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RESEARCH ARTICLE

Morphological features of perennial legumes root systems in the context of their agroecological impact on soil

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Abstract

The morphological and quantitative features of the underground architectonics of six perennial legume species (*Medicago sativa* L., *Trifolium pratense* L., *Onobrychis arenaria* Kit., *Melilotus albus* Medik., *Lotus corniculatus* L., *Galega orientalis* Lam.) were investigated during the first two years of the growing season, and their direct impact on the ecological and agrochemical parameters of grey forest soil in the Ukrainian forest-steppe was evaluated. It was established that during the second year of vegetation, a rapid multiplicative increase in individual linear plant parameters occurs; *Melilotus albus* (260 cm) and *Medicago sativa* (258 cm) lead in taproot penetration depth, while *Lotus corniculatus* leads in the accumulation of regeneration buds (51 units). It was found that from a coenotic perspective, the peak agroecological effect per hectare occurs specifically during the second year of vegetation. During this period, *Galega orientalis* forms a record fresh biomass of underground organs (322.40 t/ha), ensuring the highest rates of humus accumulation in the soil (up to 2.05% compared to the baseline 2.0%). The study proved the existence of clear functional links between the architecture of the underground sphere of grasses and the transformation of nutrients: The deep branching and thick central root of *Onobrychis arenaria* (7 mm) maximize the content of available phosphorus (413 mg/kg) and potassium (84 mg/kg), while the powerful lateral roots of *Melilotus albus* (3.0 mm) stimulate calcium accumulation (135 mg/kg). Two-year cultivation of legumes optimizes soil acidity, reducing hydrolytic acidity by 24.5-28.3% and shifting the pH toward neutral values (7.2-7.3). The results reveal the phytomorphological mechanisms of fertility restoration and the improvement of the soil chemical profile in agroecosystems.

Keywords: Root system architecture, Perennial legumes, Agroecological impact, Humus accumulation, Available phosphorus, Soil bioremediation

Introduction

Modern agroecology and plant biology are paying increasing attention to the study of the underground sphere of phytocoenoses (Kaletnik, et al. 2020). The architectonics of root systems is a key factor that determines not only the adaptive capacity of plants to environmental conditions (Kaletnik, et al. 2024) but also serves as a powerful tool for biological land reclamation and the regulation of soil processes (Petrychenko, et al. 2025). Perennial legume grasses play a unique role in agroecosystems due to their ability to radically transform the physicochemical and biological properties of the soil (Petrychenko, et al, 2024, Razanov, et al. 2024). They act as natural depositors of organic matter, optimize nitrogen and phosphorus regimes, and improve the structure of the soil profile (Butenko, et al. 2025).

Most classical studies focus on the assessment of aboveground biomass and the economic value of legume crops, while the phytomorphological features of their underground organs often remain overlooked due to the high labor intensity of direct root study methods (Razanov, et al. 2024). However, modern global trends in phytomorphology prove that it is specifically the linear parameters, volume, branching architecture, and thickness of the root skeletal axes that determine the depth and intensity of their ecological impact on the soil environment (Didur and Pantsyreva, 2025). In the context of global climate change and the degradation of agricultural landscapes in the Ukrainian forest-steppe, there is an urgent need for a detailed comparative analysis of the underground sphere of various perennial grass species to optimize crop rotations and restore the fertility of grey forest soils (Kaletnik, et al. 2025, Bulgakov, et al. 2023).

The aim of the study was a comprehensive investigation of the dynamics of morphological and quantitative parameters of the root systems of six leading perennial legume species during a two-year vegetation cycle and the establishment of their direct impact on the ecological and agrochemical indicators of grey forest soil in the conditions of the right-bank forest-steppe of Ukraine.

Materials and Methods

Field research was conducted during 2023-2025 at the "Agronomichne" Research Farm (Vinnytsia district, Vinnytsia region, Ukraine; geographical coordinates: 49°11′ N, 28°22′ E). The research region belongs to the zone of the Right-Bank Forest-Steppe of Ukraine with a temperate continental climate. The soil cover of the experimental field is represented by grey forest, deep, surface-gleyed loamy soils. The objects of the study were six species of perennial legume grasses in their first and second years of vegetation: Alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), sand sainfoin (*Onobrychis arenaria* Kit.), white sweet clover (*Melilotus albus* Medik.), birdsfoot trefoil (*Lotus corniculatus* L.), and fodder galega (*Galega orientalis* Lam.). Sowing was carried out in the spring period in accordance with generally accepted zonal recommendations.

