

*Review*

## **Benefits of grass-legume inter-cropping in livestock systems**

**Unathi Gulwa<sup>1\*</sup>, Nobulungisa Mgujulwa<sup>1</sup> and Solomon T. Beyene<sup>2</sup>**

<sup>1</sup>Eastern Cape Department of Rural Development and Agrarian Reform, Dohne ADI, South Africa.

<sup>2</sup>Department of Livestock and Pasture Science, University of Fort Hare, South Africa.

Received 5 April, 2018; Accepted 7 May, 2018

**Legumes (Fabaceae) are plants with the distinct ability to fix atmospheric nitrogen; the atmospheric nitrogen fixation by legumes is known as biological nitrogen fixation. Biological nitrogen fixation is the process whereby atmospheric nitrogen is reduced to ammonia in the presence of the enzyme nitrogenase. Nitrogen fixation in legumes starts with the formation of nodules. Inside the nodules, nitrogen fixation done by the bacteria (*Rhizobia*), and the ammonia (NH<sub>3</sub>) produced is absorbed by plant. The symbiotic relationship between a bacterium and a plant makes legumes special plants, which offer benefits when included in farming systems. These benefits are ecosystem, economic and environmental benefits. Inclusion of forage legumes in the form of intercropping in low-input grassland mixtures improves forage quantity, quality and soil fertility through addition of nitrogen (N) from N<sub>2</sub>-fixation. Intercropping is a multiple cropping practice, which involves growing two or more crops in proximity. Legumes also improve the nutritive value of the low quality native pastures grown with them and are important component of farming system since they have high nutritive value and able to rehabilitate nutrient depleted soil. There are various factors affecting legume growth and development and these factors need to be taken into account when planning to grow legumes. These factors include pedoclimatic factors especially those associated with the soil acid complex. These factors are known as physical, chemical, biological and environmental factors. The improvement of forage quantity and quality through forage legume inclusion is crucial for improved animal performance, which is a goal of all livestock farmers. The inclusion of forage legumes in low-input grassland mixtures is vital to improve biomass production, forage quality and ultimately soil fertility. The improvement of forage quantity and quality is crucial for improved animal performance, which is a goal of every livestock farmer. Forage legumes have the potential to improve the diets of ruminants because they increase the crude protein (CP) concentration of the herbage mixture relative to that of grass monocultures.**

**Key words:** Legumes, biological nitrogen fixation, forage quality, forage quantity.

### **INTRODUCTION**

Legumes (Fabaceae) are plants with the special ability to fix atmospheric nitrogen, the process of atmospheric nitrogen fixation by legumes is known as biological nitrogen fixation. Legumes can supply up to 90% of their

own nitrogen (N) when inoculated with a proper strain of *Rhizobia*. Even though legumes fix nitrogen from the atmosphere they can also take up large quantities of soil nitrogen if it is available (Weisany et al., 2013). Biological

nitrogen fixation is a natural process that is of significant importance in the world of agriculture (Herridge et al., 2008). Biological nitrogen fixation is the process whereby atmospheric nitrogen is reduced to ammonia in the presence of nitrogenase (Herridge et al., 2008).

Nitrogenase is an enzyme found naturally only in microorganisms such as symbiotic *Rhizobium*, *Frankia*, or the free-living *Azospirillum* and *Azotobacter*. In this case, the focus is on nitrogen fixation by legume plants in association with *Rhizobia*. In symbiotic relations, microorganism infects the plant root through the infection thread and lives in the nodule forming structure. The plant supplies component of nitrogenase and organic compounds to microorganisms while the microorganisms supply reduced nitrogen to the plant. The symbiotic relationship between the bacteria and the legume plant allows them to both grow and produce a high protein seed or forage crop (Coskan and Dogan, 2011). Although, biological nitrogen fixation is dependent on host cultivar and *rhizobia* it is also limited by pedoclimatic factors especially those associated with the soil acid complex of high aluminium (Al) and manganese (Mn), low calcium (Ca) and phosphorus (P). These factors are categorized as physical, chemical, biological and environmental factors (Bordeleau and Prevost, 1994).

Legume production in relation to biological nitrogen fixation also offers a number of benefits and these benefits are characterized as ecosystem, economic and environmental benefits. Inclusion of forage legumes in the form of intercropping in low-input grassland mixtures improves biomass production, forage quality and soil fertility through addition of nitrogen (N) from N<sub>2</sub>-fixation. Intercropping is a multiple cropping practice, which involves growing two or more crops in proximity. Legumes also improve the nutritive value of the low quality native pastures grown with them and are important component of farming system since they have high nutritive value and able to rehabilitate nutrient depleted soil. Diverse mixtures of plant species can use resources more efficiently in nutrient-poor environments (Hector, 1998), and can produce more biomass than communities of one or few species (Cardinale et al., 2007; Hector et al., 1999). For example, if plants differ in depth of rooting they can exploit nutrients from different soil layers (Wilson, 1988). Mixtures can use the sunlight more efficiently than monocultures through improved interception of light (Spehn et al., 2005). Furthermore, components of a mixture may show nutritional complementarity. Effects of legumes can persist even if the proportion of legumes in the total biomass is small (Mulder et al., 2002; Nyfeler et al., 2011).

