Effects of Nitrogen Fertilizer on the Growth and Development of *Ceanothus velutinus*[1]

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Abstract

Snowbrush ceanothus (*Ceanothus velutinus* Dougl. Ex Hook.) is an evergreen shrub with dark green leaves and creamy white flowers. It is a nitrogen-fixing species that enhances soil fertility through nodulation. This study evaluates the effects of various nitrogen concentrations on the growth and development of *C. velutinus*. Seedlings transplanted into calcined clay and inoculated with 30 mL of soil containing the nitrogen-fixing actinobacteria *Frankia* were treated with 0.0 to 8.4 $g{\cdot}L^{-1}$ of controlled released fertilizer (CRF, 15N-3.9P-10K) or a nitrogen-limited nutrient solution with or without 2mM ammonium nitrate (NH_4NO_3). Plant growth and photosynthesis increased linearly or quadratically with increasing CRF application rates. Visual score, plant growth index, number of shoots, leaf area, SPAD, leaf and root dry weights, and net photosynthetic rate of inoculated plants treated with 2.1 $g{\cdot}L^{-1}$ of CRF were similar to those of uninoculated or plants grown in autoclaved native soil and treated with the manufacturer-recommended application rate of 3.2 $g{\cdot}L^{-1}$ of CRF. Nodules were observed only in plants receiving 0.0, 0.3, 0.5, 1.1, or 2.1 $g{\cdot}L^{-1}$ of CRF, and the number of nodules were too small to analyze statistically. The study indicates that CRF significantly boosts *C. velutinus* growth and development. However, the nodulation of the plant and nitrogen-fixing capacity of the nodules remain unknown. Further investigation is needed to determine the effect of nitrogen on the nodulation of *C. velutinus*.

Species used in this study: *Ceanothus velutinus* Dougl. Ex Hook., *Frankia sp.*

Index words: snowbrush ceanothus, *Frankia*, native plant, nodule.

Significance to the Horticulture Industry

Snowbrush ceanothus (*Ceanothus velutinus* Dougl. Ex Hook.) is an evergreen shrub with shiny leaves and creamy white flowers. It is an actinorhizal plant that forms a symbiosis with nitrogen-fixing actinobacteria (*Frankia*) and plays a crucial ecological role in enhancing soil fertility. Understanding the nutritional requirements of snowbrush ceanothus is essential for its nursery production and landscape use. This research evaluated the growth and development of snowbrush ceanothus under various nitrogen concentrations and provides valuable insights for developing best practices to manage the nutrition required for plant production while maintaining environmental stewardship.

Introduction

Native plants are becoming increasingly important in the green industry, driven by growing demand from consumers, landscape architects, and environmental conservation efforts. They support local biodiversity, provide food and habitat for wildlife such as birds and pollinators, require less water and maintenance once established, and help prevent soil erosion (Brzuszek and Harkess 2009, Rihn et al. 2022). However, many native species are unavailable or in limited supply from nurseries due to localized markets and small native nurseries lacking capital (Brzuszek and Harkess 2009). As the demand for native plants continues to grow, the green industry is eager to develop new native cultivars, improve production methods, and raise awareness about the ecological importance of incorporating native species into landscapes.

Actinobacteria is one of the largest phyla within the bacterial domain, encompassing a diverse array of organisms that are crucial for ecological balance across both terrestrial and aquatic environments. The majority of actinobacteria are free-living organisms that are widely distributed in both aquatic (including marine) and terrestrial ecosystems (Barka et al. 2015). Their saprophytic nature makes them vital for organic matter decomposition in alkaline soils, where they form a significant portion of the microbial population and are often found both on the soil surface and at depths exceeding 2 m (∼ 7 ft) (Barka et al. 2015). They are crucial for decomposing organic matter, particularly under nutrient-limited conditions. Actinobacteria are remarkable for their ability to form mycelium and reproduce by sporulation, similar to filamentous fungi, allowing them to exist as semi-dormant spores. *Frankia* strains, a subgroup within the actinobacteria, are notable for their gram-positive, filamentous characteristics and their ability to fix atmospheric nitrogen (N). They establish symbiotic relationships with actinorhizal plants across 24 genera (Oakley et al. 2004). They are known to thrive on marginal soils and have current and potential applications in reclaiming and conditioning soil (Benson and Silvester 1993).

Received for publication August 14, 2024; in revised form November 20, 2024.

[1]This research was supported in part by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) Hatch project UTA01381 and UTA01666, Horticulture Research Institute, the Center for Water-Efficient Landscaping, and the Utah Agricultural Experiment Station (UAES) at Utah State University. It is approved as UAES journal paper number 9842. In addition, funding for this project was made possible by the USDA's Agricultural Marketing Service through the Utah Department of Agriculture and Food Specialty Crop Block Grant. We are grateful for the technical assistance from Paul Harris, Alyssa Fenstermaker, Abby McKee, Megan Kelly, and Abigail Porter, and valuable comments from anonymous reviewers. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

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Ceanothus velutinus, commonly known as snowbrush ceanothus, is an evergreen shrub native to the western North America, ranging from British Columbia to California and eastward to South Dakota and Colorado. It is an actinorhizal plant known to form nodules with actinobacteria *Frankia*, enabling it to fix nitrogen (Jeong and Myrold 2001, Stein et al. 2010). This species grows 0.9-1.5 m (3-5 ft) tall, forming dense, impenetrable thickets with tangled branches bearing sticky, dark green leaves that are oval shaped with finely toothed edges and velvety undersides, and large pyramidal clusters of aromatic creamy white flowers (Inland NW routes n.d.). It plays a vital role in the ecosystem by improving soil fertility through nodulation, a symbiotic process with bacteria that fixes atmospheric nitrogen (Philbin et al. 2021). The effect of nitrogen on the nodulation of *C. velutinus* is an important area of study, as it can provide insights into the plant's adaptations and strategies for thriving in diverse soil conditions.

Research on *Ceanothus velutinus* for specialty crop development and its nodulation with native *Frankia* bacteria remains limited. Expanding studies on its cultivation and symbiotic relationships could enhance sustainable agricultural practices, reducing synthetic fertilizer use through its nitrogen-fixing abilities. This gap in research presents a significant opportunity to explore environmentally friendly cultivation techniques and improve the understanding of plant-microbe interactions essential for ecosystem health. We hypothesize that increasing concentrations of nitrogen will significantly increase the growth and development of *C. velutinus*. Increased nitrogen levels are expected to enhance the plant's physiological and morphological traits, such as growth rate, leaf size, net photosynthetic rate, and overall biomass. The objective of this study was to understand the effects of various nitrogen concentrations on the growth and development of *C. velutinus*.

Materials and Methods

Ceanothus velutinus seeds were purchased from the Native Seed Foundation (Polson, MO, USA) and stored in a refrigerator at 2 C (36 F) until use. On 21 April 2022, the seeds were scarified in hot water at 90 C (194 F), followed by immersion in cold water with ice at 6 C (43 F) for 1 h, and then the seeds were wrapped in wet paper towels for stratification inside the refrigerator at 2 C (36 F) (Paudel et al. 2020). On 15 August 2022, the stratified seeds were sown in a 1:1 (v/v) mixture of peatmoss (SunGro Horticulture, Agawam, MA, USA) and perlite (Hess perlite, Malad City, ID, USA) and placed on a mist bench. Upon germination on 5 September 2022, the seedlings were transferred to a Utah Agricultural Experiment Station's research greenhouse. Two sets (blocks) of seedlings were prepared for the experiment because of the variation in seedlings likely resulting from genetic differences and individual growth responses.

