

## Perennial forage legumes as an element of sustainable systems

Dalibor D. TOMIĆ<sup>1\*</sup>, Vladeta I. STEVOVIĆ<sup>1</sup>, Dragan ĐUROVIĆ<sup>1</sup>,  
Miloš MARJANOVIĆ<sup>1</sup>, Milomirka R. MADIĆ<sup>1</sup>, Nenad PAVLOVIĆ<sup>1</sup>,  
Đorđe LAZAREVIĆ<sup>2</sup>, Mirjana PETROVIĆ<sup>2</sup>,  
Mirjana RADOVANOVIĆ<sup>1</sup>

<sup>1</sup>University of Kragujevac, Faculty of Agronomy, 34 Cara Dušana, 32000 Čačak, Serbia; [dalibort@kg.ac.rs](mailto:dalibort@kg.ac.rs) (\*corresponding author);  
[vladeta@kg.ac.rs](mailto:vladeta@kg.ac.rs); [dtaf84@gmail.com](mailto:dtaf84@gmail.com); [milosm@kg.ac.rs](mailto:milosm@kg.ac.rs); [mmadic@kg.ac.rs](mailto:mmadic@kg.ac.rs); [nenadpavlovic@kg.ac.rs](mailto:nenadpavlovic@kg.ac.rs); [mira.radovanovic@kg.ac.rs](mailto:mira.radovanovic@kg.ac.rs)

<sup>2</sup>Institute for Forage Crops, Globoder bb., 37251 Kruševac, Serbia; [djordje.lazarevic@ikbks.com](mailto:djordje.lazarevic@ikbks.com); [mirjana.petrovic@ikbks.com](mailto:mirjana.petrovic@ikbks.com)

### Abstract

In the current intensive systems of agricultural production, many important features, i.e., functions of the agroecosystem have been degraded and disrupted. The intensification of agricultural production inevitably leads to land degradation in terms of its physical, chemical, and biological properties. The increasing presence of monocultures, reduced crop rotation, and excessive use of mineral nutrients, lead to several negative phenomena in such agroecosystems. Along with efforts to reduce energy consumption, and environmental pollution, intensify sustainable agriculture systems, and maintain biodiversity, the possibility of increasing the area under perennial forage legumes should be considered. As nitrogen fixers, these plants are minimally fertilized with nitrogen fertilizers whose residues in the soil are lost by leaching, causing pollution of groundwater as well as surface watercourses. The introduction of perennial legumes in the crop rotation can provide numerous benefits, such as increased and more stable yields of protein-rich biomass, conservation, and repair of land resources, increased yield stability, better utilization of nutrients, water, and light, as well as weed, disease, and pest control. The introduction of legumes in production systems would limit the increasingly pronounced land degradation. In order to develop sustainable agriculture, market policy should recognize the value of products obtained from leguminous plants through certain agricultural policy measures.

**Keywords:** biodiversity; environment protection; perennial legumes; sustainable agricultural systems

### Introduction

The trend in the number of certain species of domestic animals at the global level is in the direction of decreasing the number of ruminants and increasing the number of monogastric animals (Lassaletta *et al.*, 2014). Consumption of meat and other products from monogastric animals leads to a change in the sowing structure, i.e., the way of using agricultural land (Pelletier and Tyedmers, 2010). The intensification of agriculture has led to the fact that, instead of using the pasture method, there is an increase in the use of the stable method of growing ruminants, which in itself leads to greater use of concentrated nutrients (Vicenti *et al.*, 2009). It also

Received: 08 Jun 2023. Received in revised form: 01 Jul 2023. Accepted: 23 Aug 2023. Published online: 31 Aug 2023.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

leads to an increased concentration of certain species of domestic animals in certain regions, especially pigs and poultry, which results in pollution of the agroecosystem, as well as the environment as a whole (Szogi *et al.*, 2015). Increased concentrations, mainly of nitrogen, and also of phosphorus compounds, make agricultural areas less suitable for growing legumes (Murphy-Bokern *et al.*, 2017). At the expense of reducing the area under legumes, as a rule, the areas under cereals and to some extent oil plants are becoming increasingly, and in that way, production systems are increasingly homogenized (Stevović *et al.*, 2020). This, together with the fact of a generally small and irregular application of organic fertilizers on arable land, inevitably leads to accelerated degradation of the agroecosystem to worrying levels.

Recently, there has been a problem in less intensive feed production systems, which largely depend on natural, semi-natural, and sown grasslands. Such grasslands are exposed to overexploitation in many parts of the world, especially in conditions of temporally and spatially unevenly distributed precipitation, which is a consequence of climate change (FAO, 2010). This increases the risk of degradation and erosion of grasslands and leads to a lack of protein nutrients. These problems can be solved by the more intensive introduction of perennial forage legumes into production systems. Legumes belong to the family Fabaceae, subfamily Papilionoideae. The most crucial perennial forage legumes are alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), and birdsfoot trefoil (*Lotus corniculatus* L.). They represent one of the most important groups of plants in the systems of conventional agricultural production and an indispensable component of the system of sustainable agriculture, i.e., organic production (Foyer *et al.*, 2018). In the Republic of Serbia, perennial legumes are grown on about 170,000 ha as pure crops (SYS, 2020), sometimes as combined crops with perennial or annual grasses, other legumes, or other plants. This paper aims to analyze the importance and possibilities and provide suggestions for growing perennial forage legumes to form and develop sustainable agricultural production systems, which would contribute to efforts to reduce the degradation of agroecosystems in current agricultural production systems.

### **How important forage legumes are?**

Due to the high variability in the most important agronomic properties, perennial forage legumes can be grown in different agroecological conditions, used in different ways, and grown for different purposes. They are distinguished with high protein content in the forage, with a favourable amino acid composition. In addition to quality proteins, fibers, folic acid, B vitamins, minerals (iron, zinc), and secondary bioactive compounds are present in legumes also (Wolf-Hall *et al.*, 2017). That makes this group of forage plants indispensable in meeting the needs of domestic animals for high-quality bulky food, in an economically, ecologically, and healthily far more acceptable way than other agricultural plants (Petrović *et al.*, 2016; Tomić, 2017) (Table 1). No less important is their role in preserving the most important properties/functions of natural, semi-natural, and controlled agroecosystems, to a much greater extent than other cultivated plants.

The increase of areas under legumes is especially important for systems of sustainable, i.e., organic agriculture (Annicchiarico *et al.*, 2017). The growing of perennial legumes provides high-protein feed, along with benefits for the ecosystem, such as efficient use of resources and energy, lower greenhouse gas emissions, and increased soil fertility (Cellier *et al.*, 2015). The biomass of legumes decomposes faster in the soil compared to the biomass of other plants, such as those from the Poaceae and Brassicaceae families, leaving nitrogen in the soil for these crops (Stevović *et al.*, 2020).

Forage legumes have great agrotechnical importance. Their value in preserving the soil structure, crop rotation, preventing erosion, and weed control is very important (Ćupina *et al.*, 2004). In addition, these plants improve the physical properties of the soil, reduce the number of pathogens and pests in the soil, and provide more nitrogen to subsequent crops (Blackshaw *et al.*, 2005). A greater presence of legumes in the crop rotation can provide not only an increase in the production of quality forage and grain but also a reduction of the pressure on natural resources (Foyer *et al.*, 2018).