Positive interactions were observed among non-legume species (van Ruijven and Berendse, 2003; Hooper and Dukes, 2004). In agricultural ecosystems, grass–legume mixtures have the potential to increase productivity, herbage nutritive value and resource efficiency (Peyraud et al., 2009). Results of a pan-European experiment, using two grasses and two forage legumes at thirty-one sites for three years, showed strong positive mixing effects. This improved livestock production considerably in addition to benefits in soil fertility (Nandi and Haque, 1986). Grass species can benefit from growing in mixtures with legume species (Temperton et al., 2007), and N fixation of legume species can be enhanced with competition from non-legume species (Nyfeler et al., 2011). Losses from weed competition represent a significant waste of resources (that is, water and nutrients) in agricultural systems, and more efficient use of resources in diverse grass–legume mixtures makes them more resistant to the invasion of weeds than communities composed of fewer species (Frankow-Lindberg et al., 2009). Greater evenness of species in a mixture further increases their resistance to weed invasion (Tracy and Sanderson, 2004).

Niche complementary and resistance to weeds and diseases can result in greater yield in mixtures than would be expected from the component species growing separately (Trenbath, 1974). Meta-analysis carried out by Cardinale et al. (2007) showed that mixtures were more productive than the average monoculture in 79% of the forty-four experiments they summarized. The most diverse mixture used in the experiments achieved 17 times the biomass of the average of the monocultures and 88 times the yield of the most productive species grown in monoculture. In 12% of the experiments, the mixtures were more productive than the most productive monoculture. While it is desirable in agronomic systems to achieve good yields, it is no less important to obtain herbage of high digestibility; low fibre content and high concentration of protein, to sustain satisfactory animal production (McDonald et al., 2002).

Forage legumes generally have higher nutritive value than grass species. Growing grasses and legumes in mixtures can improve herbage nutritive value compared with grass monocultures (Zemenchik et al., 2002). The improvement in nutritive value is due to slower decline in digestibility with advancing maturity and higher levels of protein in legumes (Dewhurst et al., 2009). Therefore, this review explores the benefits of grass-legume mixtures in forage production and ultimately livestock productivity, factors affecting legume production, benefits of legume inclusion on the soil and plants grown in

\*Corresponding author. E-mail: unathi.gulwa@drdar.gov.za. ugulwa@yahoo.com.

association with legumes.

## LITERATURE REVIEW

### Benefits of growing legumes

There are various benefits to the ecosystem function associated with growing legumes. These benefits are connected to the legumes' biological nitrogen fixation ability. They are categorized as economic, environmental and ecosystem benefits. Biological nitrogen fixation by legumes also presents benefits to the non-legume plants grown in association with legumes and the soils on which they are grown (Giller, 2001).

### Environmental benefits

The use of N fertiliser contributes substantially to environmental pollution therefore; biological alternatives have received increasing attention in the recent past in agricultural practices. Biological nitrogen fixation can act as a sustainable source of N and can complement or replace fertiliser inputs. Intercropping legumes capable of symbiotic N fixation offers an economically attractive and ecologically efficient means of reducing N inputs (Paynel et al., 2007). The assimilation of all the biologically fixed nitrogen by a legume plant, which maintains the balance of global nitrogen cycle and keeps nitrogen in a form that does not pollute the environment, is one of the well-known benefits of biological nitrogen fixation. Legumes contribute to enhanced carbon (C) sequestration and reduced greenhouse gas (GHG) emissions. The enhancement of C sequestration in the soil is related to increased biomass and hence to soil fertility. Raising soil fertility is viewed as the most effective way to rapidly increase C sink capacity (Serraj, 2004). Legumes also reduce GHG emissions from ruminant systems. The reduction of methane production has been seen in trials that were done on *Lotus corniculatus* (birdsfoot trefoil), *Lotus uliginosus* (greater trefoil) which are legumes possessing secondary metabolites known as condensed tannins (CTs) in their leaves. When household sheep were fed with these legumes, their methane production values decreased in comparison to those of the sheep that were on ryegrass pastures. The role of legumes in supplying N through fixation is crucial and beneficial in relation to GHG balance than had once been thought (Abberton, 2010).

### Ecosystem benefits

The amount of nitrogen contributed to the biosphere through biological nitrogen fixation is estimated to range between  $63 \times 10^6$  and  $175 \times 10^6$  tonnes per year. Symbiotic nitrogen fixation in legumes contributes about

30% to this amount of fixed nitrogen. Leguminous nitrogen fixation is viewed as the most efficient system as the mean yearly fixation rate ranges between 55-140 kg/ha in comparison with 0.3 30kg/ha for other nitrogen-fixing biological systems. In a study that was conducted in the United Kingdom, it was estimated that *Trifolium repens* (white clover) in mixture with grasses fixed up to 280 kg/ha annually. This introduction of white clover into the sward resulted to saving up to 45% nitrogen fertilizer (Lindstrom, 2001). The role of legumes in supplying N through fixation is crucial and beneficial in relation to GHG balance than had once been thought (Abberton, 2010). Powers et al. (2011) reported increases in soil carbon stock when forest or savanna was converted to pastures (5 to 12% and 10 to 22%, respectively).

