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Alfalfa (*Medicago sativa* L.)/Crops intercropping provides a feasible way to improve productivity under environmental constraints

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Abstract

The growing threats facing both food security and the environment require a sustainable development. The need for sustainable agricultural alternative systems is prompted by economic, environmental and biological perspectives. Producers require cultivation methods that minimize environmental damage while simultaneously preserving and improving farm profitability. One of the key solutions is intercropping. which is a cropping system where the crop can coexist in the same soil and can be irrigated simultaneously at the same time.It is used to improve crop yields, land use rates, as well as to reduce the barriers towards sustainable cultivation and the development of disease and insect pests as well as control weed growth. Besides, intercropping of legumes in a cropping system provides advantages for non-legumes due to improved nutrient distribution, to improved soil biology and to enhanced use of available resources. The biological fixation of nitrogen by legume crops makes intercropping legumes, especially alfalfa, a cost-effective solution to the problem of using artificial fertilizers. Alfalfa intercropping does not only meet the requirements of food security and forage needs, but also preserves the eco-environment, which has made this cultivation model a very promising one for agricultural development under environmental constraints.

1. INTRODUCTION

The growth of the world's population and climate change are the two major challenges which currently affect food security and safety. Due to these factors, the constraints on agricultural production have increased and we can no longer meet the increased needs. As a result, we have turned to other means of increasing productivity and maximizing yields, such as the use of chemicals (fertilizers, hormones, pesticides, etc.), but these methods have proved to be harmful to humans and the environment (Sun et al., 2014). There are long-term consequences of these approaches, such as the destruction of soils by soil acidification, heavy metals pollution, soil compaction, changes in soil microbiome, water contamination by chemicals, and the loss of biodiversity. A new approach for development of the agricultural sector is required. Natural ecosystem concepts can be brought into our practices, and we can achieve more sustainable agricultural ecosystems. One of these strategies is intercropping, where more than one species is cultivated simultaneously and at the same place and time (Iqbal et al., 2019). Intercropping of legumes and cereals represents an important model of cropping used in agricultural production.Forage intercropping of legumes with cereals can lead to enhance efficiency the resource use and to increase total productivity in the cropping system (Patel et al., 2021). In addition, the intercropping has an effect in biocontrol, increasing soil fertility, controlling pests, reducing weeds, decreasing pathogen pressure as well as minimizing several environmental stresses (drought, salinity, soil fertility, etc.) (Huss et al., 2022).

An example of this system which is used in case of low input or limited resource cropping systems, is intercropping with alfalfa (*Medicago sativa* L.) (Tang et al., 2022).

M. sativa is a perennial legume crop belonging to the subfamily *Papilionoideae*. It is mainly atetraploid species (2n = 4x = 32) and it is cross-pollinated, although a diploid form exists (Bhattarai et al., 2020). Because of its excellent adaptability, large output, high quality and tolerance to frequent harvesting, *M. sativa* is a significant fodder for livestock production industries worldwide (Bhattarai et al., 2020). *M. sativa*'s economic importance is due mainly to its considerable production capacity, with over 80 t ha^{-1} of green matter and nearly 20 t ha^{-1} of dry matter (Ramos-Ulate, 2022)). The high content of crude protein, which is well balanced in terms of amino acids, it is typical of *M. sativa* fodder. It is also rich in essential vitamins and micro-nutrients required for healthy livestock growth and development (Radovic et al., 2009). As a perennial it provides year-round soil cover, wind resistance, fixing the soil and providing a better planting environment. Its wide root system is able to improve soil fertility and physicochemical characteristics, which leads to the establishment of an efficient and sustainable win-win relationship between conservation and use (Sun et al., 2014). Because of its impact on the soil as a legume which had the potential of fixing around 230 kg N ha^{-1} year^{-1} of nitrogen (Gómez-Sagasti et al., 2021), its high productivity and its good forage quality, *M. sativa* is an excellent choice for intercropping.