The study of the morphology and architectonics of the root systems was carried out using the method of full excavation according to the modified methodology of Shalait and Stankov. To determine the maximum penetration depth and the radius of root distribution, delicate layer-by-layer washing of soil monoliths with water under low pressure was performed. At least 10 typical plants were sampled from each plot during the flowering phase. For each plant, the following linear and structural parameters were measured: Root system penetration depth and distribution radius (using a measuring tape); the depth of the main mass of branching; the thickness of the root collar, the central root, and the first-order lateral roots (using an electronic caliper with an accuracy of 0.1 mm); and the number of regeneration buds on the root collar (by direct visual counting).

Quantitative parameters of the underground sphere were determined using the following methods: Total root length was measured by the method of precision tensometric overlay; root surface area was calculated using the formula for cylindrical segments based on the average diameter and length of the roots; root system volume was estimated by the water displacement method (volumetrically) in graduated cylinders; the fresh weight of roots per plant was determined by weighing on an analytical balance immediately after washing and removing excess capillary moisture with filter paper. The calculation of root mass and volume per hectare was conducted based on the actual plant density for each year of vegetation.

Soil samples were taken from the 0-30 cm layer after two years of cultivation of each crop. Laboratory analyses were performed in the certified laboratory of the South-Western Interregional Center of the State Institution (SWIC-SI) "Institute of Soil Protection (ISP)" using standard methodologies: Humus content by the Tyurin method; hydrolyzed nitrogen by the Cornfield method; available phosphorus and exchangeable potassium by the Chirikov method; exchangeable calcium by the complexometric method; hydrolytic acidity by the Kappen method; and exchangeable acidity (pH) potentiometrically using a pH meter in a salt extract. Mathematical processing of experimental data was performed using methods of variational statistics, calculating the arithmetic Mean (M) and the standard error of the mean (m).

Results and Discussion

The study of the primary stages of morphogenesis of the underground organs of perennial legumes established a clear species-specificity in the formation of their architectonics as early as the initial stages of ontogenesis. During the first year of vegetation, *Medicago* *sativa* L. (83 cm) and *Melilotus albus* Medik. (82 cm) led in the rate of taproot deepening. Simultaneously, *Melilotus albus* was distinguished by the greatest root collar thickness (12 mm) and an abnormally high diameter of lateral roots (3.0 mm), indicating the rapid establishment of the skeletal framework of the underground sphere. In contrast, *Lotus corniculatus* L. and *Trifolium pratense* L. formed a more shallow system in the first year (44 and 51 cm, respectively), but focused on the regenerative potential of the upper zone: *Lotus corniculatus* formed 33 buds on the root collar (Tab. 1).

In the second year of vegetation, the root architectonics of the studied crops underwent significant structural expansion. *Medicago sativa* and *Melilotus albus* demonstrated the highest vertical growth dynamics, reaching penetration depths of 258 and 260 cm, respectively, which allows them to provide deep drainage of the soil profile. However, in terms of horizontal expansion parameters and the depth of the main branching zone, *Onobrychis arenaria* Kit. showed substantial agroecological value: Its branching zone depth increased to 35 cm, and the thickness of the central root reached 7 mm.

Lateral root thickness indicators stabilized in the second year, with *Melilotus albus* predominating (3.0 mm), ensuring the creation of large biopores in the soil after root decomposition. The dynamics of recovery bud accumulation on the root collar in the second year showed that *Lotus corniculatus* held the advantage (51 units), significantly outperforming *Trifolium pratense* (28 units) and *Onobrychis arenaria* (26 units); this indicates its high coenotic stability and capacity for intensive vegetative restoration of aboveground biomass.

The evaluation of the underground architectonics of legume grasses during the first two years of vegetation revealed patterns in the formation of their initial agroecological potential. During the first year of vegetation, *Onobrychis arenaria* Kit. stood out significantly for its linear growth intensity and surface contact area with the solid phase of the soil, forming a total root length of 2500.0 dm/plant and a surface area of 200.0 dm²/plant. High length values were also recorded for *Trifolium pratense* L. (1974.0 dm/plant). At the same time, in terms of underground biomass accumulation per unit area in the first year, *Galega orientalis* Lam. (80.33 t/ha) and *Medicago sativa* L. (76.31 t/ha) predominated (Tab. 2).

Table 1. Morphological parameters and architecture of the root systems of perennial legumes during the first and second years of vegetation.

Growth stage and morphological metrics	*Medicago sativa* L.	*Trifolium pratense* L.	*Onobrychis arenaria* Kit.	*Melilotus albus* Medik.	*Lotus corniculatus* L.	*Galega orientalis* Lam.
Year 1						
Root penetration depth (cm)	83	51	65	82	44	52
Root system radius (cm)	25	9	15	20	18	16
Depth of main branching zone (cm)	15	18	23	18	21	28
Root collar thickness (mm)	11	6	8	12	7	5
Central main root thickness (mm)	4	2	4	6	3	4
Lateral root thickness (mm)	1.5	0.8	1	3	1.1	1
Number of buds on the root collar (pcs)	12	14	13	6	33	8