### Economic benefits

Since adequate animal nutrition is essential for high rates of gain ample milk production, efficient reproduction and adequate profits, it is imperative to provide livestock with protein supplements when forage quality is low. The protein supplements are expensive and this results in high feed costs that compel dairy farmers to become more efficient with their farm operations. Feed accounts for approximately one-half of the total cost of providing milk, and high quality forage optimizes the productivity of the animals, therefore increasing the quality of forage available is one of the best methods of improving overall feeding efficiency. Combining the growth of cereal or grass forage with crops which are capable of increasing the protein content of the overall ration clearly has nutritional and financial benefits. Legumes are a good source of protein and can be used to compensate cereal or grass protein shortage (Eskandari et al., 2009). Thus, growing of plant mixtures with legumes, which is referred to as intercropping, can boost the forage protein content of ruminant diets. Several authors have reported higher crude protein content in grass-legume mixtures in comparison to sole cereals or grasses (Eskandari et al., 2009; Ojo et al., 2013).

### Benefits to the soil

Benefits to the soil emanating from biological nitrogen fixation by legumes include the improvement of soil organic matter content, soil porosity, soil nutrients, soil structure, soil pH, biological diversity and pest cycle (Heenan et al., 2004; Malik, 2010; Mohammadi et al., 2012; Ernst and Siri-Prieto, 2009).

### Soil organic matter

Soil organic matter is the organic fraction of the soil and

is composed of the decomposed plant and animal material, and microbial organisms. The carbon associated with the soil organic matter (SOM) is known as soil organic carbon. SOM is a key indicator of soil quality as it influences biological activity, serves as a nutrient reservoir, and impacts soil aggregation. Seeding of grasses with legumes in combination with continuous grazing resulted in increased SOC of pastures even though that did not translate into improved net returns (Heenan et al., 2004). From a long-term rotational study in Wagga Wagga in the United States of America, it was reported that stubble retention in legume-wheat rotation maintained higher levels of soil organic carbon (SOC) than stubble burning (Heenan et al., 2004). Rotations involving medics and vetch (*Vicia faba*) led to a significant increase in soil organic matter ranging between 12.5 to 13.8g/kg versus 10.9 to 11 g/kg for continuous wheat and wheat/fallow (Malik, 2010). Most crop residues contain more carbon than nitrogen but require both N and C to speed up the process of decomposition therefore the nitrogen contained by legumes facilitates the decomposition of crop residues in the soil and their conversion to soil building organic matter (Mohammadi et al., 2012). Several researchers have reported that crop mixtures greatly increased carbon inputs into the soil in comparison to monocultures. The increase in soil organic carbon was reported to be due to plant mixtures providing good soil cover, which ultimately results to continuous addition of roots and plant litter (Ernst and Siri-Prieto, 2009; Peypers et al., 2010; Tesfaye et al., 2007; Huntjes and Albers, 1978; Anders et al., 1996).

### Soil porosity

Most legumes have an aggressive taproot that opens pathways deep into the soil. Nitrogen rich legumes also encourage earthworms and burrows formation. The root channels and earthworm increase soil porosity and promote air movement and water percolation deep into the soil. Planting of white clover resulted in improvements in water percolation rate and the extraction of nutrients by plants from the soil. Transient structuring of soil and greater drainage of water through soil cores than under perennial ryegrass monocultures around the roots of white clover has also been reported (Graham and Vance, 2000).

### Nutrient recycling

Biennial and perennial legumes usually root deeply into the soil, and therefore they have the ability to recycle crop nutrients that are deep in the soil profile. This prevents nutrient loss due to leaching below the root

zone of shallower-rooted crops in rotation (Mohammadi et al., 2012).

### Improvement of soil structure

Legumes improve soil structure and stability. The protein, glomalin that symbiotically occurs along the roots of legumes serves as glue that binds soil together into stable aggregates. The aggregate stability increases pore space and tilth, reducing both soil erodibility and crusting (Mohammadi et al., 2012). Improved soil structure reduces the risk of soil compaction and water runoff and ultimately increases the soil's biological activity, seedling establishment and root penetration. Legume driven soil structure improvement may result in increased leaching of both fixed and applied nitrate in legume monocultures (Holtham et al., 2007).

### Improvement of soil pH

Due to the legumes' acquisition of nitrogen as diatomic N rather than as nitrate, they lower the pH of the soil. This in turn promotes increased plant-soil-microbial activity in soils with a pH above the range for optimum crop growth and development (Graham and Vance, 2000).

### Biological diversity

Biodiversity is a major co-benefit of an increased use of legumes. This has been proved in long-term studies conducted in Minnesota where, the net soil accumulation of C and N of 1 m was measured on agriculturally degraded soils. Five hundred to six hundred percent C and N diversity increase were observed in perennial grasslands than monocultures. In these mixtures, there was also greater root biomass accumulation especially from legumes and C4 grasses. White clover and birdsfoot trefoil biomass presence were observed to significantly increase the pools of C and N in the soil (Abberton, 2010).