2. INTERCROPPING BENEFITS

There are generally four different methods of intercropping (Li et al, 2020a) (Fig. 1). The first is row intercropping, in which the separate rows of different crops can be easily identified. The second is mixed cropping, in which seeds from different crops are mixed and planted in the same row. The third is the relay intercropping system, where a second crop is cultivated with a growing crop that has nearly achieved its production cycle, before being harvested. Lastly, there is strip intercropping systems, where two or more crops will be planted in a wide enough strip to allow for multiple rows, yet sufficiently close together to allow easy interaction (Iqbal et al., 2019).

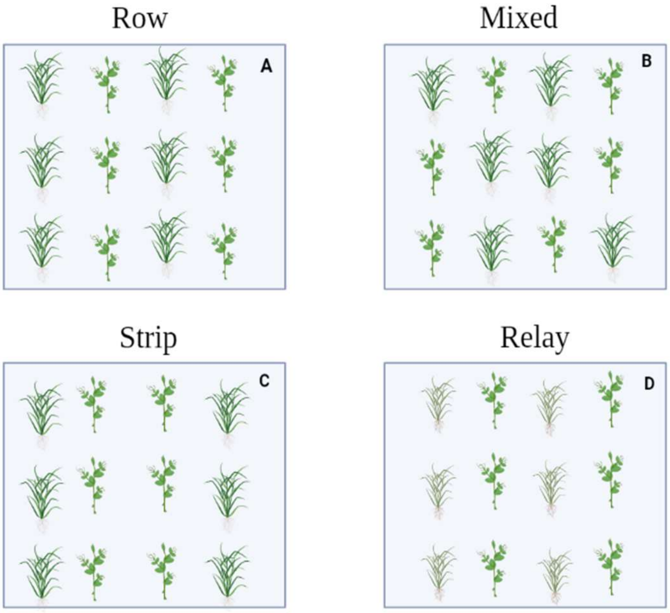

Fig. 1. Types of intercropping (Li et al., 2020a).

The higher outputs produced from intercropping, as compared to single crop yields, are mostly due to complementarity among different species. (Li et al., 2023a). The complementarity on intercropping with *M. sativa* appears when this species and other grasses differ in their root morphology and physiology. Therefore, the two species do not compete for resources and total water. *M. sativa* has a long taproot structure that provides an efficient means to obtain water and nutrients from depths, which allows it to survive under environmental constraints, such as drought and nutritional deficiency. Due to its extensive root system, the soil's fertility and physico-chemical properties can be greatly enhanced, providing an effective balance between land use and maintenance (Sun et al., 2014).

In addition, many studies have shown that intercropping has an effect on root morphology; for example, Zhang et al. (2013) showed that *M. sativa*/corn intercropping system had higher values for root density and length compared to each species monoculture. In addition, roots in the center of *M. sativa* intercrop moved to greater soil depths and also extended laterally into the associated corn rows. The complementary and compatible spatial distribution of roots between the different crops resulted in the higher productivity of biomass.

Besides as a part of this complementarity, *M. sativa* has the potential to enhance the integration of nitrogen fertilizers into the farming system and to improve other plants' nitrogen uptake through a synergistic sharing of water and nutrient absorption (Zhao et al., 2020).

On the other hand, what makes intercropping a good alternative is that animal feed is a key element in the production of milk and meat in the livestock sector. However, livestock productivity can be restricted due to lack of forage and its low quality, especially in arid and semi-arid areas (Hassen et al., 2017). Consequently, in order to provide a high-quality fodder, the use of mixed cereal-legume cultivation is an effective approach towards the amelioration of animal feed (Bo et al., 2022a) in arid areas. As well as balancing the low quality of feed derived entirely from cereals, which is too low in terms of protein (Bo et al., 2022b). Annual cereal and legume intercropping studies have shown that the intercropping improves both the nitrogen and protein concentrations of the cereal grains and could potentially improve their quality. The use of intercropping is a way to increase the concentration of nutrients in the biomass and thus improve their suitability for animal feed (Mårtensson et al., 2022).