			Year 2			
Root penetration depth (cm)	258	167	178	260	87	108
Root system radius (cm)	28	20	23	30	21	27
Depth of main branching zone (cm)	26	24	35	31	25	32
Root collar thickness (mm)	18	9	15	17	7	11
Central main root thickness (mm)	5	4	7	6	3	5
Lateral root thickness (mm)	2	0.9	1.3	3	1.2	1
Number of buds on the root collar (pcs)	25	28	26	6	51	12

Table 2. **Spatial distribution and quantitative biometric parameters of root configurations in perennial legumes across individual and coenotic levels.**

Growth stage and morphological metrics	***Medicago sativa* L.**	***Trifolium pratense* L.**	***Onobrychis arenaria* Kit.**	***Melilotus albus* Medik.**	***Lotus corniculatus* L.**	***Galega orientalis* Lam.**
			Year 1			
Total root length (dm/plant^{-1})	840	1974	2500	793.6	666.7	840
Surface contact area (dm^2/plant^{-1})	67.2	98.7	200	111.1	40	67.2
Individual root volume (dm^3)	0.42	0.37	1.25	1.25	0.2	0.42
Coenotic mass yield (t/ha^{-1})	76.31	26.08	23.52	70.43	21.06	80.33
			Year 2			
Total root length (dm/plant^{-1})	2369.1	2880	3407.7	2565	1633.3	2547.8

Surface contact area ($dm^2/plant^{-1}$)	260.6	230.4	443	359.1	98	229.3
Individual root volume (dm^3)	2.28	1.44	4.43	4.04	0.49	1.72
Coenotic mass yield (t/ha^{-1})	192.06	53.76	110.1	123.95	31.22	322.4

In the second year of vegetation, a rapid multiplicative increase in all biometric indicators of underground organs was observed across all studied species, which directly determines the peak of their ecological impact on the soil environment. The maximum individual root length per plant was demonstrated by *Onobrychis arenaria* (3407.7 dm), as well as *Trifolium pratense* (2880.0 dm). The highest active root surface area per plant was recorded in *Onobrychis arenaria* (443.0 dm^2) and *Melilotus albus* Medik. (359.1 dm^2), indicating the formation of a dense rhizosphere zone that activates microbiological processes.

However, from a coenotic perspective-in terms of the mass and volume of root systems per hectare-*Galega orientalis* held the absolute advantage in the second year of vegetation. This species formed a high fresh biomass of the underground part-322.40 t/ha. A powerful agroecological contribution to the arable soil layer was also provided by *Medicago sativa*, whose root mass reached 192.06 t/ha, and *Melilotus albus*, which accumulated 123.95 t/ha of underground phytomass with a high volume (4.04 dm^3/plant). The most moderate quantitative parameters in the second year of vegetation were observed in *Lotus corniculatus* L. (mass of 31.22 t/ha), which compensates for this with a high density of recovery buds.

A comparison of the morphometry of the underground organs of legume grasses in the second year of vegetation with changes in the ecological and agrochemical properties of the soil revealed direct functional links between root architectonics and soil formation processes. The highest increase in humus content was recorded under the crops of *Galega orientalis* (up to 2.05% compared to the baseline 2.0%) and *Medicago sativa* (2.03%). This correlates directly with the fact that in the second year of vegetation, it was these two crops that formed the most substantial fresh root mass per hectare: *Galega orientalis*- 322.40 t/ha, and *Medicago sativa*-192.06 t/ha. The large volume of underground organic matter from these species creates a reliable basis for humification processes (Tab. 3).

Table 3. Soil chemical profile modification and shift in agrochemical metrics induced by a 2-year growth cycle of diverse perennial legumes (M ± m).

Soil property metrics	**Control (baseline)**	***Medicago sativa* L.**	***Trifolium pratense* L.**	***Onobrychis arenaria* Kit.**	***Melilotus albus* Medik.**	***Lotus corniculatus* L.**	***Galega orientalis* Lam.**
Total humus content (%)	2.0 ± 0.01	2.03 ± 0.01	2.01 ± 0.01	2.02 ± 0.01	2.02 ± 0.01	2.01 ± 0.01	2.05 ± 0.03
Hydrolyzable N (mg/kg^{-1})	133 ± 2.83	130 ± 2.83	131 ± 2.83	132 ± 2.83	130 ± 4.24	133 ± 2.83	133 ± 2.83
Available P (mg/kg^{-1})	390 ± 4.24	405 ± 4.24	400 ± 1.41	413 ± 2.83	408 ± 2.83	406 ± 4.24	411 ± 1.41
Exchangeable K mg/kg^{-1})	64 ± 2.83	80 ± 4.24	77 ± 2.83	84 ± 0.86	76 ± 1.41	82 ± 2.83	82 ± 2.83
Calcium content (mg/kg^{-1})	130 ± 2.83	130 ± 2.83	131 ± 4.24	132 ± 2.83	135 ± 5.66	133 ± 4.24	132 ± 1.41