### Break pest cycle

Legumes can provide an excellent break in a crop rotation that reduces the build-up of grassy weed problems, insects and diseases. Forages and legumes also play an important role in weed control and nitrogen (N) supply for an upcoming crop. According to Malik (2010), the introduction of grain legume crops or legume-rich pastures provided N to subsequent cereal or oilseed crops when legumes were introduced into rotations. Besides, breaking pest cycles, grain legume crops also

lower infestation of non-legume crops by improving biological pest control through increased microbial diversity and activity (Lupwayi et al., 2011). Cereals rotations with legumes were useful because the legumes contributed N to the soil/ plant system and interrupted pathogen cycles. In Ethiopia, a faba bean (*Vicia faba L.*)-Wheat-wheat rotation reduced the severity of wheat take-all (*Gaeumannomyces herpotrichoides*) disease in comparison with wheat monoculture (Lupwayi et al., 2011).

## Benefits to the plant

### Improved biomass production

Legumes supply nitrogen to grass-legume mixtures, so mixtures may produce more forage yield than grasses grown alone. Generally, in grass-legume mixtures higher yields have always been achieved in comparison with sole grass plots. Several authors have reported, greater total dry matter production in grass-legume mixtures higher yields in comparison with sole grasses or cereals. In a study that was conducted by Sturludottir et al. (2013), in Northern Europe and Canada higher yield in the legume-grass mixtures than monoculture treatments were reported. The authors reported that on average, the grass-legume mixture plots had 9, 15 and 7% more DM than the most productive monoculture in the first, second and third year respectively. Sturludottir et al. (2013) reported more DM production of 9, 15 and 7% in mixture plots than the most productive monoculture in the first, second and third year respectively in a study they conducted. Gulwa et al. (2017) also reported higher total dry matter production in grass-legume mixture plots in comparison to grass only plots in a study that was conducted in the Eastern Cape Province, South Africa. The difference in growth patterns of legumes is reported to have a potential of leading to efficient use of resources such as light when grown in a mixture than when grown separately. All these different functional traits could contribute to positive interactions between the species resulting in higher yields for mixtures in comparison to monocultures. The attainment of high DM yield in the grass-legume mixture plots may also be attributed to beneficial effects of mixing grasses and legumes and also from the differences in the seasonal growth pattern between the grass and legume species (L€ uscher et al., 2005) or across years (Nyfeler et al., 2009).

### Improved nutritive value

Grasses grown in association with legumes also contain a higher percentage of protein. The protein content of legumes is typically much higher than that of grasses and

legumes fibre tends to digest faster than grass fibre, allowing the ruminant to eat more of the legume. Well nodulated legumes mostly provide an actual N supply to the subsequent crop but the net addition of this N and its availability depends on the amount of fixed N, which remains in non-harvested residues (Russelle, 2004). Grass in pure stands is common (that is, grass in natural systems), but requires high nitrogen (N) inputs. In terms of N input, two-species (grass-legume mixtures) are more sustainable than grass in pure stands and consequently dominate low N input grasslands (Nyfeler et al., 2011; Nyfeler et al., 2009; Crews and Peoples, 2004). In temperate grasslands, N is often the limiting factor for productivity (Whitehead, 1995). Plant available soil N is generally concentrated in the upper soil layers, but may leach to deeper layers, especially in grasslands that include legumes (Scherer-Lorenzen et al., 2003) and under conditions with surplus precipitation (Thorup-Kristensen, 2006). Eskandari et al. (2009) reported that grasses grown in intercropping with legumes contained a higher CP content than grasses harvested from the monoculture planted plots. This suggests that legumes grown alongside non-legume plants increase the N uptake of the companion plants by partitioning the atmospheric fixed N by legumes to the non –nitrogen fixing plants grown in association with them. Ojo et al. (2013) reported higher CP levels on *Panicum maximum* intercropped with *Lablab purpureus* in a study they conducted at the Federal University of Agriculture in Nigeria. Concentrations of nutrients in forage plants are dependent upon the interaction of a number of factors. These factors include the following: the physiology of the plant, physical and chemical compounds of the plant (tannins, cellulose and crude fibre), season and soil quality in which the forages are grown.

## Factors affecting legume development and production

Factors affecting legume production include soil related factors such as soil pH, organic carbon, and mineral contents and plant factors such as plant nutrient status (Coskan and Dogan, 2011; Weinsany et al., 2013; Serraj and Adu-Gyamfi, 2004; Sinclair and Vadez, 2002).

### Soil related factors

#### Soil pH

Soil reaction (pH) is one of the most crucial factors influencing legume and *rhizobium* symbiosis. Higher hydrogen cation (H<sup>+</sup>) concentration ions lead to increased solubility of Aluminium (Al), Manganese (Mn) and Iron (Fe) and the high amount of these elements may

become toxic to the *rhizobium*. *Rhizobium* such as *Sinorhizobium meliloti* and *Rhizobium galegae* are highly sensitive to acid pH as soil pH less than 4.6 inhibits their activity. Some of the studies conducted in the past have shown formation of efficient symbiosis and increased amounts of biological nitrogen fixation when the soil pH is between 5.6 and 6.1. Soil acidification impedes the root hair infection process and nodulation (Coskan and Dogan, 2011).