Native soil was collected from the rhizosphere of a snowbrush ceanothus plant located in Tony Grove, UT, USA (41°52′35″ N 111°34′20″ W) and stored at 4 C (39 F) until use. Soil samples collected on 1 October 2022 were used for the plants in block 1, whereas soils collected in 2019, 2021, and 2022 were mixed to create a composite sample and used for the plants in block 2. The soil was sieved through a 0.12-cm USA standard testing sieve (Cole-Parmer, Vernon Hills, IL, USA). Although the soil samples used in this study were characterized as loam, they exhibited some variations in their properties. The soil sample from block 1 had a pH of 6.5 and an electrical conductivity (EC) of 0.50 $dS \cdot m^{-1}$, with 25.1% organic matter, 7.12 $mg \cdot kg^{-1}$ nitrate (NO_3) nitrogen, 13.9 $mg \cdot kg^{-1}$ phosphorous (P), 792 $mg \cdot kg^{-1}$ potassium (K), 4.88 $mg \cdot kg^{-1}$ zinc (Zn), 107 $mg \cdot kg^{-1}$ iron (Fe), 2.39 $mg \cdot kg^{-1}$ copper (Cu), 40.9 $mg \cdot kg^{-1}$ manganese (Mn), 4.4 $mg \cdot kg^{-1}$ sulfate-sulfur (S). The soil sample from block 2 had a pH of 5.7 and an EC of 1.81 $dS \cdot m^{-1}$, with 20.7% organic matter, 165 $mg \cdot kg^{-1}$ NO_3-nitrogen, 22.2 $mg \cdot kg^{-1}$ P, 902 $mg \cdot kg^{-1}$ K, 3.7 $mg \cdot kg^{-1}$ Zn, 113 $mg \cdot kg^{-1}$ Fe, 1.43 $mg \cdot kg^{-1}$ Cu, 36.2 $mg \cdot kg^{-1}$ Mn, and 10.8 $mg \cdot kg^{-1}$ sulfate-S. A portion of each soil sample was autoclaved at 125 C (257 F) for 1 hour after a 2-day incubation period at room temperature to allow microbial growth. Post autoclaving, sterile distilled water was added to the soil to restore the desired moisture content, and the soil was incubated for an additional 2 days before use.

On 6 and 7 October 2022, nodule-free seedlings in block 1 were transplanted into 1-L (12.1 × 10.5 × 10.5 cm^3) containers (Dillen Products, Middlefield, OH, USA) filled with calcined clay (Primera, Rancho Santa Margarita, CA, USA; Paudel et al. 2025), an inorganic growing substrate. On 11 November 2022, seedlings in block 2 were transplanted into 1-L (12.1 × 10.5 × 10.5 cm^3) containers (Landmark Plastics, Akron, OH, USA) filled with calcined clay (Primera). Within each block, uniform plants were randomly assigned to 14 groups according to the soil and fertilizer treatments (Table 1). Plants in groups 1 and 2 served as uninoculated controls. Plants in groups 3 and 4 were transplanted in 30 mL of autoclaved native soil added in the center of the containers filled with calcined clay to assess the effects of sterilized soil inoculum. Plants in groups 5 to 14 were transplanted in 30 mL of native soil added in the center of the pots containing calcined clay.

On 6 June 2023, an experiment was initiated with different fertilizer treatments. Plants in groups 1 (uninoculation) and 3 (autoclaved native soil) were unfertilized. Plants in groups 2 (uninoculation) and 4 (autoclaved native soil) were top-dressed with a controlled-release fertilizer (CRF) 15N-3.9P-10K (Osmocote Plus 15-9-12; Israel Chemicals, Tel Aviv-Yafo, Israel) at 3.2 $g \cdot L^{-1}$, following the manufacturer-recommended application rate for plants. Plants in groups 5 and 6 (native soil) were irrigated with 150 mL modified nitrogen-limited nutrient solution (Bugbee 2004) supplemented with or without 2mM NH_4NO_3 at a pH of 6.0 three times a week. Plants in groups 7 to 14 (native soil) were treated with 15N-3.9P-10K CRF at 0, 0.1, 0.3, 0.5, 1.1, 2.1, 4.2, and 8.4 $g \cdot L^{-1}$, respectively. Details of soil inoculation, CRF application rates, and N applied per plant are explained in Table 1. Except for plants in group 5 and 6 (native soil), which received the nitrogen-limited nutrient solution (with or without added 2mM NH_4NO_3), all plants were irrigated with 150 mL of Logan City water (EC: 257 $\mu S \cdot cm^{-1}$; N: 2.0 $mg \cdot L^{-1}$; pH: 8) three times a week. A saucer was placed under each container before

Table 1. **Treatment and inoculation details for *Ceanothus velutinus* study.**

Group	Soil addition[z]	Treatment[y]	N applied per plant (g)
1	No	No fertilizer	0
2	No	3.2 g·L^{-1} CRF (manufacturer-recommended application rate)	0.48
3	Autoclaved native soil	No fertilizer	0
4	Autoclaved native soil	3.2 g·L^{-1} CRF (manufacturer-recommended application rate)	0.48
5	Native soil	Nitrogen-limited nutrient solution	0
6	Native soil	Nitrogen-limited nutrient solution with 2 mM NH_4NO_3	0.87
7	Native soil	0 g·L^{-1} CRF	0
8	Native soil	0.1 g·L^{-1} CRF	0.02
9	Native soil	0.3 g·L^{-1} CRF	0.05
10	Native soil	0.5 g·L^{-1} CRF	0.08
11	Native soil	1.1 g·L^{-1} CRF	0.17
12	Native soil	2.1 g·L^{-1} CRF	0.32
13	Native soil	4.2 g·L^{-1} CRF	0.63
14	Native soil	8.4 g·L^{-1} CRF	1.26

[z]Soil addition refers to treating plants with either autoclaved native soil or native soil collected from the rhizosphere of snowbrush ceanothus (*Ceanothus velutinus*) located in Tony Grove, UT, USA.

[y]Treatment refers to the application of either controlled-release fertilizer (CRF) or a nitrogen-limited nutrient solution with or without the addition of 2 mM ammonium nitrate (NH_4NO_3). The CRF used was Osmocote 15N-3.9P-10K (Osmocote Plus 15-9-12; Israel Chemicals, Tel Aviv-Yafo, Israel). Plants in groups 2 and 4 received the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF. Plants in groups 5 and 6 were treated with nitrogen-limited nutrient solutions, with group 6 receiving an additional 2 mM NH_4NO_3. Plants in groups 7 to 14 received CRF at increasing rates from 0.0 to 8.4 g·L^{-1}.

irrigation to collect leachate for subsequent measurements of NO_3 using LAQUA Twin meters (Horiba, Kyoto, Japan).

The experiment ended on 18 December 2023 and 26 January 2024 for block 1 and 2, respectively. Before all plants were destructively harvested, data was collected for visual score, plant height (cm), width (cm), number of shoots (longer than 2 cm), and leaf greenness [Soil Plant Analysis Development (SPAD)]. Visual score of *Ceanothus velutinus* was recorded using a reference scale of 0 to 5 (0 = dead plant, 1 = plant with very chlorotic leaves (> 90% leaves exhibiting yellow discoloration), 2 = plant with moderately chlorotic leaves (50 to 90% leaves exhibiting yellow discoloration), 3 = plant with green leaves (< 50% leaves exhibiting yellow discoloration), 4 = plant with acceptable quality and few chlorotic leaves (<10% leaves exhibiting yellow discoloration), 5 = plant is green and vigorous, and of good quality with no damage). Plant width was measured among the major axis (maximum width) and the minor axis (perpendicular width) to provide an accurate representation of the overall width. The leaf greenness of four to six mature leaves on each plant was measured in SPAD units using a handheld chlorophyll meter (MC-100, Apogee Instruments, Logan, UT, USA), and the average was recorded. At harvest, leaf area (cm^2) was measured using a leaf area meter (LI-3000; LI-COR Biosciences, Lincoln, NE, USA), and number of nodules was counted after washing the roots. Leaf and root samples were oven-dried at 80 C (176 F) for 7 days, and dry weights (gram) were recorded. To quantify the overall growth, plant growth index (PGI; ((plant height + (width 1 + width 2)/2))/2) was calculated (Lalk et al. 2023).