Hydrolytic acidity (meq/100g^{-1})	0.53 ± 0.014	0.38 ± 0.028	0.38 ± 0.028	0.43 ± 0.042	0.40 ± 0.028	0.38 ± 0.014	0.40 ± 0.014
Active reaction (pH_{KCl})	7.0 ± 0.14	7.3 ± 0.14	7.3 ± 0.14	6.6 ± 0.28	7.2 ± 0.56	7.3 ± 0.28	7.2 ± 0.28

All studied species ensured a significant increase in the content of available phosphorus (from 390 to 400-413 mg/kg) and potassium (from 64 to 76-84 mg/kg). *Onobrychis arenaria* left behind the maximum amount of available phosphorus (413 mg/kg) and potassium (84 mg/kg). This is explained by its deep branching zone (35 cm) and thick central root (7 mm), which allow the plant to draw poorly soluble mineral compounds from deep layers and, after the decomposition of fine roots, enrich the arable horizon with them.

The highest calcium content (135 mg/kg) was recorded under *Melilotus albus* crops. This effect is consistent with the morphological feature of the species in the second year-an abnormally high thickness of the lateral roots (3.0 mm). The powerful lateral roots of *Melilotus albus* intensively drain the soil and are capable of converting calcium from carbonate compounds into a mobile form, which improves the structure of the soil absorbing complex.

The cultivation of most grasses (except *Onobrychis arenaria*) led to the optimization of pH toward a neutral medium (from pH 7.0 to 7.2-7.3) and a sharp decrease in hydrolytic acidity from 0.53 to 0.38-0.40 meq/100 g of soil. The most pronounced reduction in acidity (down to 0.38) was observed under *Medicago sativa*, *Trifolium pratense*, and *Lotus corniculatus*. For *Lotus corniculatus* and *Trifolium pratense*, this effect is achieved not through depth, but due to the high density of the root network in the upper layer (total root length of *Trifolium pratense*-2880 dm/plant, and surface area-230.4 dm²/plant), which activates rhizosphere microflora and stabilizes the soil solution.

The transformation of the nitrogen regime (hydrolyzed nitrogen content of 130-133 mg/kg) indicates that in the second year of vegetation, legume grasses actively utilized existing soil nitrogen to form their powerful vegetative and underground mass, while the primary effect of nitrogen fixation will manifest in subsequent years following the mineralization of this biomass.

Conclusion

High species specificity in the architectonics of the underground sphere of the studied legume grasses was established during the stages of initial development and the formation of a stable stand. As early as the second year of vegetation, *Melilotus albus* Medik. (260 cm) and *Medicago sativa* L. (258 cm) predominate in terms of the vertical penetration depth of the taproot, while *Lotus corniculatus* L. forms the most shallow system (87 cm) but possesses the highest regenerative potential of the root collar (51 recovery buds).

It has been substantiated that for most perennial legume grasses, the second year of vegetation is the stage of reaching the peak agroecological effect in the coenotic dimension. Due to the optimal plant density in the second year, maximum indicators of underground biomass accumulation per unit area were recorded: In particular, *Galega orientalis* Lam. has the largest fresh mass of 322.40 t/ha and a volume of 488.5 m³/ha; high productivity was also demonstrated by *Medicago sativa* L. (192.06 t/ha) and *Melilotus albus* Medik. (123.95 t/ha).

The existence of a direct functional link between the morphological parameters of the underground organs of grasses after two years of cultivation and the improvement of the ecological and agrochemical state of the soil has been proven. The powerful root biomass of *Galega orientalis* and *Medicago sativa* ensures the highest rates of organic matter accumulation, increasing the humus content to 2.03-2.05% compared to the baseline 2.0%.

The study found that the differentiation of root architectonics determines the nature of the transformation of the soil's mineral profile: The deep branching and thick central root of *Onobrychis arenaria* Kit. (35 cm and 7 mm, respectively) contribute to the maximum mobilization and deposition of available phosphorus (413 mg/kg) and potassium (84 mg/kg) in the arable layer. Conversely, the abnormally high thickness of the lateral roots of *Melilotus albus* Medik. (3.0 mm) provides intensive loosening and the highest level of accumulation of available calcium (135 mg/kg).

Cultivating legume grasses for two years effectively optimizes the acid-base properties of grey forest soil. Due to the high density of the root network in the upper horizons (*Trifolium pratense*-2880 dm/plant, *Medicago sativa*-2369 dm/plant), hydrolytic acidity decreases by 24.5-28.3% (down to 0.38 meq/100 g of soil) and pH shifts toward neutral values (7.2-7.3), creating an optimal ecological background for rhizosphere microflora and subsequent crops in the rotation.

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