**Table 1.** The importance of forage legumes for feeding farm animals and people

The role	Conditions and Benefits	References
Feed for domestic animals	The high protein content in the forage, with a favourable amino acid composition	Petrović <i>et al.</i> (2016); Murphy-Bokern <i>et al.</i> (2017); Tomić (2017)
	Contain fibers, folic acid, B vitamins, minerals (iron, zinc), and secondary bioactive compounds	Wolf-Hall <i>et al.</i> (2017)
	High yield of forage and hay	Tomić <i>et al.</i> (2012a); Tomić <i>et al.</i> (2012b); Tomić <i>et al.</i> (2014); Tomić (2017); Tomić <i>et al.</i> (2018); Stevović <i>et al.</i> (2020)
Human food	Alternative protein for meat replacement	Dandamudi <i>et al.</i> (2018); Kaur <i>et al.</i> (2022)
	Dietary food for disease prevention	Valachovičova <i>et al.</i> (2017)
	In the food industry	Chen <i>et al.</i> (2006); Dagleish (2006) Garcia-Mora <i>et al.</i> (2015); Dandamudi <i>et al.</i> (2018)
	Sprouts of perennial legumes as human food	Gan <i>et al.</i> (2017); Xu <i>et al.</i> (2019); Chiriac <i>et al.</i> (2020)
Other	As honey plants In medicinal purposes	Stevović <i>et al.</i> (2020)

The advantages of growing leguminous plants are largely the result of their most important and unique feature: the ability to fix atmospheric nitrogen through symbiosis with root bacteria, whose common name is rhizobia, thus satisfying most of their needs for this nutrient and providing it to other organisms - soil microorganisms, non-leguminous plants in mixture crops, or cultivated plants that come after them in crop rotation (Bokan *et al.*, 2016).

In the era of the Green Revolution, the cultivation of legumes was quite limited, mainly due to the large use of synthetic mineral fertilizers. Knowledge of the damaging impact of especially synthetic nitrogen fertilizers on the physical, chemical, and biological properties of the soil, as well as increased pollution of the agroecosystem and the environment as a whole, has restored interest in growing legumes as a component of intensive crop rotations (Battye *et al.*, 2017). Annual and perennial forage legumes can be grown for very different purposes; such as protection of soil from erosion, green manure, mulching, sowing in rows in orchards and vineyards, as honey, ornamental plants, or medicinal purposes (Stevović *et al.*, 2020).

The United Nations Framework Convention on Climate Change encouraged research for adapting agricultural exploitations to the new climate change. The aim is to set up pastures with complex perennial grasses and legume mixtures alongside annual forage crops, as a means to adapt their exploitation to present-day and future climate conditions.

### Systems for producing forage from leguminous plants

Perennial forage legumes can be grown for various purposes, such as grain, green forage, hay, silage, haylage, or green manure, where the choice of production system depends on climatic and edaphic conditions, as well as the purpose of the final product (Peyraud *et al.*, 2009). They are mainly represented as a component of natural, semi-natural, and sown grasslands (long-term or short-term), or on arable land on which they are purposely based as pure crops (Vazquez *et al.*, 2022). Sown grasslands are based on arable land and are used for two to several years, by growing species such as red clover, white clover, or birdsfoot trefoil (Stevović *et al.*, 2020). Regardless of the method of utilization, grass forage is realized mainly through meat and milk as the end products of ruminants.

In terms of forage yield, agronomic quality, especially forage quality, mixtures of grasses and legumes have significant agronomic advantages, compared to pure grasses. However, there are several disadvantages of such systems, including weak growth at the beginning of vegetation (Peyraud *et al.*, 2009), less durability when using grazing compared to pure grasses, the risk of bloating, and some difficulties in ensiling or haymaking (Phelan *et al.*, 2014).

### **Use of perennial legumes for human diet**

Proteins represent a very important group of compounds used in human nutrition. Meat is the basic source of protein with high biological value. However, in recent times, there has been an increasing consumer demand for alternative protein products for various reasons (Kaur *et al.*, 2022). The so-called "vegetable meat" made of protein from leguminous plants, is especially important. They are an important source of dietary proteins of high biological value for vegetarian diets, either for cultural or health reasons (Dandamudi *et al.*, 2018). Research shows that a human diet based on plant proteins carries a significantly lower risk of the most serious diseases (Valachovičova *et al.*, 2017). Important properties of legume proteins such as hydration, gelation, or emulsification make this group of plants suitable for industrial processing. The interaction of proteins and water resulting in hydration is very important in the food industry to obtain the ability to gel, swell and retain water (Garcia-Mora *et al.*, 2015). Equally important is their gelling function, which represents the cross-linking of molecules using certain forces with uneven holding power (Chen *et al.*, 2006). The emulsifying property of proteins is one of the most commonly used techniques in the food industry for producing oil-in-water emulsions (Dagleish, 2006).

Legume seeds are used to obtain sprouts for human consumption. Sprouts of red clover and alfalfa have been used in the human diet for centuries (Silva *et al.*, 2013). Today also they are considered a functional food because they contain well-known bioactive substances that provide many health benefits (Gan *et al.*, 2017). During the germination of legumes, the activity of hydrolytic enzymes increased and it leads to an increase in basic biomolecules, mineral substances, and their bioavailability of them (Vlaisavljević *et al.*, 2017; Chiriac *et al.*, 2020). Xu *et al.* (2019) have determined the significant effect of the sprout of perennial legumes on the chemical composition, thermal, and pasting of legume flours.

### **Forage legumes in crop rotation**

In order to control the population of plant diseases, pests, and weeds, to make full use of available nutrients from the depth of the soil profile, crop rotation should include plants with different life cycles, habitus, and root architecture, as well as a sensitivity/resistance spectrum to diseases and pests (Reckling *et al.*, 2016). It is known that crop rotation contributes to the improvement of soil structure, its permeability, microbiological activity, water retention capacity, the content of organic matter in the soil, preventing erosion, and stabilizing crop yields and sustainability of production systems (Bokan *et al.*, 2016) (Table 2).

Leguminous species affect the next crop through a series of effects such as crop rotation, and nitrogen effects, as well as through a series of legume-specific effects (Peoples *et al.*, 2009). The effect of the so-called intermittent crop is pronounced when the crop rotation is not diverse, for example, species from the group of cereals change successively, and some of the broad-leaved cultivated plants are introduced into such a system, i.e., legumes or a short-term grass-legume mixture (Robson *et al.*, 2002). The most important outcome of this effect is a reduction in the number of soil pathogens and pests characteristic of, in this example, cereals (Kirkegaard *et al.*, 2008). As pre-crops for cereals in the crop rotation, forage legumes increase soil fertility, and thus grain yield, with a significant reduction in the incidence of root diseases and reduce the number and thus the harmfulness of nematodes (Ates *et al.*, 2013). The effects of nitrogen are reflected in the release of biologically fixed nitrogen from the harvest residues of legumes, where the degree of their decomposition is

limited by the relatively low value of the carbon/nitrogen ratio (Jensen *et al.*, 2004). The main outcome of legume-specific effects is an increased number of bacteria that promote plant growth (Lugtenberg and Kamilova, 2009), especially those that bind hydrogen (Maimaiti *et al.*, 2007), thus contributing to the accelerated development of the next crop. The architecture of the entire root system of legumes, which is characterized by a spindle root, with a reticulate of lateral roots, in contrast to the fibrous root system of cereals, helps infiltrate water into deeper layers, forming channels that facilitate root development and penetration of the next cultivated plant into deeper layers (Neumann *et al.*, 2011).