Net photosynthetic rate (P_n) was recorded for plants in groups 2 (uninoculation) and 4 (autoclaved native soil) treated with 3.2 g·L^{-1} of CRF and plants in groups 9 to 14 (native soil) treated with 0.3 to 8.4 g·L^{-1} of CRF on 5 and 9 December 2023 for block 1 and 15 and 17 December 2023 for block 2, respectively. P_n was not recorded for other treatments because the small-sized leaves prevented accurate measurement. Parameters were recorded using a portable photosynthesis system (LI-6800, LICOR Biosciences, Lincoln, NE, USA) with a fluorometer head (LI-6800-01A, LICOR Biosciences, Lincoln, NE, USA). The chamber settings were held constant across measurements with 25 C (77 F) air temperature, 150 µ·mol·s^{-1} flow rate, 1,000 µmol·m^{-2}·s^{-1} light intensity, 10,000 rpm fan speed, and 50% relative humidity. Throughout the experimental period, the greenhouse temperatures were maintained at 24.8 ± 0.9 C (76.6 ± 33.6 F) (mean ± SD) during the day and 21.2 ± 1.1 C (71.2 ± 34.0 F) (mean ± SD) at night. The average daily light integral inside the greenhouse was 24.0 ± 10.7 mol·m^{-2}·d^{-1} (mean ± SD) recorded using a full-spectrum quantum sensor (SQ-500-SS; Apogee Instruments, Logan, UT, USA). Supplemental light was provided using light-emitting diodes (Luxx Lighting, Jurupa Valley, CA, USA) at an average light intensity of 540.9 ± 239.7 mol·m^{-2}·d^{-1} (mean ± SD) at the plant's canopy level from 0600 to 2200 HR when light intensity inside the greenhouse was less than 500 µmol·m^{-2}·s^{-1}.

The experiment used a randomized complete block design with two blocks and 14 treatments. Plants in block 1 had seven replications per treatment, with each replication consisting of a single plant grown in an individual pot. In block 2, treatments in groups 1 to 4 had seven replications each, while treatments in groups 5 to 14 had nine replications each. The number of nodules was not analyzed due to insufficient replication. Statistical analyses were performed using the PROC GLIMMIX procedure in SAS Studio (version 3.8; SAS Institute, Cary, NC, USA). Mean separation was done using the Tukey-Kramer method, adjusting for multiplicity with a significance level specified at 0.05.

Results and Discussion

Figure 1 shows the NO_3 concentrations of leachate solution when plants were treated with 0.0 to 8.4 g·L^{-1} of

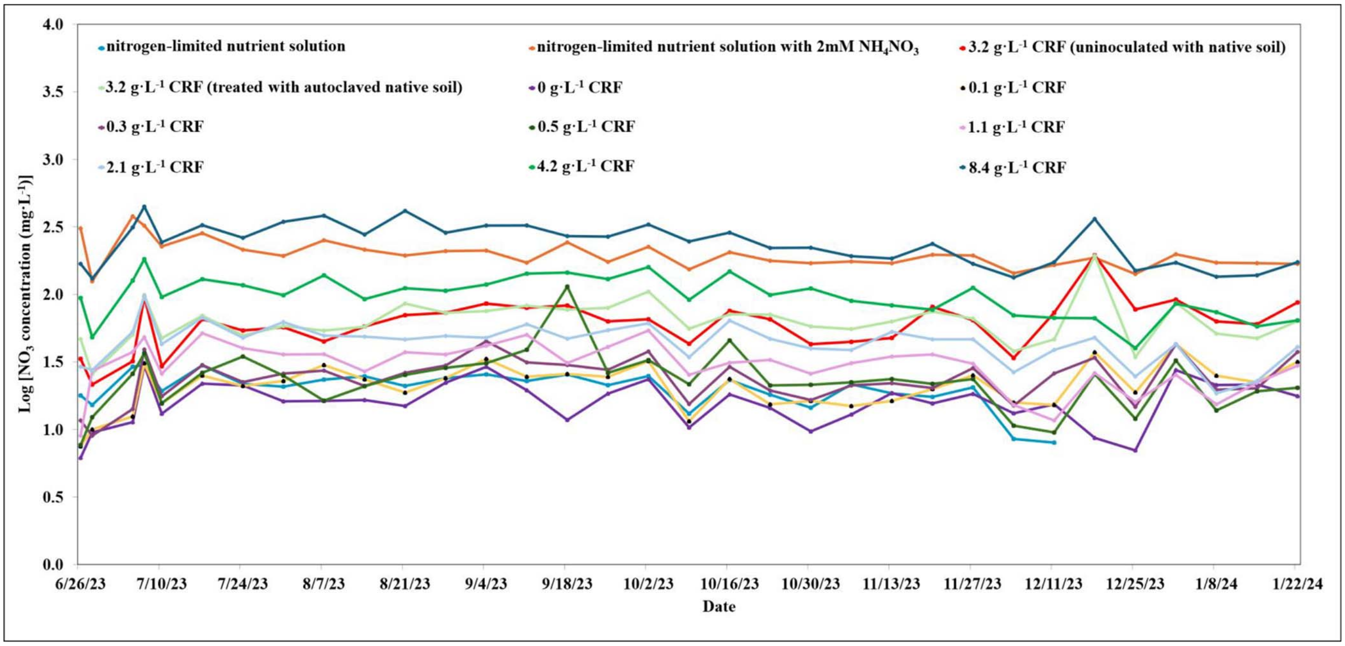

Fig. 1. Nitrate (NO_3) concentration in the leachate when *Ceanothus velutinus* was treated with 0.0 to 8.4 g·L^{-1} of controlled-released fertilizer (CRF) or a nitrogen-limited nutrient solution with or without the addition of 2 mM ammonium nitrate (NH_4NO_3). The CRF used was Osmocote 15N-3.9P-10K (Osmocote Plus 15-9-12; Israel Chemicals, Tel Aviv-Yafo, Israel). Plants without soil inoculation or with autoclaved native soil were treated with the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF. Plants inoculated with native soil were treated with a nitrogen-limited nutrient solution with and without an additional 2 mM NH_4NO_3 or received CRF at increasing rates from 0.0 to 8.4 g·L^{-1}. The figure represents data collected from two plants per block for each treatment.