## Soil macronutrient

### Nitrogen

Even though legumes can fix nitrogen from the atmosphere, they can take up large quantities of soil nitrogen if it is available. Nitrogen is an important element for the formation of soil organic matter. Nitrogen (N) release from a legume crop occurs as the aboveground plant residues, roots and nodules gradually decompose. Although there are contrasting reports of the role of starter N on BNF, it is widely agreed that, excess N inhibits nodulation and N<sub>2</sub> fixation, especially in soils with good fertility status (Serraj and Adu-Gyamfi, 2004; Unkovich et al., 2008). The negative effect of nitrate on legume nodulation and subsequent reduction in BNF is attributed to the inhibition of root infection, nodule development and nitrogenase activity (Weinsany et al., 2013).

### Phosphorus

Nodule development and function are critical sinks for phosphorous (P) therefore; nodules usually require the highest P content in the plant (Sinclair and Vadez, 2002). Adequate P fertilization has been observed to yield to enhanced nodule number, mass and greater N<sub>2</sub>-fixation activity per plant (Serraj and Adu-Gyamfi, 2004). Legumes release fixed N and build up soil organic matter during growth. The increase in soil organic carbon was reported to reach a new plateau after only three years on a clay soil on a study that was done on alfalfa (Sinclair and Vadez, 2002). The deficiency of phosphorus (P) supply and the availability poses a severe limitation on nitrogen fixation and symbiotic interactions. However, there are differences in phosphorus requirements of various *rhizobia*. The slow growing *rhizobia* are more tolerant to low P levels than the fast growing *rhizobia* (Russelle, 2004).

### Potassium

Although potassium (K) is not viewed as an integral

constituent of the metabolite, it serves to activate various enzymes, serves as a counter ion and is the major cationic cellular osmoticum. Potassium affects the growth rate of internodes. For some *rhizobia*, a qualitative requirement for K was seen when *Rhizobium Trifolii* and *Rhizobium Meliloti* revealed restricted growth when potassium was omitted from a defined medium whereas a linear response was obtained in batch culture (Russelle, 2004).

## Sulphur

Sulphur (S) is an essential element for growth and physiological functioning of the plants. The sulphur containing amino acid cysteine and methionine play an important role in the structure conformation and function of proteins and enzymes in vegetative plant tissue. As synthetic media for growth of *rhizobia* contains S, there has been very little attempt made to define quantitative requirements of sulphur. When examining S nutrition of two strains of *Bradyrhizobium japonicum* and two strains of *Bradyrhizobium sp.* using batch and chemostat cultures, high levels of contaminating S present in the media components had to be removed before S limitation occurred in the batch culture. The growth of four *Bradyrhizobia* strains was limited in the chemostat culture when the S concentration in the inflowing media was less (Unkovich et al., 2008; Weisany et al., 2013).

## Soil essential micronutrient content related factors

### Boron

Boron (B) is amongst the eight essential micronutrients that are also known as trace elements required for the normal growth of most plants. Strong alterations in nitrogen fixation in soybean plants were reported when B supply was low. Results of a study that was done on the effect of B on the *rhizobium*- legume cell surface interaction and nodule development in peas indicated that the number of *rhizobia* infecting the host cells and the number of infection threads (the infection threads developed morphological abnormalities) were reduced in boron deficient plants. The cell walls of boron deficient plants with structural aberrations lack the covalently bound proline rich proteins contributing to O<sub>2</sub> barrier, preventing inactivation of nitrogenase associated with a decline in N<sub>2</sub> fixation (Sinclair and Vadez, 2002).

### Copper

Copper (Cu) plays an important role in a protein expressed co-ordinately with nifgenes and may affect the

efficacy of bacteroid function. This element also plays an important role in the respiratory proteins that are required for the  $N_2$  fixation in *rhizobia*. Several *rhizobial* strains, particularly *R.leguminosarum* bv phaseoli make the pigment melanin. Cu deficiency in subterranean clover reduces nitrogen fixation (Abberton, 2010; Weisany et al., 2013).

### **Iron**

Iron (Fe) is required for various key enzymes of the nitrogenase complex as well as for the electron carrier ferredoxin and some hydrogenases. A particular high iron requirement exists in legumes for the heme component of haemoglobin. Iron is required in higher amount for nodule formation in legumes than in host plants, as in the case of lupins and peanuts. When Fe was limited in peanut nodules, a reduction in specific rates of nitrogenase were observed. This was an indication of a possible direct limitation by Fe deficiency on nodule function. In lupin and peanut, nodule development is much more susceptible to a shortage of iron than are other parameters such as plant shoot and root weights (Burket, 1997).

### **Manganese**

In one of the initial steps of the infection process, the binding of rhizobia to young root hairs is enhanced when *R. leguminosarum* is starved of manganese (Mn). However, it is still unknown whether Mn amounts affect the type of rhizobial exopolysaccharide (Appanna and Preston, 1987).