**Table 2.** Forage legumes' agrotechnical value, role in crop rotation and effect on soil fertility

Conditions and Benefits	References
The amount of fixed atmospheric nitrogen through symbiosis with root bacteria in forage legumes varies in a wide range - between 15 and 390 kg N ha <sup>-1</sup> per year	BNF (2013); Tomić <i>et al.</i> (2014); Bokan <i>et al.</i> (2016); Batty <i>et al.</i> (2017); Stevović <i>et al.</i> (2017); Murphy-Bokern <i>et al.</i> (2017); Tomić <i>et al.</i> (2018) Bekuzarova <i>et al.</i> (2020)
Maintaining the soil's structure and avoiding erosion	Ćupina <i>et al.</i> (2004); Holtham <i>et al.</i> (2007); Prahara and Maitra (2020)
Improve the physical properties of the soil, reduce the number of pathogens and pests in the soil, and provide more nitrogen to subsequent crops	Blackshaw <i>et al.</i> (2005); Stevović <i>et al.</i> (2020); Prahara and Maitra (2020)
Increase the content of organic matter in the soil	Schjønnning <i>et al.</i> (2007)
Legumes biomass as green manure	Baddeley <i>et al.</i> (2017);
Reduction in the incidence of root diseases and reduce the number and thus the harmfulness of nematodes	Ates <i>et al.</i> (2013)
Mulching, sowing inter rows in orchards and vineyards	Lorin <i>et al.</i> (2015); Stevović <i>et al.</i> (2020)
Helps infiltrate water into deeper layers, facilitates root development	Neumann <i>et al.</i> (2011); Huang <i>et al.</i> (2017)
Weed control	Ćupina <i>et al.</i> (2004); Bilalis <i>et al.</i> (2010) Farooq <i>et al.</i> (2011); Tomić <i>et al.</i> (2014); Tomić <i>et al.</i> (2018); Melandera <i>et al.</i> (2020)
Some legumes can reduce the amount of water in too moist soil through greater transpiration	Tello-Gracia <i>et al.</i> (2020)

### Impact on soil fertility

In intensive agricultural systems, the presence of monocultures, reduced crop rotations, and excessive use of mineral nutrients, lead to an expedited process of soil degradation (Stevović *et al.*, 2020). The extent to which the introduction of perennial forage legumes in production systems will be able to mitigate such negative phenomena depends on several factors. It is certain that the correct choice of species, methods of production, and use, as well as the degree of representation of perennial legumes on total agricultural land, can significantly reduce the disadvantages of intensive systems.

By cultivating legumes, quite large amounts of nitrogen can be provided in the soil, taking into account its content in biomass (Bekuzarova *et al.*, 2020). However, due to unsynchronized processes of decomposition of organic matter, mineralization, and losses, on the one hand, and the needs of cultivated plants in certain stages of development, on the other, its utilization by the next crop is realistically reduced (Andrews *et al.*, 2007).

The accelerated development of agriculture, especially in the last few decades, has led to a very rapid decrease in the content of organic matter in the soil, which is now alarmingly low in highly developed agricultural production systems. By cultivating (especially for a longer period) and plowing leguminous crops, even grass-leguminous mixtures increase the content of organic matter in the soil, as well as its quality (Schjønning *et al.*, 2007). The rhizosphere of legumes, unlike other plants, is characterized by a higher content of organic matter, and thus a greater possibility of storing mineral nutrients (Angers and Caron, 1998). The decomposition of dead parts of plants, as well as roots after the decomposition of legume crops, has a favourable effect on the formation of soil aggregates, which can be maintained in a stable form over a long period of time. Plants differ in terms of their impact on soil structure, with differences not only between species but also between cultivars within a species, even between genotypes within a cultivar (Macleod *et al.*, 2007). Most studies have shown that the effect of legumes on the aggregation of soil particles is much more pronounced compared to plants belonging to grasses (Holtham *et al.*, 2007).

The richness of the soil in terms of diversity and the number of useful groups of microorganisms is far greater in soils used for growing legumes, especially perennials, compared to soils where other types of plants are cultivated. The results of Spehn *et al.* (2000) showed that the degree of decomposition of organic matter and the total biomass of microbes depends more on whether legumes are present in the plant cover than on the diversity which confirms the positive impact of legumes on microbial biomass and their activity.

Land fauna is also of great importance for the functioning of the soil system. The presence of earthworms, the most useful representatives of this group, in numerous studies is incomparably higher in soils under leguminous plants, especially perennials, as well as grass-leguminous mixtures, compared to soils under high-intensive annual cultivated plants (Cooledge *et al.*, 2022). The reason for their higher presence in such areas, in relation to intensive production systems, is mainly the higher nutritional value and taste of plant parts, as well as the exudates of leguminous plant roots (Huxham *et al.*, 2005).

### **Influence of legumes on nitrogen status in soil**

The amount of fixed atmospheric nitrogen in forage legumes varies in a wide range - between 15 and 390 kg N ha<sup>-1</sup> per year, depending on the type of legumes, as well as biotic and abiotic factors to which plants are exposed throughout the year (Stević *et al.*, 2017). Only 8–14% of the amount of nitrogen in the aboveground part is found in the underground parts of the plant (BNF, 2013). It is considered that 80% of soil nitrogen in the zone of the root system is deposited through root secretions, as well as dead rhizobium cells, i.e., root tissue (Mayer *et al.*, 2003).

Biotic and abiotic factors that adversely affect the amount of fixed N<sub>2</sub> include: stress caused by diseases, inadequate soil fertility, soil acidity, salinity, drought, high temperatures, and defoliation (removal of the aboveground part). These processes/conditions affect the molecular communication between legumes and rhizobia and/or reduce the photosynthetic activity of the plant, thus determining the amount of fixed N<sub>2</sub> (Tomić *et al.*, 2012a). The high nitrogen content in the soil also reduces the activity of rhizobium (Tomić *et al.*, 2014). Usually, one part of the nitrogen in plants originates from nitrogen fixation, while the rest comes from soil reserves. For example, it is estimated that in alfalfa in the year of sowing about 50% of nitrogen comes from nitrogen fixation, and in subsequent years about 80% (Sheaffer *et al.*, 1989).

Legumes provide some of the nitrogen needed by grasses in grass-legume mixtures. Biologically fixed nitrogen can be provided to grasses in mixtures in several ways: by releasing legume root exudates in the grass root zone, decomposing parts of roots and nodules, using mycorrhizal fungi as carriers, and by the excrement of the grazing animals (Paynel *et al.*, 2001). Here, too, the data on the amount of nitrogen delivered are highly variable and range from 5 to 80 kg ha<sup>-1</sup>; it is considered that this amount satisfies 20-70% of the grasses need

for nitrogen. The amounts of nitrogen delivered to grasses mostly depend on the type of legumes, agroecological factors, as well as the status of nitrogen in the soil (Murphy-Bokern *et al.*, 2017).

The amount of fixed nitrogen in grass-legume mixtures varies greatly and usually decreases during the growing season. The reason for these oscillations is precisely the very dynamic and complex nature of such an agroecosystem (Louarn *et al.*, 2015). In the short term, grasses promote nitrogen fixation by absorbing nitrogen from the soil, encouraging leguminous plants, i.e., rhizobia, to be more active (Pankiewicz *et al.*, 2015). However, this stimulates the growth of grasses, which disrupts the grass-legumes relationship, due to the greater competitive ability of grasses in relation to legumes (Tomić *et al.*, 2018). As a consequence, productivity and persistence, as well as nitrogen fixation of legumes, are significantly reduced. The reduced share of legumes, as well as the lower activity of rhizobium, as a feedback effect, also reduces the amount of nitrogen introduced into the mixture crop through nitrogen fixation, so that the growth of grasses is slowed down (Staniak *et al.*, 2014).

### **Perennial forage legumes and biodiversity**

The negative consequences that accompany today's high-intensity grasslands are reflected in the drastically reduced biodiversity. Agricultural ecosystems are highly dependent on living organisms that are responsible for the decomposition of organic matter, maintaining soil fertility, its structure, pollinating of cultivated plants, etc. Ecological studies, in the recent past, have indicated the extent to which the provision of such ecosystem services is dependent on plant diversity (Zabala *et al.*, 2021) (Table 3). For these reasons, it would be necessary to apply methods that could increase the biodiversity of agroecosystems, especially in agricultural areas intended for forage production, where it would be possible to maintain an acceptable level of productivity. Therefore, but also from the point of view of nutritional value, i.e., maximum utilization of nutrient components of forage, it is necessary to include several types of legumes in such production systems (Sauvadet *et al.*, 2021). With the maximum utilization of natural resources, the positive sides of such production systems are reflected in lower inputs, as well as in the preservation of the physical, chemical, and biological properties of the soil.

