Esta diferencia limitó la capacidad del indicador para discriminar con claridad las estrategias hídricas en esta fase del experimento.

En síntesis, los resultados respaldan la importancia de evaluar de manera integrada los rasgos morfológicos y fisiológicos durante la viverización. Asimismo, demuestran que los atributos que predicen el desempeño, ya sea en campo o bajo condiciones restrictivas, son altamente especie-específicos, como lo reveló el análisis de importancia. En *A. chilensis*, las variables más explicativas fueron tanto morfológicas como fisiológicas, mientras que en *Q. saponaria* la arquitectura radical tuvo un peso relativamente mayor en la predicción de su desempeño. Para ambas especies, la poda radical fue el factor con mayor contribución porcentual; sin embargo, el tratamiento sin poda fue consistentemente el que generó mejor desempeño. Este tipo de análisis facilita la identificación de los rasgos clave de vivero que deberían priorizarse para mejorar la calidad de planta y ajustar las prácticas de producción según las estrategias de cada especie.

Si bien esta investigación muestra los efectos de la fertilización fosfórica y la poda química durante la viverización y su influencia sobre el desempeño, es importante reconocer que estos resultados constituyen un proxy de las condiciones que las plantas enfrentarán en ambientes naturales. Las evaluaciones se realizaron bajo condiciones mayormente controladas, tales como la capacidad física de los contenedores, los

regímenes de riego aplicados y las condiciones impuestas durante la fase de restricción hídrica. En este sentido, una proyección clave de esta y futuras investigaciones es evaluar el desempeño de las plantas directamente en campo, donde la supervivencia, el crecimiento y la estabilidad hídrica permitirían validar de manera más robusta los efectos de los tratamientos aplicados durante la viverización.

Otra limitación relevante de este estudio se relaciona con el momento en que se evaluó la arquitectura radical. Las mediciones se realizaron al final de la etapa de viverización, cuando las plantas habían alcanzado un desarrollo aéreo y radical cercano al máximo permitido por la capacidad del contenedor, lo que pudo haber atenuado o enmascarado algunos efectos tempranos de los tratamientos sobre la dinámica del sistema radical. En este contexto, estudios futuros deberían considerar evaluaciones secuenciales de la arquitectura radical a lo largo de la viverización. Si bien este enfoque implica un mayor esfuerzo metodológico, permitiría capturar con mayor precisión los efectos temporales de las prácticas de manejo y fortalecer la capacidad predictiva de los atributos de vivero sobre el desempeño y la supervivencia de las plantas en escenarios de restauración mediterránea sometidos a creciente estrés hídrico.

Finalmente, estos resultados ponen de manifiesto la necesidad de incorporar de manera más explícita enfoques de la ecología funcional y el uso de atributos funcionales, con el fin de comprender de forma integrada cómo los rasgos morfológicos y fisiológicos interactúan para determinar el desempeño de las plantas a lo largo del continuo vivero-campo, reforzando así el concepto de TPC. En este contexto, el desarrollo de

aproximaciones interdisciplinarios, que integren ecología funcional, fisiología vegetal, silvicultura y restauración ecológica, permitiría avanzar hacia una evaluación más robusta de las implicancias de las prácticas de vivero y de sus respuestas especie-específicas. Este tipo de aproximaciones resulta especialmente relevante para especies mediterráneas, donde la creciente variabilidad climática y la intensificación del estrés hídrico imponen la necesidad de ajustar los criterios de producción de plantas no solo en función de estándares morfológicos, sino también de estrategias funcionales asociadas a la supervivencia y al desempeño a largo plazo.

CONCLUSIONES GENERALES

Los mayores niveles de fertilización fosfórica generaron aumentos en atributos morfológicos, nutricionales y de arquitectura radical, así como respuestas fisiológicas asociadas a una mayor capacidad de generación radical (PCR) y un mejor desempeño frente al déficit hídrico. Estos resultados indican que una adecuada disponibilidad de P durante la viverización fortalece rasgos y estrategias relevantes para el establecimiento en ambientes mediterráneos con marcada estacionalidad hídrica.

En contraste, la poda química de raíces no generó beneficios consistentes sobre los atributos morfofisiológicos en vivero, así como sobre el desempeño en el PCR o la resistencia al déficit hídrico. Si bien indujo modificaciones en la arquitectura radical, estas se asociaron con una menor capacidad de exploración del suelo y con respuestas fisiológicas más conservadoras, lo que podría limitar el desempeño en escenarios de disponibilidad hídrica restringida. No obstante, esta práctica continúa siendo utilizada en viverización debido a su efecto positivo sobre otros objetivos de producción, como la estabilidad mecánica de las plantas en terrenos con pendiente.

Asimismo, la comparación entre *A. chilensis* y *Q. saponaria* evidencia que especies que coexisten en un mismo ambiente pueden presentar estrategias hídricas contrastantes frente a condiciones restrictivas. *A. chilensis* exhibe un comportamiento más conservador o isohídrico, mientras que *Q. saponaria* manifiesta una estrategia más adquisitiva o anisohídrica. Estos resultados refuerzan la importancia de una adecuada selección de

especies en función de las características del sitio de restauración, así como de la aplicación de prácticas de vivero que favorezcan el desarrollo de atributos especie-específicos, capaces de mejorar su desempeño bajo condiciones de restricción hídrica.

En conjunto, los resultados permiten aceptar parcialmente la hipótesis general de esta tesis, dado que la fertilización fosfórica modificó la arquitectura radical y se asoció a respuestas fisiológicas que favorecieron el desempeño bajo déficit hídrico, mientras que la poda química de raíces, pese a inducir cambios en el sistema radical, no se tradujo en mayor desempeño frente al déficit hídrico.

En síntesis, esta tesis evidencia que la calidad de planta destinada a ecosistemas mediterráneos no puede definirse a partir de criterios universales, sino que emerge de la interacción entre las prácticas de viverización y las estrategias morfofisiológicas propias de cada especie. La incorporación explícita de la arquitectura radical y el análisis de importancia permitieron avanzar hacia una evaluación más integral y con mayor capacidad predictiva del establecimiento bajo déficit hídrico. No obstante, el desafío venidero se direcciona en integrar la evaluación de atributos funcionales en el desempeño, con el fin de fortalecer el enfoque del TPC, especialmente bajo escenarios de cambio climático.

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