### **Molybdenum**

Molybdenum (Mo) is a micronutrient precisely for plants forming root nodules with nitrogen-fixing bacteria, even though non-nodule forming plants also use small amounts of Mo in a protein involved with nitrogen metabolism uptake. Molybdenum in iron (Fe) molybdenum (Mo) cobalt (Co) co-factor is at the heart of the nitrogen reduction process. The role therefore clearly depicts the relevance of this micronutrient on the  $N_2$  fixation process. Foliar application of Mo was reported to increase the levels on  $N_2$  fixation and nodule mass in grain legumes in field conditions and this has led to higher overall N content and seed yield. The bacteria; *B. japonicum* strain deficient in molybdenum transport indicated impaired nitrogen fixation activity when inoculated to soybean roots. In studies that were conducted in the laboratory, various legumes that were severely starved of Mo displayed more intense signs of

deficiency (Allen et al., 1999).

### **Nickel**

Soil nickel (Ni) application to field-grown soybean (*Glycine max* Merr.) resulted in a significant increase in nodule weight and seed yield. In some legumes, small amounts of Ni are essential for root nodule growth and hydrogenase activation. The efficiency of nitrogen fixation immediately depends on hydrogenase activity because the oxidation of hydrogen by the latter provides ATP required for the reduction to ammonia (Bertrand and de Wolf, 1967)).

### **Cobalt**

Cobalt (Co) is essential for the nitrogen-fixing microorganisms, including the cyanobacteria. Co is essential for symbiotic nitrogen fixation by legumes and non-legumes. For example, soybeans, grown with only atmospheric nitrogen and no mineral nitrogen have rapid nitrogen fixation and growth with 1.0 or 0.1..g Co ml<sup>-1</sup> but have minimal growth without Co additions. Cobalt has also been shown to be essential for *rhizobial* growth and is required as a part of bacterial enzyme complex. Cobalt deficiency affects nodule development and function at different levels and to different degrees (Ahmed and Evans, 1960).

### **Conclusion**

The inclusion of forage legumes in low-input grassland mixtures is vital to improve biomass production, forage quality and ultimately soil fertility. The improvement of forage quantity and quality is crucial for improved animal performance that is a goal of all livestock farmers. Mixing legumes and grasses serves as the forage supplementary alternative since pure grasses or cereals provide poor quality fodder due to their inherent lower crude protein content. Legume species like *Trifolium species* whose CP levels remain higher even during the driest seasons, while simultaneously partitioning the fixed nitrogen to the companion grasses are highly recommended for grass-legume mixtures. Mixing legumes with grasses increases the CP concentration of the herbage mixture relative to that of grass monocultures. This suggests that legumes have the potential to improve the diets of ruminants.

Crude protein concentrations of grasses are usually lower during the dry season (winter), therefore, forage legumes should be incorporated in sole grass stands to increase forage quantity and quality during dry seasons. Forage legumes must be used to supplement the nutritive

value of natural grasses. Legume species like *Lespedeza cuneata* can be recommended for production and baling for hay during summer and autumn for utilization during winter or early spring as, it's fibre content increases with advancing maturity. Species like *T.repens*, which, partition more of the fixed nitrogen to the companion non-legume plant and remain palatable throughout the growing seasons should be produced and grazed as standing hay. Legumes enhance carbon (C) sequestration and reduce greenhouse gas (GHG) emissions.