Research related to the impact of legumes on biodiversity has in the past been more focused on their impact on natural or semi-natural ecosystems (Altieri and Rogé, 2010). Today, several key traits, such as nitrogen fixation, pollination, weed control, and soil improvement, which provide legumes, are important for sustainable agroecosystems (Sauvadet *et al.*, 2021). Microorganisms that decompose the organic matter of legumes, certain types of fauna found in the soil, as well as organisms that feed on legumes, play an important role in the nitrogen cycle, thus making it available to plants located near legumes (Sugiyama and Yazaki, 2012). The morphology of the roots of most legumes enables production systems with less or no-tillage, because, after their cultivation, the soil structure is more stable, and the soil surface is covered with plant material (Prahara and Maitra, 2020). With the increased activity of microorganisms, the absence of soil mixing has a favorable effect on the activity of beneficial fauna, especially earthworms, as well as on the preservation of organic matter in the soil. In the soil without cultivation, the presence of other useful fauna has also increased (Jordan *et al.*, 2004).

**Table 3.** The role of forage legumes in environment protection

Conditions and Benefits	References
For systems of sustainable, i.e., organic agriculture	Huxham <i>et al.</i> (2005); Huang <i>et al.</i> (2017); Stevović <i>et al.</i> (2020)
Reduction of the pressure on natural resources	Peoples <i>et al.</i> (2009); Reckling <i>et al.</i> (2016); Foyer <i>et al.</i> (2018)
Efficient use of resources and energy, lower greenhouse gas emissions, and increased soil fertility	Cellier <i>et al.</i> (2015); Huang <i>et al.</i> (2017)
Diversity and the number of useful groups of microorganisms	Spehn <i>et al.</i> (2000); Jordan <i>et al.</i> (2004)
Protection of soil from erosion	Nyawade <i>et al.</i> (2019); Li <i>et al.</i> (2019); Stevović <i>et al.</i> (2020)
Increase the number of earthworms	Huxham <i>et al.</i> (2005); Cooledge <i>et al.</i> (2022)
Increase the biodiversity of agroecosystems	Sauvadet <i>et al.</i> (2021); Zabala <i>et al.</i> (2021)
Bioremediation	Fester <i>et al.</i> (2014); Teng <i>et al.</i> (2015); Ansari <i>et al.</i> (2018); Ding <i>et al.</i> (2021)
Source of bioenergy	Sanderson <i>et al.</i> (2020); Kukharets <i>et al.</i> (2023)

### The role of perennial forage legumes in weed control

Due to the high leaf area index, as well as the rapid stem growth in some species, legumes are basically far better competitors to weed species compared to narrow-leaved cultivated plants (Bilalis *et al.*, 2010). Perennial species of legumes that are used several times during the year (mowing/grazing) are, thanks to their rapid regeneration, even better competitors so the presence of weeds in areas where they are grown is very small (Tomić *et al.*, 2018). Perennial forage legumes remain on the same surface for several years, are mowed periodically in that way, weeds are mostly removed before seed formation. Thanks to the highly competitive ability of perennial forage legumes, the emergence of new weeds is prevented (Tomić *et al.*, 2012b).

Mixtures of small-grained legumes and other plants are also very effective in weed control (Tomić *et al.*, 2014), but also production systems known as live mulch (Lorin *et al.*, 2015), in which legumes are sown in the inter-row space of widely grown crops and by competing for food, water, and light, reduce the appearance of weeds. Biomass of plants grown for green manure, as well as harvest residues of some legumes, can greatly reduce the number of certain weed species, preventing their emergence due to disturbed a ratio of the active part of the sunlight spectrum, as well as the temperature regime on the soil surface (Melandera *et al.*, 2020). Laboratory tests have shown that plant residues of some legumes with their chemical ingredients can reduce the emergence and development of certain weeds (Farooq *et al.*, 2011).

### Soil and water conservation

In forage production systems, perennial forage legumes as dense crops can reduce soil erosion by reducing surface water runoff and increasing precipitation infiltration (Nyawade *et al.*, 2019). Live plants and their remains on the soil surface protect the soil from raindrops, prevent compaction of the surface layer and the formation of crusts, and reduce the rate of surface water runoff (Li *et al.*, 2019). The remains of the aboveground part and the roots of legumes increase the content of organic carbon in the soil, stabilize the soil particles, increase macroporosity, and thus increase the degree of infiltration and water retention in the soil (Huang *et al.*, 2017).



Legumes that increase the content of organic matter in the soil and provide its protection with their residues on the surface can preserve water in the soil by increasing infiltration and increasing the water content in the zone of the root system. In contrast, some legumes can reduce the amount of water in too moist soil through greater transpiration (Tello-Gracia *et al.*, 2020).

### **Perennial forage legumes for green manure**

The most common way to increase the amount of nitrogen introduced into the soil in the temperate climate zone is to cultivate of legumes, either as the main crop or sub-crop and to plow them as green manure at a certain stage of development (Baddeley *et al.*, 2017). The same authors state that in order to avoid nitrogen losses as much as possible, the next crop should have at its disposal a sufficient amount of mineralized nitrogen from green manure in the development phase, which is the most demanding in terms of its availability. Perennial crops for green manure (usually two to three years) are part of the crop rotation on arable land. Alfalfa, red clover, birdsfoot trefoil, as well as mixtures of grass and legumes, are often used for this purpose, with the last growth in autumn, before plowing, generally not being mowed (Stevović *et al.*, 2020).

In addition to the mentioned leguminous crops, mixtures of annual or perennial legumes can be used as green manure (Ćupina *et al.*, 2017). The diversity of the chemical composition of the species in the mixture affects the different rates of biomass decomposition after plowing, so that the availability of nitrogen is prolonged for a longer period and thus increases its utilization by the next crop, while reducing its losses. Due to many limitations in agricultural technology, the maximum number of legumes in mixtures for any purpose should not be more than three (Storkey *et al.*, 2015).

The degree of total mineralization of green manure and nutrient loss by leaching mainly depends on the chemical composition of the plant mass, soil temperature, its physical and chemical properties, and water content (Campiglia *et al.*, 2010). Data on the amount of nitrogen delivered to the land, i.e. the next cultivated plant, are highly variable and range from almost zero to an incredible 500 kg ha<sup>-1</sup> N. In practice, it is calculated that in optimal conditions these amounts should correspond to the amount of nitrogen from synthetic fertilizer of 100-200 kg ha<sup>-1</sup> (O'Dea *et al.*, 2013).

### **Role in soil phytoremediation**

Phytoremediation or bioremediation implies the cultivation of plants on contaminated soil (Ansari *et al.*, 2018). In that way, such soils would be indirectly used for the production of bioenergy or other industrial products, because the cultivation of plants for the production of food for humans and animals on them is unacceptable. In legumes, the process of nitrogen fixation, as well as the growth and development of plants stimulated by rhizobia, increase the content of harmful compounds in plants, the ability to decompose harmful organic substances, and indirectly help in photo stabilization and translocation of harmful substances from soil to plant (Teng *et al.*, 2015). In this way, the symbiosis of Rhizobium and legumes enhances the ability to remove harmful substances from the soil (Meena *et al.*, 2014). Legumes, for example, have almost no direct effect on the decomposition of oil residues, but their symbionts-rhizobial microorganisms are responsible for this (Kaksonen *et al.*, 2006). Recently, rhizobia have been used to remove harmful substances from the soil, such as active substances of pesticide, aromatic and acyclic hydrocarbons, chlorine substances, and phenolic substances (Jin *et al.*, 2013).