3. *M. SATIVA*IN INTERCROPPING

Numerous studies on the use of *M. sativa* as an intercrop point out the impact of this system on increasing accompanying crop productivity. *M. sativa* intercropping with maize boost both maize grain production as well as *M. sativa* forage biomass production, while preserving more natural resources as compared to either crop alone (Sun et al., 2014). It is approximately double the amount of M*. sativa* grown in rotation after maize or in monoculture systems. Similarly, the crude protein, water-soluble carbohydrate, and lactic acid content of the alfalfa-corn intercrop can be significantly improved (Berti et al, 2021a; Zhang et al., 2015; Xu et al., 2022).

Bo et al. (2022b) proved that themixed cropping of cereals and alfalfa was the most preferred cropping method comparing to monoculture for its optimized quality forage production and nutritional content in animal feed. they revealed that the mixed cropping of cereals and alfalfa had an impact on dry matter, neutral detergent fiber, and on acid detergent fiber concentrations, while it induced a highest value of water-soluble carbohydrate, crude protein, ether extract, crude fiber, and ash%. Similarly, mineral compositions analysis of Ca, Na, K, P, and Mg demonstrated that the higher mineral compositions resulted in the mixed ryegrass-alfalfa crop.

4. BENEFITS OF INTERCROPPING WITH *M. SATIVA* UNDER ENVIRONMENTAL CONSTRAINTS

The main common stresses affecting crops are biotic and abiotic. Abiotic stresses involve environmental factors such as heat, drought and/or high salinity levels. Biotic stresses, on the other hand, involve living organisms such as diseases, insects and other pests. Such constraints have several negative consequences in terms of plant health and performance (Fig. 2) (Umar et al., 2021).

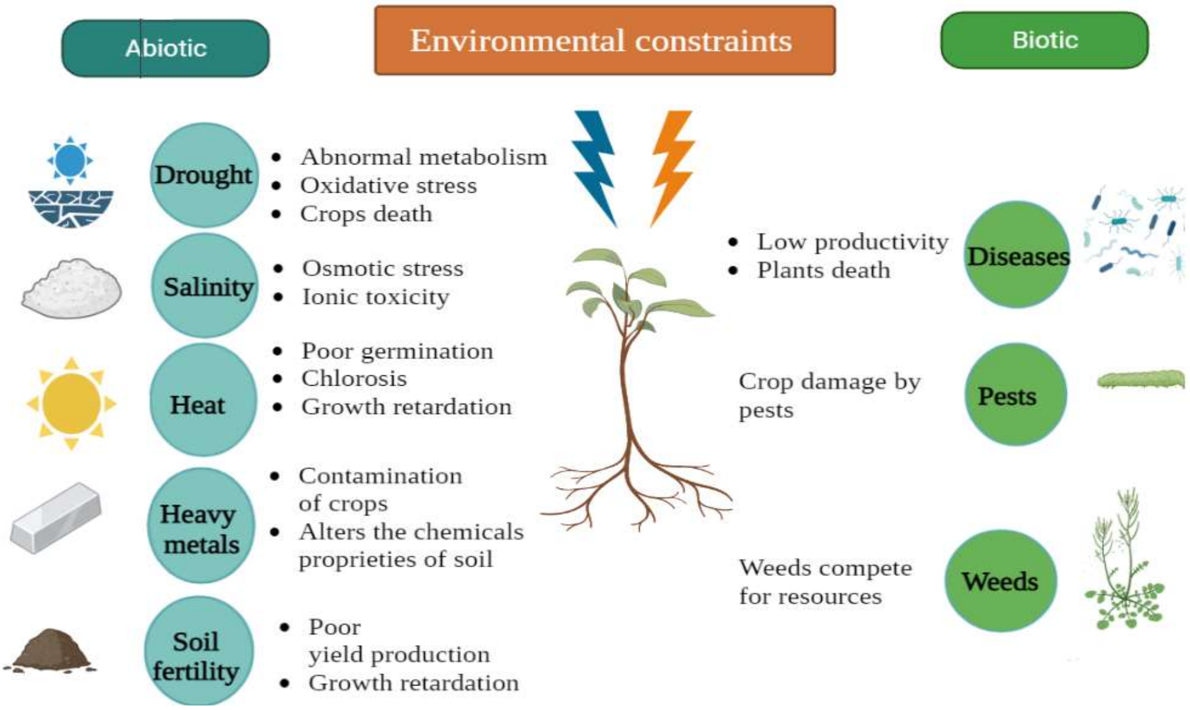

Fig. 2. Environmental constraints and their effects on crops (Umar et al., 2021).This figure is created with BioRender.com.