The C sequestration enhancement in the soil is linked to increased biomass and hence to soil fertility. The assimilation of all the biologically fixed nitrogen by a legume plant, which maintains the balance of global nitrogen cycle and keeps nitrogen in a form that does not pollute the environment, is one of the well-known benefits of biological nitrogen fixation. Intercropping forage legumes with cereals or grasses is one of the climate smart option offering a potential for increasing forage and, consequently, livestock production in many parts of the world. When developing a fodder production plan incorporating legume production the physical, chemical, biological and environmental factors affecting legume growth and development should be taken into account. These factors may impede optimum legume growth and development if not properly addressed.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Abberton M (2010). Enhancing the role of legumes: potential and obstacles. *Journal Integrated Crop Management* 11:178-187.
- Anders MM, Potdar MV, Francis CA (1996). Significance of intercropping in cropping systems. In: Ito, O., Katayama, K., Johansen, C., Kumar Rao, JVDK., Adu- Gyamfi, JJ., Rego TJ, (eds). *Roots and nitrogen in cropping systems of the semi-arid tropics*. JIRCAS. Ohwashi. Ibaraki. Japan pp. 1-18.
- Appanna VD, Petterson CM (1987). Manganese elicits the synthesis of a novel exopolysaccharide in arctic Rhizobium. *FEBS Letter* 215:79-82.
- Allen R, Roll J, Rangari P, Shah V, Roberts G, Ludden P (1999). Incorporation of molybdenum into the iron-molybdenum cofactor of nitrogenase. *Journal Biology and Chemistry* 274:15869-15874.
- Ahmed S, Evans HJ (1960). Cobalt: A micronutrient for the growth of soybean plants under symbiotic conditions. *Soil Science* 90:205-210.
- Bertrand D, de Wolf A (1967). Nickel, a dynamic trace element for higher plants. *C R Academic Science* 265:1053-1055.
- Bordeleau LM, Prevost D (1994). Nodulation and nitrogen fixation in extreme environments. *Plant and Soil* 161:115-125. Netherlands.
- Burket J, Hemphill D, Dick R (1997). Winter cover crops and nitrogen management in sweet corn and broccoli rotations. *Horticultural Science* 32:664-668.
- Cardinale BJ, Wright JP, Cadotte MW, Carroll IT, Hector A, Srivastava DS, Loreau M, Weis JJ (2007). Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proceedings of the National Academy of Sciences of the United States of America* 104:18123-18128.
- Coskan A, Dogan K (2011). *Symbiotic Nitrogen Fixation in Soybean. Soybean Physiology and Biochemistry*, Edited by Hany, A., El-Shemy, ISBN, 978-953-307-534-1.
- Crews TE, Peoples MB (2004). Legume versus fertilizer sources of nitrogen: ecological Trade-offs and human needs. *Agriculture Ecosystems and Environment* 102:279-297.
- Dewhurst RJ, Delaby L, Moloney A, Boland T, Lewis E (2009). Nutritive value of forage legumes used for grazing and silage. *Irish Journal of Agricultural and Food Research* 48:167-187.
- Eskandari H, Ghanbari A, Javanmard A (2009). Intercropping of Cereals and Legumes for Forage Production. *Notulae Scientia Biologicae* 1(1):07-13.
- Frankow-Lindberg BE, Brophy C, Collins RP, Connolly J (2009). Biodiversity effects on yield and unsown species invasion in a temperate forage ecosystem. *Annals of Botany* 103:913-921.
- Giller FE (2001). *Nitrogen Fixation in Tropical Cropping Systems*. British library. London. United Kingdom, 2<sup>nd</sup> edition.
- Graham PH, Vance CP (2000). Nitrogen Fixation in perspective: an overview of research and extension needs. *Field Crops Research* 65:93-106.
- Gulwa U, Mgujulwa N, Beyene ST (2017). Effect of Grass-legume intercropping on Dry Matter Yield and Nutritive Value of Pastures in the Eastern Cape Province, South Africa. *Universal Journal of Agricultural Research* 5(6):355-362.
- Hector A (1998). The effect of diversity on productivity detecting the role of species complementarity. *Oikos* 82:597-599.
- Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Hogberg P, Huss-danell K, Joshi J, Jumpponen A, Korner C, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Pereira JS, Prinza RDJ, Schererlorenzen M, Schulze ED, Siamantziouras ASD, Spehn EM, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH (1999). Plant diversity and productivity experiments in European Grasslands. *Science* 286:1123-1127.
- Heenan DP, Chan KY, Knight PG (2004). Long-term impact of rotation, tillage and stubble management on the loss of soil organic carbon and nitrogen from a Chromic Luvisol. *Soil and Tillage Research* 76:59-68.
- Herridge DF, Peoples MB, Boddey RM (2008). Global inputs of Biological nitrogen Fixation in agricultural systems. *Plant and Soil* 311:1-18.
- Hooper DU (1998). The role of complementarity and competition in ecosystem responses to variation in plant diversity. *Ecology* 79:704-719.
- Holtham DAL, Matthews GP, Scholefield D (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.
- Huntjes JLM, Albers RAJM (1978). A model experiment to study the influence of living plants on the accumulation of soil organic matter in pastures. *Journal Plant and Soil* 50(2):411-418.
- Lindstrom B (2013). *Micronutrients in Temperate Forage Crops Grown in Sweden: Species Differences and Effects of Phenological Development and Soil*. Doctoral Thesis, pp. 11-58.
- Lupwayi NZ, Kennedy AC, Rowland MC (2011). Grain legume impacts on soil biological processes in sub-Saharan Africa. *African Journal of Plant Science* 5(1):1-7.
- L€ uscher A, Fuhrer J, Newton PCD (2005). Global atmospheric change and its effect on managed grassland systems. In: McGilloway, DA., (ed.). *Grassland: a global resource*. Wageningen, Academic Press: pp. 251-264.
- Mulder CPH, Jumpponen A, Hogberg P, Hussdanell K (2002). How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. *Oecologia* 133:412-421.
- Malik R (2010). The introduction of grain legume crops or legume-rich pastures provided N to a subsequent cereal or oilseed crops. *Proceedings of the 19<sup>th</sup> World Congress of Soil Science, Soil Solutions for a Changing World*. Australia.
- McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA (2002). *Animal*