Rhizobia are also thought to control the bioremediation of heavy metals (Fester *et al.*, 2014). Potential metabolic systems involved in these processes are bioactive metabolites (Jin *et al.*, 2013), adsorption and accumulation of heavy metals, and secretion of microbial enzymes that alter the chemical properties of metal compounds (Teng *et al.*, 2015), volatilization of heavy metals by activity of microbes. The intensive formation

of legume biomass in such agroecosystems is a necessary precondition for more efficient phytoremediation (Hao *et al.*, 2012).

Leguminous species that have a highly developed and deep root system, such as alfalfa, can absorb the nitrate form of nitrogen from deeper soil layers and thus prevent its leaching into groundwater (Ding *et al.*, 2021).

### **Perennial forage legumes and bioenergy**

The search for renewable energy sources, such as bioenergy, and their practical use are becoming increasingly important today (Aidonojic *et al.*, 2023). An approach that involves the use of alternative energy sources, while providing quality animal feed in the last few decades has been increasingly pronounced (Kukharets *et al.*, 2023). Biorefining offers a different way of combining food production and bioenergy (Sanderson *et al.*, 2020). For this purpose, alfalfa forage is especially interesting, due to its economical production, i.e., low inputs. Energy production is based on the technological process of extracting proteins from legumes or whole plants, which are used in the feeding of domestic animals or as a food supplement for humans, and then the residues, which contain mainly polysaccharides, are utilized as bioenergy raw materials (Lamb *et al.*, 2003).

### **Conclusions**

Thanks to several positive characteristics, perennial forage legumes have, to a certain extent, exceeded their basic purpose in agricultural production systems. The advantages of production systems that include perennial forage legumes are numerous, but the most important are: increasing the value of agroecosystems by providing nitrogen to the next crop, reducing or eliminating the use of synthetic nitrogen, improving soil fertility, increasing biodiversity, weed control, reducing pesticide use. Recently, the importance of perennial legumes in the process of green energy production as well as in the procedures of phytoremediation of soil has been growing. The cost-effectiveness of production of perennial forage legumes at the farm level is often misjudged as negative, because their positive, economically invisible advantages are generally not taken into account. Therefore, today there is an urgent need to provide support for the cultivation of perennial forage legumes so that this production is comparable to the cultivation of leading cultivated plants. Although the yield potential of the leading species of cereals is constantly increasing, their yields are stagnating or even declining in real terms. The reason for that, in addition to the possible consequences of climate change, is largely related to the increasingly pronounced problems caused by diseases and weeds, as well as soil degradation. Therefore, the advantages of introducing legumes into systems that include only the most profitable cultivated plants are becoming more and more pronounced. Recognizing such advantages, market policy should recognize the value of products obtained from leguminous plants with certain incentives, i.e., premiums. The introduction of legumes in greater amounts in production systems would limit the increasingly pronounced deterioration of the quality of the environment in the broadest sense and thus improve the systems of sustainable agriculture.

### **Authors' Contributions**

Conceptualization: DT, VS, DĐ. Methodology: DT, MP. Writing—review and editing: DT, VS, MR, MRM. Resources: MM, ĐL. Supervision: NP. All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

**Acknowledgements**

The research presented in this article is part of Project Ref. No. 451-03-47/2023-01/200088 was funded by the Ministry of Science, Technological Development and Innovation, Republic of Serbia.

**Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

**References**

- Aidonojie AP, Ukhurebor EK, Oaihimore EI, Ngonso FB, Egielewa EP, Akinsehinde OB, Kusuma SH, Darmokoesoemo H (2023). Bioenergy revamping and complimenting the global environmental legal framework on the reduction of waste materials: A facile review. *Heliyon* 9(1):e12860. <https://doi.org/10.1016/j.heliyon.2023.e12860>
- Altieri MA, Rogé P (2010). The ecological role and enhancement of biodiversity in agriculture. In: Lockie S, Carpenter D (Eds). *Agriculture, Biodiversity and Markets*. Earthscan, London pp 15-32.
- Andrews M, Scholefield D, Abberton MT, McKenzie BA, Hodge S, Raven JA (2007). Use of white clover as an alternative to nitrogen fertiliser for dairy pastures in nitrate vulnerable zones in the UK: productivity, environmental impact and economic considerations. *Annals of Applied Biology* 151:11-23. <https://doi.org/10.1111/j.1744-7348.2007.00137.x>
- Angers DA, Caron J (1998). Plant-induced changes in soil structure: processes and feedbacks. *Biogeochemistry* 42:55-72.
- Annicchiarico P, Thami Alami I, Abbas K, Pecetti L, Melis RAM, Porqueddu C (2017). Performance of legume-based annual forage crops in three semi-arid Mediterranean environments. *Crop and Pasture Science* 68:932-941. <https://doi.org/10.1071/CP17068>
- Ansari AA, Gill SS, Gill R, Lanza RG, Newman L. (2018). *Phytoremediation, Management of Environmental Contaminants*. (6<sup>th</sup> Ed). Springer Nature, Switzerland. <https://doi.org/10.1007/978-3-319-99651-6>
- Ates F, Moneim D, El A, Ryan J (2013). Annual forage legumes in dryland agricultural systems of the West Asia and North Africa Regions: research achievements and future perspective. *Grass and Forage Science* 69:17-31. <https://doi.org/10.1111/gfs.12074>
- Baddeley AJ, Pappa AV, Pristeri A, Bergkvist G, Monti M, Reckling M, Schläfke N, Watson AC (2017). Legume-based Green Manure Crops. In: Murphy-Bokern D, Stoddard F, Watson AC (Eds). *Legumes in Cropping systems*. CAB International, Willingford, UK pp 125-138. <https://doi.org/10.1079/9781780644981.0125>
- BNF (2013). Biological nitrogen fixation (BNF) by legume crops in Europe. Legume Futures Report 1.5, Scotland Rural College. Retrieved 2014 February from: [https://www.academia.edu/15728456/Biological\\_nitrogen\\_fixation\\_BNF\\_by\\_legume\\_crops\\_in\\_Europe](https://www.academia.edu/15728456/Biological_nitrogen_fixation_BNF_by_legume_crops_in_Europe)
- Battye W, Aneja PV, Schlesinger HW (2017). Is nitrogen the next carbon? *Earth's Future* 5:894-904. <https://doi.org/10.1002/2017EF000592>.
- Bekuzarova AS, Kozyrev KA, Shabanova AI, Lushenko VG, Weissfeld IL (2020). Enhancing of nitrogen fixation by legumes. In: Solovyev D, Burygin G (Eds). *Proceedings of PLAMIC2020, BIO Web of Conferences, II International Scientific Conference "Plants and Microbes: The Future of Biotechnology"*, 14 August, Saratov 23:02006. <https://doi.org/10.1051/bioconf/20202302006>