controlled-released fertilizer (CRF) or a nitrogen-limited nutrient solution supplemented with or without the addition of 2 mM NH_4NO_3 over the experimental period. The results indicate a clear trend that increasing the CRF rate led to increased NO_3 concentrations in the leachate, suggesting greater nutrient leaching with higher fertilizer inputs. Initially, plants treated with a lower CRF rate, ranging from 0 to 2.1 g·L^{-1}, exhibited numerically lower NO_3 concentrations (1.4-25.8 N mg·L^{-1}) in their leachate compared with those treated with the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF without soil inoculation (4.9-44.1 N mg·L^{-1}) (Fig. 1). The NO_3 concentrations in the leachate increased in the plants receiving 4.2 or 8.4 g·L^{-1} of CRF (9.0-100.9 N mg·L^{-1}) (Fig. 1). Additionally, plants treated with a nitrogen-limited nutrient solution showed lower NO_3 concentrations (1.8-6.8 N mg·L^{-1}) in the leachate compared with those receiving nitrogen-limited nutrient solution supplemented with 2 mM NH_4NO_3 (28.2-85.5 N mg·L^{-1}) over the experimental period. Despite the absence of nitrogen fertilizer in groups 1 (uninoculation), 3 (autoclaved native soil) and 5 (native soil), detectable NO_3 levels were observed in the leachate. This may be attributed to the nitrogen content in Logan City water (2.0 mg·L^{-1} of N, in the autoclaved native soil, or in native soil). These findings underscore the necessity of optimizing fertilizer application rates to balance plant nutrient requirements with environmental sustainability. Higher CRF rates can significantly increase nitrate runoff, potentially leading to detrimental effects on surrounding ecosystems. This study is consistent with Gruhn et al. (2000), who highlight the importance of balanced nutrient availability in mitigating environmental impacts. Therefore, effective management of fertilizer inputs is essential for maximizing plant growth while minimizing ecological risks.

The application of CRF and soil inoculation significantly influenced the visual quality, plant growth index, number of shoots, leaf area, SPAD, and leaf and root dry weights of *Ceanothus velutinus* (all P values < 0.0001) (Table 2). *C. velutinus* without soil inoculation nor fertilizer (Group 1), with autoclaved native soil and no fertilizer (Group 3), and with native soil inoculation and nitrogen-limited nutrient solution (Group 5) resulted in the lowest visual score of 2, 2, and 0, respectively. Despite the inoculation of native soil in Group 5, the available nitrogen may not have been sufficient to support the plant's overall health and growth. In contrast, plants without soil inoculation and treated with 3.2 g·L^{-1} CRF (Group 2), with autoclaved native soil and treated with 3.2 g·L^{-1} CRF (Group 4), with native soil inoculation and the nitrogen-limited nutrient solution supplemented with 2 mM NH_4NO_3 (Group 6) and those inoculated with native soil and treated with 0.0, 0.1, 0.3, 0.5, 1.1, 2.1, or 4.2 g·L^{-1} CRF (Groups 7-13) exhibited an average visual score of 3 or 4, where plants had less than 50% of leaves with yellow discoloration and were of acceptable quality with less than 10% of leaves showing chlorosis, respectively (Table 3; Fig. 2.). The moderate CRF application could have provided a nutrient-rich environment that facilitated plant's growth and development. However, plants inoculated with native soil and treated with 8.4 g·L^{-1} CRF decreased the visual score to 3, where less than 50% of leaves were chlorotic. Although CRFs are designed to minimize nutrient losses by slowly releasing nutrients, when applied at higher rates, these

Table 2. **A summary of the analysis of variance for the effect of treatments on visual score (VS), plant growth index (PGI), number (No.) of shoots, leaf area, SPAD (Soil Plant Analysis Development), leaf dry weight (DW) and root dry weight of *Ceanothus velutinus* in a greenhouse at harvest.**

Treatment[z]	VS	PGI[y]	No. of shoots	Leaf area	SPAD	Leaf DW	Root DW
***P* value**	****[x]	****	****	****	****	****	****

[z]*Ceanothus velutinus* was treated with 0.0 to 8.4 g·L^{-1} of controlled released fertilizer (Osmocote 15N-3.9P-10K, Osmocote Plus 15-9-12; Israel Chemicals, Tel Aviv-Yafo, Israel) or a nitrogen-limited nutrient solution with or without the addition of 2 mM ammonium nitrate (NH_4NO_3) at harvest.

[y]PGI was calculated as [plant height + {(width 1 + width 2)/2}]/2.

[x]****: significant at $P < 0.0001$.

fertilizers can cause salt accumulation, increasing the risk of high salinity in the root zone (Pasian 2013). These conditions can reduce water uptake and lead to the accumulation of toxic ions like sodium and chloride in plant tissues, resulting in symptoms like leaf chlorosis and necrosis (Munns and Tester 2008). These findings underscore the critical importance of nitrogen and tailored CRF applications in enhancing plant health and growth. The results demonstrated that nutrient availability plays a crucial role in promoting optimal growth and development (Chauhan et al. 2023), hence preserving the esthetic appearance of plants.

Ceanothus velutinus without soil inoculation nor fertilizer (Group 1) and with autoclaved native soil and no fertilizer (Group 3) exhibited limited growth, with plant growth indices (PGI) of 2.2 cm and 3.4 cm, respectively (Table 4). In contrast, plants receiving the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF without soil inoculation (Group 2) or with autoclaved native soil (Group 4) showed significantly improved growth, with PGIs reaching 12.9 cm and 13.7 cm, respectively. Plants inoculated with native soil and receiving nitrogen-limited nutrient solution (Group 5) had a PGI of 2.8 cm. In contrast, plants receiving nitrogen-limited nutrient solution supplemented with 2mM NH_4NO_3 (Group 6) had the significantly highest growth, with a PGI of 22.2 cm. All these results highlighted the critical role of nitrogen in plant health and development. Statistical analysis revealed significant quadratic trends in PGI ($P < 0.0001$) when plants were inoculated with native soil and treated with CRF ranging from 0 to 8.4 g·L^{-1} (Group 7 to 14), indicating consistent increase with higher concentration of CRF. The highest PGI of 24.9 cm was recorded when plants were inoculated with native soil and treated with 8.4 g·L^{-1} CRF (Group 14) compared with those treated with 0, 0.1, 0.3, 0.5, 1.1, or 2.1 g·L^{-1} CRF which recorded significantly lower PGIs of 3.7, 4.0, 5.2, 7.0, 8.0, and 14.3 cm, respectively. The growth responses observed with increasing CRF concentrations, including enhanced growth rate and biomass accumulation, highlight the importance of an adequate nutrient supply for supporting plant growth processes (Römheld 2012).

Ceanothus velutinus grown without soil inoculation (Group 1), with autoclaved native soil (Group 3), or with native soil (Group 5 and 7) recorded minimal shoot development, with only one shoot produced when no fertilizer or the nitrogen-limited nutrient solution was

Table 3. **Visual score (VS) of *Ceanothus velutinus* treated with 0.0 to 8.4 g·L^{-1} of controlled-release fertilizer (CRF) or a nitrogen-limited nutrient solution with or without the addition of 2 mM ammonium nitrate (NH_4NO_3) at harvest.**

Group	Soil addition	Treatment[z]	VS[y]
1	No	No fertilizer	2 c[x]
2	No	3.2 g·L^{-1} CRF (manufacturer-recommended application rate)	3 ab
3	Autoclaved native soil	No fertilizer	2 bc
4	Autoclaved native soil	3.2 g·L^{-1} CRF (manufacturer-recommended application rate)	4 a
5	Native soil	Nitrogen-limited nutrient solution	0 d
6	Native soil	Nitrogen-limited nutrient solution with 2 mM NH_4NO_3	3 abc
7	Native soil	0 g·L^{-1} CRF	3 abc
8	Native soil	0.1 g·L^{-1} CRF	3 abc
9	Native soil	0.3 g·L^{-1} CRF	3 abc
10	Native soil	0.5 g·L^{-1} CRF	4 ab
11	Native soil	1.1 g·L^{-1} CRF	4 ab
12	Native soil	2.1 g·L^{-1} CRF	4 a
13	Native soil	4.2 g·L^{-1} CRF	4 ab
14	Native soil	8.4 g·L^{-1} CRF	3 ab

[z]Treatment refers to the application of either controlled-release fertilizer (CRF) or a nitrogen-limited nutrient solution with or without the addition of 2 mM NH_4NO_3. The CRF used was Osmocote 15N-3.9P-10K (Osmocote Plus 15-9-12; Israel Chemicals, Tel Aviv-Yafo, Israel). Plants in groups 2 and 4 received the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF. Plants in groups 5 and 6 were treated with nitrogen-limited nutrient solutions, with group 6 receiving an additional 2 mM NH_4NO_3. Plants in groups 7 to 14 received CRF at increasing rates from 0.0 to 8.4 g·L^{-1}.