- nutrition. Harlow: Prentice Hall.
- Mohammadi KSY, Heidari GKS, Majidi M (2012). Effective factor on Biological Nitrogen Fixation. *African Journal of Agricultural Research* 7(12):1782-1788.
- Ernst O, Siri-Prieto G (2009). Impact of perennial pasture and tillage systems on carbon input and soil quality indicators. *Soil and Tillage Research* 2698:1-9.
- Nyfeler D, Huguenin-eli EO, Suter M, Frossard DE, Luscher A (2011). Grass-Legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture Ecosystems and Environment* 140:155-163.
- Nyfeler D, Huguenin-Elie O, Suter M, Frossard E, Connolly J, Lüscher A (2009). Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal Applied Ecology* 46:683-691.
- Nandi LA, Haque I (1986). Forage legume cereal systems: Improvements of soil fertility and Agricultural production with special reference to sub-seharan Africa In: Haquel. pp. 145-187.
- Ojo VOA, Dele TA, Amole UY, Adeoye SA, Hassan JAO, Idowu OJ (2013). Effect of Intercropping Panicum Maximum var. Ntchisi and Lablab purpureus on the growth, yield and chemical composition of Panicum maximum var. Ntchisi at different harvesting times. *Pakistan Journal of Biological Sciences* 10:1-4.
- Paynel F, Lesuffleur F, Bigot J, Diquelou S, Cliquet JB (2007). Study of <sup>15</sup>N transfer between legumes and grasses. *Agronomy for Sustainable Development*. Springer Verlag (Germany) 28(2):281-290.
- Peyraud JL, Le Gall A, Luscher A (2009). Potential food production from forage legume-based systems in Europe: an overview. *Irish Journal of Agricultural and Food Research* 48:115-135.
- Peypers P, Sanginga J, Kasereka B, Walangululu M, Vanlauwe B (2010). Increased productivity through integrated soil fertility management in cassava-legume intercropping systems in the highlands of Sud-Kivu, DR Congo. *Journal of Field Crops Research*, 120:76:85.
- Powers JS, Corre MD, Twine TE, Veldkamp E (2011). Geographic bias of field observations of soil carbon stocks with tropical land-use changes precludes spatial extrapolation. *PNAS* 108:6318-6322.
- Russelle M (2004). The environmental impacts of N<sub>2</sub> fixation by alfalfa. *Ext. and Alfalfa. Workgroup* pp. 57-62.
- Serraj R, Adu-Gyamfi J (2004). Role of symbiotic nitrogen fixation in the improvement of legume productivity under stressed environments. *West African. Journal Applied Ecology* 6:95-109.
- Scherer-Lorenzen M, Palmberg C, Prinz A, Schulze ED (2003). The role of plant diversity and composition for nitrate leaching in grasslands. *Ecology* 84:1539-1552.
- Spehn EM, Hector A, Joshi J, Scherer-lorenzen M, Schmid B, Bazeley-white E, Beierkuhnlein C, Caldeiram C, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Hogberg P, Huss-danell K, Jumppone NA, Koricheva J, Leadley PW, Lorea UM, Minn SA, Mulder CPH, O'Donovan G, Otway SJ, Palmberg C, Pereiraj S, Pfisterera B, Prin ZA, Readd J, Schulze ED, Siamantziouras ASD, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH (2005). Ecosystem effects of biodiversity manipulations in European grasslands. *Ecological Monographs* 75:37-63.
- Sturludottir E, Brophy C, Elanger GB, Gustavsson AM, Jørgensen M, Lunnan T, Helgadotti A (2013). Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. *Journal of the British Grassland Society* 12037:1-12.
- Tesfaye M, Liu J, Allan DL, Vance CP (2007). Genomic and Genetic Control of Phosphate Stress in Legumes. *Plant Physiology* 144(2):594-603.
- Temperton VM, Mwangi PN, Scherer-Lorenzen M, Schmid B, Buchmann N (2007). Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. *Oecologia* 151:190-205.
- Tracy BF, Sanderson MA (2004). Forage productivity, species evenness and weed invasion in pasture communities. *Agriculture, Ecosystems and Environment* 102:175-183.
- Trenbath BR (1974). Biomass productivity of mixtures. *Advances in Agronomy* 26:177-210.
- Thorup-Kristensen K (2006). Effect of deep and shallow root systems on the dynamics of soil inorganic N during 3-year crop rotations. *Journal of Plant Soil* 288:233-248.
- Unkovich M, Herridge D, Peoples M, Cadisch G, Boddey B, Giller K, Alves CP (2008). Measuring plant-associated nitrogen fixation in agricultural systems. *The Australian Centre for International Agricultural Research (ACIAR)*, 136 (258).
- Sinclair TR, Vadez V (2002). Sensitivity of N<sub>2</sub> fixation traits in soybean cultivar Jackson to manganese. *Journal Crop Science* 42:791-796.
- Van Ruijven J, Berendse F (2003). Positive effects of plant species diversity on productivity in the absence of legumes. *Ecology Letters* 6:170-175.
- Weisany W, Raei Y, Allahverdipoor KH (2013). Role of Some of the Nutrients in the Biological Nitrogen Fixation. *Bulletin of Environment Pharmacology and Life Sciences* 2(4):77-84.
- Wilson JB (1988). Shoot competition and root competition. *Journal of Applied Ecology* 25:279-296.
- Whitehead DC (1995). *Legumes Biological Nitrogen Fixation and interaction with grasses*. Whitehead: DC (Ed) *Grassland Nitrogen*, CAB International. Wallingford. UK: pp. 35-57.
- Zemenchik RA, Albrecht KA, Shaver RD (2002). Improved nutritive value of Kura Clover and birdsfoot trefoil-grass mixtures compared with grass monocultures. *Agronomy Journal* 94:1131-1138.