- Bilalis D, Papastylianou P, Konstantas A, Patsiali S, Karkanis A, Efthimiadou A (2010). Weed-suppressive effects of maize–legume intercropping in organic farming. *International Journal of Pest Management* 56(2):173-181. <https://doi.org/10.1080/09670870903304471>
- Blackshaw R, Moyer J, Huang H (2005). Beneficial effects of cover crops on soil health and crop management. *Recent Research Developments in Soil Science* 1:15-35.
- Bokan N, Dugalić G, Tomić D, Vasiljević S, Karagić Đ, Milić D, Milošević B, Katanski S (2016). The importance of legumes for organic agriculture. In: Stevović V (Ed). *Proceedings of the XXI Conference on Biotechnology with International Participation*, Čačak, Serbia, University in Kragujevac, Faculty of Agronomy 21(23):123-128.
- Campiglia E, Mancinelli R, Radicetti E, Marinari S (2010). Legume cover crops and mulches: effects on nitrate leaching and nitrogen input in a pepper crop (*Capsicum annuum* L.). *Nutrient Cycling in Agroecosystems* 39:399-412. <https://doi.org/10.1007/s10705-010-9404-2>
- Cellier P, Schneider A, Thiébeau P, Vertès F (2015). Environmental impacts of introducing legumes into production systems. In: Schneider A, Huyghe C (Eds). *Pulses for sustainable agricultural and food systems*. Editions Quae: Versailles, France, pp 297-338.
- Chen L, Remondetto GE, Subirade M (2006). Food protein-based materials as nutraceutical delivery systems. *Trends in Food Science and Technology* 17(5):272-283. <https://doi.org/10.1016/j.tifs.2005.12.011>
- Chiriac ER, Chițescu CL, Sandru C, Geană EI, Lupoae M, Dobre M., Borda D, Gird CE, Boscencu R (2020). Comparative study of the bioactive properties and elemental composition of red clover (*Trifolium pratense*) and alfalfa (*Medicago sativa*) sprouts during germination. *Applied Sciences* 10(20):7249. <http://dx.doi.org/10.3390/app10207249>
- Cooledge CE, Chadwick RD, Smith MJL, Leake RJ, Jones LD (2022). Agronomic and environmental benefits of reintroducing herb- and legume-rich multispecies leys into arable rotations: a review. *Frontiers of Agricultural Science and Engineering* 9(2):245-271. <https://doi.org/10.15302/J-FASE-2021439>
- Ćupina B, Erić P, Krstić Đ, Vučković S (2004). Forage catch crops in sustainable agriculture and organing farming. *Acta Agriculturae Serbica* 9:451-459.
- Ćupina B, Mikić A, Krstić Đ, Vujić S, Zorić L, Đorđević V, Erić P (2017). Mixtures of Legumes for Forage Production. In: Murphy-Bokern D, Stoddard FL, Watson CA (Eds). *Legumes in Cropping Systems*. CABI, Oxfordshire pp 193-208.
- Dalgleish DG (2006). Food emulsions—their structures and structure-forming properties. *Food Hydrocolloids* 20(4):415-422. <https://doi.org/10.1016/j.foodhyd.2005.10.009>
- Dandamudi A, Tommie J, Nommsen-Rivers L, Couch S (2018). Dietary patterns and breast cancer risk: A systematic review. *Anticancer Research* 38(6):3209-3222. <https://doi.org/10.21873/anticancer.12586>
- Ding Y, Huang X, Li Y, Zhang Q, Liu X, Xu J, Di H (2021). Nitrate leaching losses mitigated with intercropping of deep-rooted and shallow-rooted plants. *Journal of Soils and Sediments* 21:364-375. <https://doi.org/10.1007/s11368-020-02733-w>
- FAO (2010). *The state of food and agriculture*. Food and Agriculture Organization of the United Nations, Rome, pp 147. Retrieved 2023 May 30<sup>th</sup> from: <http://www.fao.org/3/a-i2050e.pdf>
- Farooq M, Jabran K, Cheema AZ, Wahidb A, Siddique HMK (2011). The role of allelopathy in agricultural pest management. *Pest Management Science* 67:493-506. <https://doi.org/10.1002/ps.2091>
- Fester T, Giebler J, Wick LY, Schlosser D, Kästner M (2014). Plant-microbe interactions as drivers of ecosystem functions relevant for the biodegradation of organic contaminants. *Current Opinion Biotechnology* 27:168-175. <https://doi.org/10.1016/j.copbio.2014.01.017>
- Foyer HC, Nguyen H, Lam HM (2018). Legumes-The art and science of environmentally sustainable agriculture. *Plant, Cell and Environment* 42(1):1-5. <https://doi:10.1111/pce.13497>
- Gan RY, Lui WY, Wu K, Chan CL, Dai SH, Sui ZQ, Corke H (2017). Bioactive compounds and bioactivities of germinated edible seeds and sprouts: An updated review. *Trends in Food Science and Technology* 59:1-14. <https://doi.org/10.1016/j.tifs.2016.11.010>
- Garcia-Mora P, Penas E, Frias J, Gomez R, Martinez-Villaluenga C (2015). High-pressure improves enzymatic proteolysis and the release of peptides with angiotensin I converting enzyme inhibitory and antioxidant activities from lentil proteins. *Food Chemistry* 171:224-232. <https://doi.org/10.1016/j.foodchem.2014.08.116>

- Hao XL, Lin YB, Johnstone L, Baltrus DA, Miller SJ, Wei GW (2012). Draft genome sequence of plant growth-promoting rhizobium *Mesorhizobium amorphae*, isolated from zinc-lead mine tailings. *Journal of Bacteriology* 194:736-737. <https://doi.org/10.1128/jb.06475-11>
- Holtham DAL, Matthews GP, Scholefield DS (2007). Measurement and simulation of void structure and hydraulic changes caused by root-induced soil structuring under white clover compared to ryegrass. *Geoderma* 142:142-151. <https://doi.org/10.1016/j.geoderma.2007.08.018>
- Huang Z, Tian FP, Wu GL, Liu Y, Dang ZQ (2017). Legume grasslands promote precipitation infiltration better than gramineous grasslands in arid regions. *Land degradation and development* 28:309-316. <https://doi.org/10.1002/ldr.2635>
- Huxham SK, Sparkes DL, Wilson P (2005). The effect of conversion strategy on the yield of the first organic crop. *Agriculture, Ecosystems and the Environment* 106:345-357. <https://doi.org/10.1016/j.agee.2004.09.002>
- Jensen CR, Joernsgaard B, Andersen MN, Christiansen JL, Mogensen VO, Friis P, Petersen CT (2004) The effect of lupins as compared with peas and oats on the yield of the subsequent winter barley crop. *European Journal of Agronomy* 20:405-418. [https://doi.org/10.1016/S1161-0301\(03\)00057-1](https://doi.org/10.1016/S1161-0301(03)00057-1)
- Jin QJ, Zhu KK, Cui WT, Xie YJ, Han B, Shen WB (2013). Hydrogen gas acts as a novel bioactive molecule in enhancing plant tolerance to paraquat-induced oxidative stress via the modulation of heme oxygenase-1 signaling system. *Plant Cell and Environment* 36:956-969. <https://doi.org/10.1111/pce.12029>
- Jordan D, Miles RJ, Hubbard VC, Lorenz T (2004). Effect of management practices and cropping systems on earthworm abundance and microbial activity in Sanborn Field: a 115-year-old agricultural field. *Pedobiologia* 48(2):99-110. <https://doi.org/10.1016/j.pedobi.2003.06.001>
- Kaksonen AH, Jussila MM, Lindström K, Suominen L (2006). Rhizosphere effect of *Galega orientalis* in oil-contaminated soil. *Soil Biology and Biochemistry* 38:817-827. <https://doi.org/10.1016/j.soilbio.2005.07.011>
- Kaur L, Boning M, Beniwal SA, Abhilasha, Kaur R, Chain MF, Singh J (2022). Alternative proteins vs animal proteins: The influence of structure and processing on their gastro-small intestinal digestion. *Trends in Food Science and Technology* 122:275-286. <https://doi.org/10.1016/j.tifs.2022.02.021>
- Kirkegaard JA, Christen O, Krupinsky J, Layzell D (2008) Break crop benefits in temperate wheat production. *Field Crops Research* 107:185-195. <https://doi.org/10.1016/j.fcr.2008.02.010>
- Kukharets V, Juočiušienė D, Hutsol T, Sukmaniuk O, Česna J, Kukharets S, ... Shevtsova A (2023). An algorithm for managerial actions on the rational use of renewable sources of energy: determination of the energy potential of biomass in Lithuania. *Energies* 16:548-565. <https://doi.org/10.3390/en16010548>
- Lamb JFS, Sheaffer CC, Samac DA (2003). Population density and harvest maturity effects on leaf and stem yield in alfalfa. *Agronomy Journal* 95:635-641. <https://doi.org/10.2134/agronj2003.6350>
- Lassaletta L, Billen G, Romero E, Garnier J, Aguilera E (2014). How changes in diet and trade patterns have shaped the N cycle at the national scale: Spain (1961–2009). *Regional Environmental Change* 14:785-797. <https://doi.org/10.1007/s10113-013-0536-1>
- Li G, Wan L, Cui M, Wu B, Zhou J (2019). Influence of canopy interception and rainfall kinetic energy on soil erosion under forests. *Forests* 10:1-15. <https://doi.org/10.3390/f10060509>
- Lorin M, Jeuffroy HM, Butier A, Valantin-Morison M (2015). Undersowing winter oilseed rape with frost-sensitive legume living mulches to improve weed control. *European Journal of Agronomy* 71:96-105. <https://doi.org/10.1016/j.eja.2015.09.001>
- Louarn G, Pereira-Lopès E., Fustec J., Mary B., Voisin AS, Cesar FCP, Gastal F (2015). The amounts and dynamics of nitrogen transfer to grasses differ in alfalfa and white clover-based grass-legume mixtures as a result of rooting strategies and rhizodeposits quality. *Plant Soil* 389:289-305. <https://doi.org/10.1007/s11104-014-2354-8>
- Lugtenberg B, Kamilova F (2009). Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology* 63:541-556. <https://doi.org/10.1146/annurev.micro.62.081307.162918>
- Macleod CJA, Binley A, Hawkins SL, Humphreys MW, Turner LB, Whalley WR, Haygarth PM (2007). Genetically modified hydrographs: what can grass genetics do for temperate catchment hydrology? *Hydrological Processes* 21:2217-2221. <https://doi.org/10.1002/hyp.6780>
- Maimaiti J, Zhang Y, Yang J, Cen YP, Layzell DB, Peoples M, Dong Z (2007). Isolation and characterization of hydrogen-oxidizing bacteria induced following exposure of soil to hydrogen gas and their impact on plant growth. *Environmental Microbiology* 9:435-444. <https://doi.org/10.1111/j.1462-2920.2006.01155.x>