[y]Visual score (VS) reference scale from 0 to 5 (0 = dead plant, 1 = plant with very chlorotic leaves (> 90% of leaves exhibiting yellow discoloration), 2 = plant with moderately chlorotic leaves (50 to 90% of leaves exhibiting yellow discoloration), 3 = plant with green leaves (< 50% of leaves exhibiting yellow discoloration), 4 = plant with acceptable quality and few chlorotic leaves (<10% of leaves exhibiting yellow discoloration), 5 = plant is green, vigorous, and of good quality with no damage.

[x]Means with same lowercase letters within a column are not significantly different among treatments according to the Tukey-Kramer method of multiplicity at $\alpha \leq 0.05$.

Fig. 2. ***Ceanothus velutinus*** **treated with 0.0 to 8.4 g·L^{-1} of controlled-released fertilizer (CRF) or a nitrogen-limited nutrient solution supplemented with or without 2 mM ammonium nitrate (NH_4NO_3) at harvest (i.e. 25 January 2024). The CRF used was Osmocote 15N-3.9P-10K (Osmocote Plus 15-9-12; Israel Chemicals, Tel Aviv-Yafo, Israel).**

applied (Table 4), highlighting the necessity of nitrogen. Plants receiving the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF without soil inoculation (Group 2) or with autoclaved native soil (Group 4) produced two and three shoots, respectively (Table 4). Plants inoculated with native soil and treated with the nitrogen-limited nutrient solution plus 2 mM NH_4NO_3 (Group 6) significantly boosted the shoot count to four, underscoring the crucial role of nitrogen. An increase in the application rate of CRF led to a linear increase in the number of shoots ($P < 0.0001$), ranging from two shoots at 0.1 g·L^{-1} CRF (lowest) to five shoots at 8.4 g·L^{-1} CRF.

Ceanothus velutinus without soil inoculation (Group 1), with autoclaved native soil (Group 3), or with native soil (Group 5) exhibited significantly smaller leaf areas, measuring 1.4, 2.5, and 3.0 cm^2, respectively, when no fertilizer or the nitrogen-limited nutrient solution was applied (Table 5). In contrast, plants receiving the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF without soil inoculation (Group 2) or with autoclaved native soil

Table 4. Plant growth index (PGI) and number (No.) of shoots of *Ceanothus velutinus* treated with 0.0 to 8.4 g·L^{-1} of controlled-release fertilizer (CRF) or a nitrogen-limited nutrient solution with or without the addition of 2 mM ammonium nitrate (NH_4NO_3) at harvest.

Group	Soil addition	Treatment[z]	PGI (cm)[y]	No. of shoots
1	No	No fertilizer	2.2 g[x]	1 c
2	No	3.2 g·L^{-1} CRF (manufacturer-recommended application rate)	12.9 c	2 bc
3	Autoclaved native soil	No fertilizer	3.4 f	1 c
4	Autoclaved native soil	3.2 g·L^{-1} CRF (manufacturer-recommended application rate)	13.7 c	3 bc
5	Native soil	Nitrogen-limited nutrient solution	2.8 fg	1 c
6	Native soil	Nitrogen-limited nutrient solution with 2 mM NH_4NO_3	22.2 a	4 ab
7	Native soil	0 g·L^{-1} CRF	3.7 f	1 c
8	Native soil	0.1 g·L^{-1} CRF	4.0 f	2 c
9	Native soil	0.3 g·L^{-1} CRF	5.2 ef	2 c
10	Native soil	0.5 g·L^{-1} CRF	7.0 de	3 bc
11	Native soil	1.1 g·L^{-1} CRF	8.0 d	2 bc
12	Native soil	2.1 g·L^{-1} CRF	14.3 bc	3 abc
13	Native soil	4.2 g·L^{-1} CRF	18.9 ab	4 ab
14	Native soil	8.4 g·L^{-1} CRF	24.9 a	5 a
	Trend for plants treated with 0.0 to 8.4 g·L^{-1} CRF		**[w]Q******	**L******

[z]Treatment refers to the application of either controlled-release fertilizer (CRF) or a nitrogen-limited nutrient solution with or without the addition of 2 mM NH_4NO_3. The CRF used was Osmocote 15N-3.9P-10K (Osmocote Plus 15-9-12; Israel Chemicals, Tel Aviv-Yafo, Israel). Plants in groups 2 and 4 received the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF. Plants in groups 5 and 6 were treated with nitrogen-limited nutrient solutions, with group 6 receiving an additional 2 mM NH_4NO_3. Plants in groups 7 to 14 received CRF at increasing rates from 0.0 to 8.4 g·L^{-1}.

[y]PGI = ((plant height + (width 1 + width 2)/2))/2.

[x]Means with same lowercase letters within a column are not significantly different among treatments according to the Tukey-Kramer method of multiplicity at $\alpha \leq 0.05$.

[w]****: significant at $P \leq 0.0001$; L: linear; Q: quadratic.

Table 5. Leaf area and SPAD (Soil Plant Analysis Development) of *Ceanothus velutinus* treated with 0.0 to 8.4 $g\cdot L^{-1}$ of controlled-release fertilizer (CRF) or a nitrogen-limited nutrient solution with or without the addition of 2 mM ammonium nitrate (NH_4NO_3) at harvest.

Group	Soil addition	Treatment[z]	Leaf area (cm^2)	SPAD
1	No	No fertilizer	1.4 f[y]	5.0 c
2	No	3.2 $g\cdot L^{-1}$ CRF (manufacturer-recommended application rate)	94.5 b	38.3 a
3	Autoclaved native soil	No fertilizer	2.5 ef	9.5 b
4	Autoclaved native soil	3.2 $g\cdot L^{-1}$ CRF (manufacturer-recommended application rate)	113.9 b	33.7 a
5	Native soil	Nitrogen-limited nutrient solution	3.0 def	19.5 ab
6	Native soil	Nitrogen-limited nutrient solution with 2 mM NH_4NO_3	241.7 a	40.7 a
7	Native soil	0 $g\cdot L^{-1}$ CRF	5.7 de	11.4 b
8	Native soil	0.1 $g\cdot L^{-1}$ CRF	6.6 de	10.6 b
9	Native soil	0.3 $g\cdot L^{-1}$ CRF	13.1 d	19.6 a
10	Native soil	0.5 $g\cdot L^{-1}$ CRF	32.6 c	24.0 a
11	Native soil	1.1 $g\cdot L^{-1}$ CRF	35.1 c	23.1 a
12	Native soil	2.1 $g\cdot L^{-1}$ CRF	112.2 b	32.1 a
13	Native soil	4.2 $g\cdot L^{-1}$ CRF	186.6 ab	42.4 a
14	Native soil	8.4 $g\cdot L^{-1}$ CRF	161.3 ab	46.0 a
Trend for plants treated with 0.0 to 8.4 $g\cdot L^{-1}$ CRF			**[x]Q******	**L******

[z]Treatment refers to the application of either controlled-release fertilizer (CRF) or a nitrogen-limited nutrient solution with or without the addition of 2 mM NH_4NO_3. The CRF used was Osmocote 15N-3.9P-10K (Osmocote Plus 15-9-12; Israel Chemicals, Tel Aviv-Yafo, Israel). Plants in groups 2 and 4 received the manufacturer-recommended application rate of 3.2 $g\cdot L^{-1}$ CRF. Plants in groups 5 and 6 were treated with nitrogen-limited nutrient solutions, with group 6 receiving an additional 2 mM NH_4NO_3. Plants in groups 7 to 14 received CRF at increasing rates from 0.0 to 8.4 $g\cdot L^{-1}$.