4.1. Benefits of intercropping with *M. sativa*underdrought stress

As water scarcity has become a major limitation to agricultural development, there is a strong need to increase crop productivity per unit area. Intercropping has been widely applicable in both arid and semi-arid regions based on its high and stable level of productivity and resource efficient use (Yin et al., 2020). Drought causes a restriction in crops growth, which is not only due to physiological limitations, but also to the plant's limited ability to utilize nutrients due to insufficient rainfall and low soil moisture levels. Nevertheless, *M. sativa* has maintained its growth despite drought conditions, most probably through its ability to biologically fix atmospheric N_2 (Mårtensson et al., 2022). In drought conditions, alfalfa was more productive when grown in association with winter wheat (*Triticumaestivum*) (Skelton et al., 2005). *M. sativa* intercropping is feasible and may provide farmers with an alternative to diversify their cropping system under even restricted soil moisture conditions (Berti et al., 2021b).

Alfalfa potentially provides a shading effect over the soil surface, decreasing the evaporation and providing an improvement in soil water status (Mårtensson et al., 2022). For example, intercropping wheat and alfalfa can both conserve and enhance soil water content, as well as improving the efficiency of soil water use (Qiong et al., 2022).

A study conducted by Al-Shareef et al. (2018) on the response of blue panicum (*Panicumantidotale* L) - alfalfa (*Medicago sativa* L. cv. Cuf 101 USA) intercropping under drought conditions, revealed its sustainability. In fact, under limited water resources, utilization of only 60% of water demand is convenient and practical in terms of productivity, as well as obtaining greater protein levels.

Another good example of the potential of intercropping with *M. sativa* to increase crop productivity under drought conditions is shown by the intercropping with wheat (Martensson et al, 2022). Here, it was shown the ability of this system to provide better growing conditions due to nitrogen and water availability

4.2. Benefits of intercropping with *M. sativa*undersalinity constraint

Over the last few decades, the salinization of soils has emerged and become among the world's most severe environmental problems. About one billion hectares of the world's land have been impacted by soil salinization (Litalien and Zeeb, 2020). Soil salinity levels already account for 20% of all cropland and about 33% of irrigated agricultural land in the world, and are likely to rise faster than today by 2050. Obviously, climate change worsens soil salinity because of high air temperature, low relative humidity, and less precipitation (Mukhopadhyay et al., 2021).

M. sativa has historically been categorized as being a salt-tolerant plant that grow well in salt soils. In particular, *M. sativa* can contribute to the removal of salt and Na^+ from saline-sodic soils (Liang and Shi, 2021). It reduces soil salt storage and enhances Na^+ elimination from the soil (Qadir et al., 2003). These properties of alfalfa are valorized by intercropping; for example, the intercropping of alfalfa and spring wheat is a great cropping system to conserve water, promote the soil stability and ion balance, and minimize soil salinity in saline areas (Su et al., 2022).

4.3. Benefits of intercropping with *M. sativa* undernutritional deficiency

Contemporary agricultural practice relies on applying high amounts of reactive nitrogen in order to maintain a high production of crops for use as food, fodder, and feedstocks. But approximately 50% of applied nitrogen (N) is wasted from cropping systems either as nitrate leaching to groundwater and waterways or nitrous oxide (N_2O) (Pannu et al., 2019).