- Mayer J, Buegger F, Jensen ES, Schloter M, Hess J (2003). Estimating N rhizodeposition of grain legumes using a <sup>15</sup>N in situ stem labeling method. *Soil Biology and Biochemistry* 35:21-28. [https://doi.org/10.1016/S0038-0717\(02\)00212-2](https://doi.org/10.1016/S0038-0717(02)00212-2)
- Meena VS, Maurya BR, Meena RS, Meena SK, Singh NP, Malik VK (2014). Microbial dynamics as influenced by concentrate manure and inorganic fertilizer in alluvium soil of Varanasi, India. *African Journal of Microbiology Research* 8(1):257-263. <https://doi.org/10.5897/AJMR2013.5448>
- Melandera B, Rasmussen AI, Olesen EJ (2020). Legacy effects of leguminous green manure crops on the weed seed bank in organic crop rotations. *Agriculture, Ecosystems and Environment* 302:1-9. <https://doi.org/10.1016/j.agee.2020.107078>
- Murphy-Bokern D, Peeters A, Westhoek H (2017). The Role of Legumes in Bringing Protein to the Table. In: Murphy-Bokern D, Stoddard F, Watson C (Eds). *Legumes in Cropping systems*, CAB International, Wallingford, UK, pp 18-36. <https://doi.org/10.1079/9781780644981.0018>
- Neumann A, Torstensson G, Aronsson H (2011). Losses of nitrogen and phosphorus via the drainage system from organic crop rotations with and without livestock on a clay soil in southwest Sweden. *Organic Agriculture* 1:217–229. <https://doi.org/10.1007/s13165-011-0017-0>
- Nyawade OS, Gachene KKC, Karanja NN, Gitari IH, Schulte-Geldermann E, Parker LM (2019). Controlling soil erosion in smallholder potato farming systems using legume intercrops. *Geoderma regional* 17:1-11. <https://doi.org/10.1016/j.geodrs.2019.e00225>
- O’Dea JK, Miller PR, Jones CA (2013). Greening summer fallow with legume green manures: on-farm assessment in north-central Montana. *Journal of Soil and Water Conservation* 68:270-282. <https://doi.org/10.2489/jswc.68.4.270>
- Pankiewicz CSV, Fernanda P, Santos FDNK, Agtuca B, Xu Y, Schueller JM, ... Ferrieri AR (2015). Robust biological nitrogen fixation in a model grass–bacterial association. *The Plant Journal* 81:907-919. <https://doi.org/10.1111/tpj.12777>
- Paynel F, Murray J, Cliquet B (2001). Root exudates: a pathway for short-term N transfer from clover and ryegrass. *Plant and Soil* 229:235-243. <https://doi.org/10.1023/A:1004877214831>
- Pelletier N, Tyedmers P (2010). Forecasting potential global environmental costs of livestock production 2000–2050. In: Vitousek P (ed) *Proceedings of the National Academy of Sciences of the USA*, PNAS, Stanford, CA, 107:18371–18374. <https://doi.org/10.1073/pnas.1004659107>
- Peoples MB, Brockwell J, Herridge DF, Rochester IJ, Alves BJR, Urquiaga S, ... Jensen ES (2009). The contributions of nitrogen fixing crop legumes to the productivity of agricultural systems. *Symbiosis* 48:1-17. <https://doi.org/10.1007/BF03179980>
- Petrović PM, Stanković SM, Anđelković SB, Babić ŽS, Zornić GV, Vasiljević LjS, Dajić-Stevanović PZ (2016). Quality parameters and antioxidant activity of three clover species in relation to the livestock diet. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 44(1):201-208. <https://doi.org/10.15835/nbha44110144>
- Peyraud JL, Le Gall A, Lüscher A (2009). Potential food production from forage legume-based systems in Europe: an overview. *Irish Journal of Agricultural and Food Research* 48:115-135.
- Phelan P, Moloney AP, McGeough EJ, Humphreys J, Bertilsson J, O’Riordan EG, O’Kiely P (2014). Forage legumes for grazing and conserving in ruminant production systems. *Critical Reviews in Plant Sciences* 34:281-326. <https://doi.org/10.1080/07352689.2014.898455>
- Praharaj S, Maitra S (2020). Importance of legumes in agricultural production system: an overview. *Agro Economist* 7(2):69-71.
- Reckling M, Hecker JM, Bergkvist G, Watson CA, Zander P, Schläpke N, ... Bachinger J (2016). A cropping assessment framework – evaluating effects of introducing legumes into crop rotations. *European Journal of Agronomy* 76:186-197. <https://doi.org/10.1016/j.eja.2015.11.005>
- Robson MC, Fowler SM, Lampkin NH, Leifert C, Leitch M, Robinson D, Watson CA, Litterick AM (2002). The agronomic and economic potential of break crops for ley/arable rotations in temperate organic agriculture. *Advances in Agronomy* 77:369-427. [https://doi.org/10.1016/S0065-2113\(02\)77018-1](https://doi.org/10.1016/S0065-2113(02)77018-1)
- Sanderson M, Martin N, Adler P (2020). Biomass, energy, and industrial uses of forages. *Forage Harvesting and Utilization* 8:635-647. <https://doi.org/10.1002/9781119436669.cb43>