[y]Means with same lowercase letters within a column are not significantly different among treatments according to the Tukey-Kramer method of multiplicity at $\alpha \leq 0.05$.

[x]****: significant at $P \leq 0.0001$; L: linear; Q: quadratic.

(Group 4) showed significantly greater leaf areas of 94.5 and 113.9 cm^2, respectively, compared with the unfertilized groups (Group 1, 3, and 5). These results underscore the critical role of nitrogen. Conversely, plants receiving the nitrogen-limited nutrient solution supplemented with 2 mM NH_4NO_3 (Group 6) exhibited the statistically highest leaf area of 241.7 cm^2, illustrating the substantial benefit of nitrogen supplementation. A quadratic ($P < 0.0001$) increase in leaf area was observed with rising CRF concentrations. Leaf area initially increased gradually at lower CRF levels (0 to 0.3 $g\cdot L^{-1}$), followed by a steeper rise from 0.5 to 2.1 $g\cdot L^{-1}$. Leaf areas were 186.6 and 161.3 cm^2, respectively, when the CRF application rate was increased to 4.2 and 8.4 $g\cdot L^{-1}$. The quadratic increase in the leaf area highlights the importance of precise nutrient management for the optimal growth and development of *C. velutinus*. When nutrient supply meets or exceeds plant demand, nutrient uptake and utilization efficiency decrease, leading to a plateau or decline in growth performance (Römheld 2012). Supporting this finding, Agro and Zheng (2014) observed a similar quadratic trend in container-grown woody ornamental plants, where leaf area increased and then plateaued or decreased at higher CRF application rates.

Ceanothus velutinus without soil inoculation nor fertilizer (Group 1) or with autoclaved native soil and without fertilizer (Group 3) recorded the lowest SPAD readings of 5.0 and 9.5, respectively (Table 5). In contrast, plants treated with the manufacturer-recommended application rate of 3.2 $g\cdot L^{-1}$ CRF but without soil inoculation (Group 2) or with autoclaved native soil (Group 4) had significantly higher SPAD values of 38.3 and 33.7, respectively, demonstrating the substantial positive impact of balanced fertilizer application on leaf greenness. Inoculated plants showed varying SPAD readings depending on nitrogen availability. Plants inoculated with native soil and treated with the nitrogen-limited nutrient solution (Group 5) had a SPAD reading of 19.5, suggesting lower leaf greenness. Conversely, plants inoculated with native soil and treated with the nitrogen-limited nutrient solution supplemented with 2mM NH_4NO_3 (Group 6) resulted in a significantly greater SPAD reading of 40.7. The increase in CRF levels from 0 to 8.4 $g\cdot L^{-1}$ led to steady linear ($P < 0.0001$) increase in SPAD readings, with the highest SPAD of 46.0 observed in plants treated with 8.4 $g\cdot L^{-1}$ CRF compared with those treated with 0 and 0.1 $g\cdot L^{-1}$ CRF with the lowest SPAD readings of 11.4 and 10.6, respectively.

Plants without soil inoculation nor fertilizer (Group 1) or with autoclaved native soil and without fertilizer (Group 3) exhibited the lowest leaf dry weights of 0.1 and 0.1 g and the lowest root dry weights of 0.2 and 0.3 g, respectively (Table 6). In contrast, plants treated with the manufacturer-recommended application rate of 3.2 $g\cdot L^{-1}$ CRF and without soil inoculation (Group 2) had significantly greater leaf and root dry weights of 3.1 and 2.6 g, respectively. Similarly, plants with autoclaved native soil (Group 4) also showed significantly greater leaf and root dry weight of 3.9 and 3.3 g, respectively, when compared with untreated controls (Groups 1 and 3). Nitrogen availability influenced plant growth, with plants inoculated with native soil and treated with the nitrogen-limited nutrient solution (Group 5) showing significantly lower leaf and root dry weights of 0.1 and 0.4 g, respectively. In contrast, plants inoculated with native soil and treated with the nitrogen-limited nutrient solution supplemented with 2 mM NH_4NO_3 (Group 6) had leaf and root dry weights of 7.6 and 3.9 g, respectively. The application of CRF resulted in quadratic increase in leaf dry weight ($P < 0.0001$). Plants receiving 0, 0.1, 0.3, 0.5, 1.1, and 2.1 $g\cdot L^{-1}$ CRF recorded

Table 6. **Leaf and root dry weights (DW) of *Ceanothus velutinus* treated with 0.0 to 8.4 g·L^{-1} of controlled-release fertilizer (CRF) or a nitrogen-limited nutrient solution with or without the addition of 2 mM ammonium nitrate (NH_4NO_3) at harvest.**

Group	Soil addition	Treatment[z]	Leaf DW (g)	Root DW (g)
1	No	No fertilizer	0.1 d[y]	0.2 e
2	No	3.2 g·L^{-1} CRF (manufacturer-recommended application rate)	3.1 b	2.6 bc
3	Autoclaved native soil	No fertilizer	0.1 d	0.3 e
4	Autoclaved native soil	3.2 g·L^{-1} CRF (manufacturer-recommended application rate)	3.9 b	3.3 ab
5	Native soil	Nitrogen-limited nutrient solution	0.1 cd	0.4 de
6	Native soil	Nitrogen-limited nutrient solution with 2 mM NH_4NO_3	7.6 a	3.9 ab
7	Native soil	0 g·L^{-1} CRF	0.3 cd	0.7 de
8	Native soil	0.1 g·L^{-1} CRF	0.3 cd	0.5 de
9	Native soil	0.3 g·L^{-1} CRF	0.5 cd	0.8 de
10	Native soil	0.5 g·L^{-1} CRF	1.0 c	1.4 d
11	Native soil	1.1 g·L^{-1} CRF	1.3 c	1.7 cd
12	Native soil	2.1 g·L^{-1} CRF	3.8 b	4.0 ab
13	Native soil	4.2 g·L^{-1} CRF	7.0 a	4.4 a
14	Native soil	8.4 g·L^{-1} CRF	9.4 a	4.0 ab
Trend for plants treated with 0.0 to 8.4 g·L^{-1} CRF			[x]**Q******	**L******

[z]Treatment refers to the application of either controlled-release fertilizer (CRF) or a nitrogen-limited nutrient solution with or without the addition of 2 mM NH_4NO_3. The CRF used was Osmocote 15N-3.9P-10K (Osmocote Plus 15-9-12; Israel Chemicals, Tel Aviv-Yafo, Israel). Plants in groups 2 and 4 received the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF. Plants in groups 5 and 6 were treated with nitrogen-limited nutrient solutions, with group 6 receiving an additional 2 mM NH_4NO_3. Plants in groups 7 to 14 received CRF at increasing rates from 0.0 to 8.4 g·L^{-1}.