Sustainable agriculture-based resources will involve a better control of N. A relevant option to nitrogen fertilization is to provide bioavailable nitrogen by intercropping with a legume, which, thanks to symbiotic nitrogen-fixing bacteria, called rhizobia, is able to increase soil nitrogen content, then made available to the perennial cereals (Martensson et al., 2022). Moreover, the intercropping improves also the legume symbiotic interaction with rhizobia. For example, interspecific facilitation in the alfalfa/triticale intercropping system has improved the formation of symbiotic root nodules and the capacity of nitrogen fixation (Zhao et al., 2020). The changes in the root morphology enhance N use and provide evidence for the optimum management of nitrogen fertilization in intercropping systems (Shao et al., 2021). As expected, most N transfer occurred in one direction, from *M. sativa* to the other plant (Lai et al., 2022). In this sense, *M. sativa* -wheat intercropping offers opportunities to reduce the competitive pressure through complementary use of resources as well as facilitating the development of the wheat crop by providing nitrogen from the legume (Skelton et al., 2005).

Global P supply is limited and P is frequently identified as the most limiting nutrient in long-term biological systems (Tautges et al., 2018). Intercropping of legumes and cereals increases grain yields when legume and cereal rhizosphere are mixed, due to increased soil P availability from acidification of the legume rhizosphere (Sun et al., 2020). Wang et al. (2020) showed that *M.* sativa is adapted to low P levels morphologically through a higher proportion of fine roots as well as physiologically by increasing ATPase activity and carboxylate secretion into the rhizosphere soil in order to mobilize poorly soluble P from the soil. These results were more significant in intercrops than in *M. sativa*monocrops, suggesting that alfalfa root properties are affected by both soil P supply and neighbor identity. Therefore, the increased availability of soil P through alfalfa root exudation and the improved rhizosphere environment due to alfalfa root fineness, led to better shoot growth and P acquisition by maize in the alfalfa/maize mixture.

Root management is a key strategy to improve P acquisition and yield production. A challenge to improve the availability of food, particularly in infertile soils, might be overcome through the optimization of root shape and distribution to improve phosphorus acquisition (Sun et al., 2019). Consequently, the inter-cropping of alfalfa has the potential to improve the availability of nitrogen, phosphorus and potassium in the soil in the intercropping area (Liu et al., 2021).

4.4. Benefits of intercropping with *M. sativa* for the soil microbial community

Microorganisms in the soil are a key part of soil fertility and have an important contribution to biogeochemical cycling. They are involved in the formation and decomposition of organic matter, respiration, mineralization, as well as nutrient cycling and water infiltration. Changes in soil microbial abundance or diversity can have an impact on soil nutrient uptake, total nitrogen, organic matter, available phosphorus, and soil pH (Fu et al,, 2019). Both the structure and diversity of soil microbial communities are affected by the composition and diversity of plant species (Fahey et al., 2020). In continuously cropped systems, the microbial communities show a reduced density (Pang et al., 2021). Conversely, intercropping is a major way of increasing microbial diversity and quantity, which in turn leads to an increase in productivity (Zhang et al., 2018)

Many examples show that intercropping with *M. sativa* has promise to increase microbial activity as well as diversity of microorganisms in the other plant's rhizosphere. For example, Mulberry and alfalfa intercropping affected soil pH and water content of the rhizosphere of mulberry and alfalfa as well as microbial community and growth changes, which led to different carbon use patterns and capacity (Zhang et al., 2018 Li et al., 2023b). Also, Sun et al. (2009) showed that the intercropping of *M. sativa* and Siberian wild rye (*Elymussibiricus* L.), has a beneficial impact on the maintenance and improvement of soil nutrient status as well as on microbial activity and community composition. This impact was demonstrated by the increase in microbial biomass in *M. sativa*rhizosphere. Similarly, regarding of soil enzymes which mainly originate from soil fungi, bacteria and plant roots and their activities. Notably, they noted that urease, invertase and alkaline phosphatase activities were increased in the rhizosphere of *M. sativa* due to intercropping. This finding demonstrates that these practices can boost microbial activities as well as biochemical cycling of N, C and P in *M. sativa*rhizosphere and can therefore improve nutrient supply and consequently plant performance.