- Sauvadet M, Trap J, Plassard C, Van den Meersche K, Achard R, Allinne C (2021). Agroecosystem diversification with legumes or non-legumes improves differently soil fertility according to soil type. *Science of the Total Environment* 795:1-11. <https://doi.org/10.1016/j.scitotenv.2021.148934>
- Schjøning P, Munkholm LJ, Elmholt S, Olesen JE (2007). Organic matter and soil tilth in arable farming: management makes a difference within 5-6 years. *Agriculture, Ecosystems and Environment* 122:157-172. <https://doi.org/10.1016/j.agee.2006.12.029>
- Sheaffer CC, Barnes K, Heichel H (1989). Annual Alfalfa in Crop Rotation. Minnesota Agriculture Experiment Station, Bulletin, St. Paul, MN, pp 588.
- Silva LR, Pereira MJ, Azevedo J, Gonçalves RF, Valentão P, De Pinho PG, Andrade PB (2013). *Glycine max* (L.) Merr., *Vigna radiata* L. and *Medicago sativa* L. sprouts: A natural source of bioactive compounds. *Food Research International* 50:167-175. <https://doi.org/10.1016/j.foodres.2012.10.025>
- Spehn EM, Joshi J, Schmid B, Alphei J, Körner C (2000). Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. *Plant and Soil* 224:217-239. <https://doi.org/10.1023/A:1004891807664>
- Staniak M, Ksiezak J, Bojarszczuk J (2014). Mixtures of Legumes with Cereals as a Source of Feed for Animals. In Pilipavicius V (Ed). *Organic Agriculture Towards Sustainability*. InTech, BoD–Books on Demand pp 123-146. <http://doi.org/10.5772/58358>
- Stevović V, Lazarević Đ, Tomić D, Delić D, Đurović D (2017). Pre-sowing seed inoculation in the birdsfoot trefoil seed production. *AGROFOR International Journal* 2(1):41-47. <https://doi.org/10.7251/AGRENG1701041S>
- Stevović V, Đurović D, Tomić D (2020). Forage legumes in agricultural production systems. In: Pržulj N, Trkulja V (Eds). *From genetics and environment to food*. Academy of Sciences and Arts of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina 41:313-437.
- Storkey J, Döring T, Baddeley J, Collins R, Roderick S, Jones H, Watson C (2015). Engineering a plant community to deliver multiple ecosystem services. *Ecological Applications* 25:1034-1043. <https://doi.org/10.1890/14-1605.1>
- Sugiyama A, Yazaki K (2012). Root exudates of legume plants and their involvement in interactions with soil microbes. In: Vivanco M, Baluska (Eds). *Secretions and Exudates in Biological Systems*. Springer, Heidelberg, Germany pp 27-48. [https://doi.org/10.1007/978-3-642-23047-9\\_2](https://doi.org/10.1007/978-3-642-23047-9_2)
- SYS (2020). Statistical Yearbook of the Republic of Serbia. Statistical Office of the Republic of Serbia, Belgrade, Serbia, pp 450. <https://www.stat.gov.rs/en-us/publikacije/> 30.05.2023.
- Szogi AA, Vanotti BM, Ro SK (2015). Methods for treatment of animal manures to reduce nutrient pollution prior to soil application. *Tropical Collection on Land Pollution* 1:47-56. <https://doi.org/10.1007/s40726-015-0005-1>
- Tello-García E, Huber L, Leitinger G, Peters A, Newsely C, Ringler ME, Tasser E (2020). Drought- and heat-induced shifts in vegetation composition impact biomass production and water use of alpine grasslands. *Environmental and Experimental Botany* 169: 1-39. <https://doi.org/10.1016/j.envexpbot.2019.103921>
- Teng Y, Wang X, Li L, Li Z, Luo Y (2015). Rhizobia and their bio-partners as novel drivers for functional remediation in contaminated soils. *Frontiers in Plant Science* 6(32):1-11. <https://doi.org/10.3389/fpls.2015.00032>
- Tomić D, Stevović V, Đurović D, Lazarević Đ, Stanisavljević R (2012a). Effect of rainfall amounts on forage yield and water content in red clover (*Trifolium pratense* L.) grown for combined forage and seed production. In: Marietta H (Ed) *Proceedings of the 11th Alps-Adria Scientific Workshop*. Slovak Academy of Sciences Institute of Hydrology, Smolenice, Slovakia 61:129-132.
- Tomić D, Stevović V, Đurović D, Lazarević Đ (2012b). The impact of soil liming on the productivity of grass-legume mixture of red clover (*Trifolium pratense* L.) and Italian ryegrass (*Lolium italicum* L.). *Acta Agriculturae Serbica* 17(33):21-29.
- Tomić D, Stevović V, Đurović D, Bokan N, Stanisavljević R, Lazarević Đ (2014). Effect of additional fertilizing with nitrogen on forage yield in red clover-Italian ryegrass grass-legume mixture. In: Kovačević D (Ed). *Book of Proceedings, Fifth International Scientific Agricultural Symposium „Agrosym 2014“*. East Sarajevo, Faculty of Agriculture pp 175-180.
- Tomić D (2017). Foliar application of mineral nutrients in red clover seed production on acidic soil. PhD thesis. The University of Kragujevac, Faculty of Agronomy in Čačak, Serbia.
- Tomić D, Stevović V, Đurović D, Bokan N, Popović B, Knežević J (2018). Forage yield of a grass-clover mixture on an acid soil in the third year after soil liming. *Journal of Central European Agriculture* 19(2):482-489. <https://doi.org/10.5513/JCEA01/19.2.2149>

- Valachovičova M, Příbojova J, Urbanek V, Bírošova L (2017). Selected cardiovascular risk markers in vegetarians and subjects of general population. *Central European Journal of Public Health* 25(4):299-302. <https://doi.org/10.21101/cejph.a4529>
- Vazquez E, Schless PM, Borer TE, Bugalho NM, Caldeira CM, Eisenhauer N, ... Spohn M (2022). Nitrogen but not phosphorus addition affects symbiotic N<sub>2</sub> fixation by legumes in natural and semi-natural grasslands located on four continents. *Plant and Soil* 478:689-707. <https://doi.org/10.1007/s11104-022-05498-y>
- Vicenti A, Toteda F, Di Turi L, Cocca C, Perrucci M, Melodia L, Ragni M (2009). Use of sweet lupin (*Lupinus albus* L. var. *multitalia*) in feeding for Podolian young bulls and influence on productive performances and meat quality traits. *Meat Science* 82:247-251. <https://doi.org/10.1016/j.meatsci.2009.01.018>
- Vlaisavljević S, Kaurinović B, Popović M, Vasiljević S (2017). Profile of phenolic compounds in *Trifolium pratense* L. extracts at different growth stages and their biological activities. *International Journal of Food Properties* 20:3090-3101. <https://doi.org/10.1080/10942912.2016.1273235>
- Wolf-Hall C, Hillen C, Robinson JG (2017). Composition, nutritional value, and health benefits of pulses. *Cereal Chemistry* 94:11-31. <https://doi.org/10.1094/CCHEM-03-16-0069-FI>
- Xu M, Jin Z, Simsek S, Hall C, Rao J, Chen B (2019). Effect of germination on the chemical composition, thermal, pasting, and moisture sorption properties of flours from chickpea, lentil, and yellow pea. *Food Chemistry* 295:579-587. <https://doi.org/10.1016/j.foodchem.2019.05.167>
- Zabala AJ, Martínez-Paz MJ, Alcon F (2021). A comprehensive approach for agroecosystem services and disservices valuation. *Science of the Total Environment* 768:1-14. <https://doi.org/10.1016/j.scitotenv.2020.144859>



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



**License** - Articles published in *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

**Notes:**

- **Material disclaimer:** The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- **Maps and affiliations:** The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- **Responsibilities:** The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.