[y]Means with same lowercase letters within a column are not significantly different among treatments according to the Tukey-Kramer method of multiplicity at $\alpha \leq 0.05$.

[x]****: significant at $P \leq 0.0001$; L: linear; Q: quadratic.

leaf dry weights of 0.3, 0.3, 0.5, 1.0, 1.3, and 3.8 g, respectively. Plants receiving 4.2 and 8.4 g·L^{-1} CRF exhibited significantly higher leaf dry weights of 7.0 and 9.4 g, respectively, suggesting that nutrient availability primarily drives above-ground growth. The application of CRF resulted in linear increase in root dry weight ($P < 0.0001$). Plants treated with 0, 0.1, 0.3, 0.5 and 1.1 g·L^{-1} CRF recorded lower root dry weights of 0.7, 0.5, 0.8, 1.4, and 1.7 g, respectively. The highest root dry weight of 4.4 g was recorded when plants were inoculated with native soil and treated with 4.2 g·L^{-1} CRF. This result indicates that higher CRF levels promote plant root growth, although the benefits may plateau at a CRF level of 4.2 g·L^{-1}. Cabrera (2003) also found that nitrogen supply promoted root growth up to a certain threshold when two container-grown woody ornamental plants (*Ilex opaca* 'Hedgeholly' and *Lagerstroemia* × 'Tonto') were evaluated. This pattern suggests that while moderate nutrient additions enhance root growth, excessive fertilizer application may hinder root development, likely due to nutrient imbalance or increased salt stress (Cabrera 2003). Ågren and Franklin (2003) further explained that as nutrient availability increases, plants tend to allocate relatively less resources to their roots. Furthermore, Ristvey et al. (2007) reported that higher nitrogen rates promoted shoot growth over root development in young azalea (*Rhododendron* 'Hinodegiri' × *R. yedoense* var. *poukhanense* 'Karen') plants. This observation indicates that nutrient-rich environments can lead to a preferential allocation of resources toward leaf and shoot development to optimize photosynthetic capacity and overall plant productivity (Table 4 and 6); however, excessive fertilizer application may hinder root growth (Table 6).

The substantial increases in the PGI, number of shoots, leaf area, SPAD, and leaf and root dry weights of plants at the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF without soil inoculation underscore the critical role of balanced nutrient availability (Gruhn et al. 2000). In our study, although the morphological parameters of *Ceanothus velutinus* increased linearly or quadratically with increasing CRF levels, these parameters did not change significantly when fertilizer levels exceeded 2.1 g·L^{-1}. This may be because plant growth responses do not correlate well with nutrient availability when substrate nutrient concentration exceeds a certain range (Taiz et al. 2015). For example, a study by Chen et al. (2020) reported that morphological parameters of *Shepherdia* × *utahensis* 'Torrey' did not change significantly when Osmocote 15N-3.9P-10K exceeded 2.1 g·L^{-1}. Similar results were reported by Beddes and Kratsch (2010), where leaf area, shoot dry weight, and root dry weight of *Alnus maritima* (Marsh.) Muhl. ex Nutt did not increase when Osmocote 15N-3.9P-10K was more than 3.6 g·L^{-1}. The contrast between Group 5 and Group 6, which received the same solution but with or without an additional 2 mM NH_4NO_3, highlights the critical role of nitrogen in plant growth and development. The addition of NH_4NO_3 in Group 6 resulted in better plant performance across all measured parameters, including visual score, plant growth index, number of shoots, leaf area, SPAD, leaf DW, and root DW.

A positive quadratic relationship was observed between CRF and the P_n of *Ceanothus velutinus* ($P < 0.0001$; $r^2 = 0.34$) (Fig. 3). The P_n of *C. velutinus* varied significantly across CRF levels. Plants inoculated with native soil and treated with 0.3, 0.5, and 1.1 g·L^{-1} CRF exhibited the lowest P_n of 5.6, 8.1, and 9.0 μmol·m^{-2}·s^{-1}, respectively, compared with plants treated with 4.2 g·L^{-1} CRF which had a P_n of 13.0 μmol·m^{-2}·s^{-1}. Plants without soil inoculation and plants with autoclaved native soil exhibited a P_n of

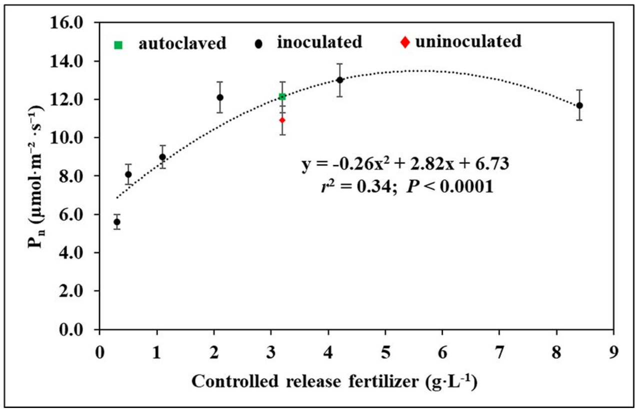

Fig. 3. Net photosynthetic rate (P_n) of *Ceanothus velutinus* seedlings treated with 0.3 to 8.4 g·L^{-1} of controlled-release fertilizer (CRF). The CRF used was Osmocote 15N-3.9P-10K (Osmocote Plus 15-9-12; Israel Chemicals, Tel Aviv-Yafo, Israel). The black round markers represent the plants inoculated with native soil and treated with CRF at different rates, whereas the red diamond marker represents the uninoculated plants that were treated with the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF, and green square marker represents plants treated with autoclaved native soil and the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF. A regression analysis was conducted for the plants inoculated with native soil. The error bars represent the SE of four to nine replications. There was no statistical difference in the P_n of *C. velutinus* inoculated with native soil and treated with 0.5, 1.1, 2.1, 4.2, or 8.4 g·L^{-1} CRF, compared with the uninoculated plants treated with manufacturer-recommended application rate of 3.2 g·L^{-1} CRF or plants with autoclaved native soil and treated with manufacturer-recommended application rate of 3.2 g·L^{-1} CRF, based on Tukey-Kramer method for multiplicity at α ≤ 0.05.

10.9 and 12.1 µmol·m^{-2}·s^{-1}, respectively, when they were treated with the manufacturer-recommended application rate of 3.2 g·L^{-1} CRF. Notably, plants treated with 2.1 and 8.4 g·L^{-1} CRF exhibited P_n of 12.1 and 11.7 µmol·m^{-2}·s^{-1}, respectively. The quadratic increase in net photosynthetic rate with increasing CRF levels up to 2.1 g·L^{-1} reflects the well-established principle that plant growth is positively correlated with nutrient availability up to an optimal range, beyond which further increases yield diminishing returns.