Another example shows that intercropping of *M. sativa* and corn increased microbial functions associated with the improvement of plant-soil nutrient accessibility. Intercropping was also shown to increase both denitrification (*napA*, *norC* gene abundance) as well as nitrogen fixation (*nifD*, *nifK*, *nifH* gene abundance) significantly compared to nitrogen fertilizer application, along with a reduction in nitrate uptake (*nasA* gene abundance) (Zhang et al., 2022). The same results were reported for wheat and alfalfa (Li et al, 2020b) and alfalfa and oat intercropping (Liu et al., 2021).

4.5. Benefits of intercropping with *M. sativa* under heavy metals

Cumulative heavy metal contamination is an environmental challenge, representing a significant risk to both human health and ecosystems (Cui et al., 2018). The use of intercrops in soils contaminated with heavy metals is an attractive approach for the phytoremediation. The intercropping of low accumulation cash crops and hyperaccumulators could be a reasonable and economical way to remediate soils and possibly maintain crop production and yields during the phytoremediation process (Ma et al., 2018).

M. sativa has been used for phytoremediation of soils polluted with heavy metals, petroleum hydrocarbons as well as aromatic hydrocarbons. *M. sativa* requires minimal maintenance (no annual plowing and seeding) and increases fixed nitrogen which can decrease oxidative stress caused by heavy metal accumulation (Jeder et al., 2021).

Moreover, Cui et al. (2018) experiment proved that the intercropping of ryegrass and alfalfa can enhance the growth of plants and increases the biomass of plants in heavy metal-contaminated soil. Crops cultivated in the intercropping treatment exhibited significantly lower Pb concentration in shoots and roots of plants in comparison with those in the monoculture treatments. Consequently, the intercropping of both forage species may provide a promising phytoremediation model for heavy metal accumulation and enhanced yields at large rates under heavy metal-contaminated soils.

4.6. Benefits of intercropping with *M. sativa* under polycyclic aromatic hydrocarbons

Many species of plants, including alfalfa, have proven to be a valuable candidate for the phytoremediation of PAHs. Sun et al. (2011) assessed the potential of tall fescue and alfalfa intercropping in comparison to monoculture, intercropped plants significantly improve the rate of PAH degradation in the soil, particularly high molecular weight PAHs. In this work, intercropping increased also the number of PAH-degrading bacteria and microbial activities in the soil. As a result, intercropping alfalfa and tall fescue offers an interesting strategy for phytoremediation of PAH-contaminated soils.

4.7. Benefits of intercropping with *M. sativa*against pests

Pests are the most troublesome, inducing defoliation and resulting in lower yield, and lower quality of forage (Pellissier et al., 2017). Recently, attention has switched over to using cultural methods to combat pests, since pesticides frequently lead to negative impacts on biodiversity and ecosystem function, including killing of beneficial insects. Pest infestation has been demonstrated to be more intense in monocultures than in mixed crop systems (Skelton et al., 2005). Intercropping is an alternative strategy for sustainable pest management without using chemical pesticides. This form of pest management reduces pest numbers by adding more diversity to the ecosystem. Also, through intercropping can be based on planting of crops that either kill or repel pests, attract natural enemies, or provide anti-bacterial properties that can help to reduce disease and pest damage and pesticide use (Mir et al, 2022). In fact, *M. sativa* and grass intercrops decrease the population of insect pests versus monocultures (Degooyer et al., 1999). Along with many natural benefits, alfalfa is an insect-friendly plant which provides pollen, nectar and alternative prey for both predators and parasitoids, as well as offering a potential use in the management of pest control (Tajmiri et al., 2017).Tajmiri et al. (2017) revealed that in the intercropping of canola and *M. sativa*, reduced the egg, larvae and pupae densities compared to the monoculture. Also, they proved that intercropping provided the most optimum system with greater advantages over traditional insecticide use.

5. CONCLUSIONS

Intercropping has proven to be one of the most successful cropping systems in terms of achieving high crop outputs with high quality, under environmental constraints. Similarly, *M. sativa* has also become a valuable support for other crops and consequently for producers. Owing to its perennial, leguminous nature and its tolerance to numerous constraints, *M. sativa* is becoming a joker crop that can rescue the development of sustainable and low input agriculture by using it as an intercrop.

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