The formation of nodules is essential for nitrogen-fixing activity in plants. Notably, no nodules were formed in plants without native soil inoculation or with autoclaved native soil, regardless of fertilizer application. The complete absence of nodules on plant roots without native soil inoculation or with autoclaved native soil confirms the necessity of viable *Frankia* populations in the soil for successful nodulation. Soil from actinorhizal plant habitats has dense populations of infective *Frankia*, and host plants are the primary factor amplifying *Frankia* populations in the soil (Benson and Silvester 1993, Liu et al. 2024, Schwencke and Carú 2001). Several factors can influence *Frankia* populations in soil, thereby affecting nodulation. Zimpfer et al. (2002) demonstrated that *Casuarina cunninghamiana* cladode extracts increased *Frankia* infectivity in the soil, while amendments like leaf litter enhanced nodule formation (Nickel et al. 2001). However, the abundance and diversity of *Frankia* within root nodules often differs from that in the soil, suggesting plants selectively form nodules with specific *Frankia* strains rather than based on their prevalence in soil (Tekaya et al. 2017). In a study by Yamanaka et al. (2009) inoculation with *Frankia* improved the growth and nodulation of *Alnus sieboldiana* seedlings, with prenodulated seedlings exhibiting enhanced performance. In our study, treatments involving native soil and CRF exhibited significant variations in nodule formation relative to nitrogen levels. Specifically, for treatments involving native soil and CRF, nodule formation varied. No nodules were observed when plants were treated with 0.1 g·L^{-1} CRF, and only one nodule was recorded at both 0 and 0.3 g·L^{-1} CRF (Fig. 4). Nodule formation peaked at three and four nodules at 0.5 and 1.1 g·L^{-1} CRF, respectively. However, as CRF levels increased further (high N rate: Table 1), nodule formation declined, with only two nodules at 2.1 g·L^{-1} and no nodules were observed at 4.2 and 8.4 g·L^{-1}. The peak in nodule formation at 0.5 and 1.1 g·L^{-1} CRF followed by a decline is likely associated with the significant impact of nitrogen fertilizer on the infection process of actinorhizal plants (Chen et al. 2020).

In our study, soil was collected from the rhizosphere of *Ceanothus velutinus* in Tony Grove, UT, USA, where root nodules were observed (Fig. 4A). The soil samples collected from wild populations are known to have significant effects on nodulation on actinorhizal plants (Chen et al. 2020). The use of autoclaved native soil in our study served as an important control to evaluate the role of microbial communities, particularly *Frankia*, in the growth and development of *C. velutinus*. Autoclaving is a widely accepted method for soil sterilization to eliminate microbial populations, including actively growing organisms and resistant structures such as spores (Wolf and Skipper 1994). In this study, a portion of native soil was autoclaved at 125 C (257 F) for one hour after a preincubation period, aiming to destroy viable *Frankia* and other soil microbes while preserving the soil properties. Plants grown in autoclaved native soil (Groups 3 and 4) did not exhibit significantly better growth compared with uninoculated controls (Groups 1 and 2). This observation aligns with the understanding that, while autoclaving does not significantly alter macronutrient levels, including nitrogen (Ding et al. 2023), the absence of microbial activity hinders nutrient mineralization and uptake processes crucial for plant growth. Additionally, the lack of nodules in plants grown in autoclaved native soil treatments confirmed that successful nitrogen-fixing symbiosis depends on the presence of viable *Frankia* (Schwencke and Carú 2001). These results emphasize the critical role of live microbial populations in establishing effective symbiosis and enhancing plant growth through nitrogen fixation (Benson and Silvester 1993). The comparison between autoclaved native soil treatments and those with native soil inoculation highlights the significant impact of soil microbiota on *C. velutinus* growth and

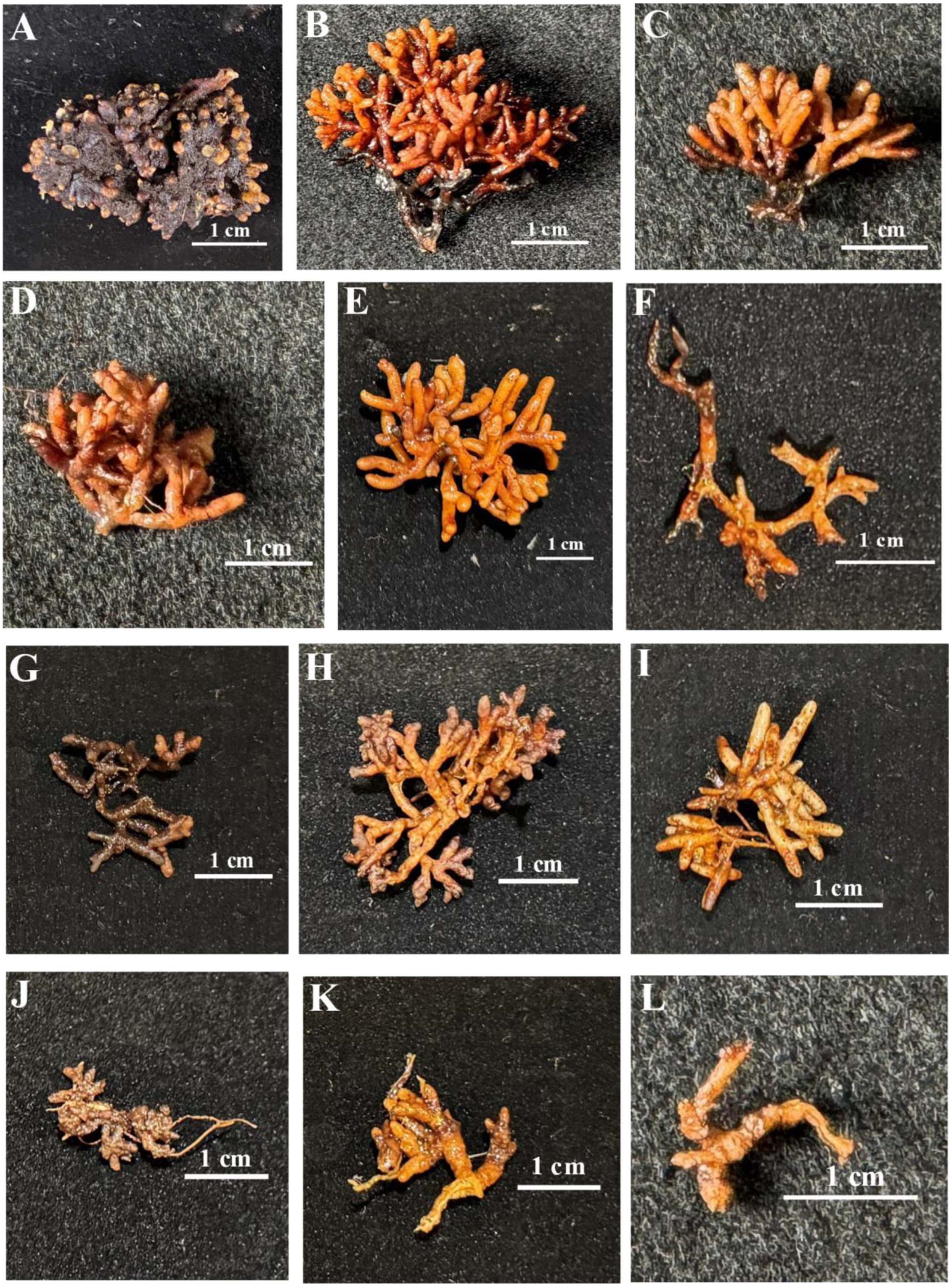

Fig. 4. Root nodules observed in the soil collected from the rhizosphere of *Ceanothus velutinus* (A) at Tony Grove, UT, USA, and nodules collected from *C. velutinus* roots when inoculated with native soil and treated with 0 g·L^{-1} (B), 0.3 g·L^{-1} (C), 0.5 g·L^{-1} (D, E, F), 1.1 g·L^{-1} (G, H, I, J), and 2.1 g·L^{-1} (K, L) of controlled-release fertilizer (CRF). The CRF used was Osmocote 15N-3.9P-10K (Osmocote Plus 15-9-12; Israel Chemicals, Tel Aviv-Yafo, Israel).

development. However, further investigations are required to confirm the mechanism of *Frankia* strains infecting *C. velutinus*.

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