Chapter 1 : Review of Literature

"*Look deep into nature, and then you will understand everything better*"-

Albert Einstein

1. Introduction

Even with the various initiatives taken to reduce food insecurity, undernutrition, and hunger, there are still some serious problems faced globally (Sibhatu and Qaim, 2017). As per the latest record by the Food and Agriculture Organization of the United Nations, 13% of the population living in developing countries are afflicted with malnutrition, and by 2050, a huge increase in food demand is predicted, projection of 59% to 102% rise. According to some estimates, global agricultural production needs to be increased by about 60% –70% to palliate the problem of food insecurity (Pawlak, 2020). Therefore, to mitigate this issue of food security and nutrition, sustainable agriculture schemes need to be promoted, keeping in mind a balanced trade-off between productivity and ecosystem services (Struik & Kuyper, 2017; Calicioglu et al., 2019). Some of the sustainable practices that can help agricultural intensification include the management of natural resources through intercropping, crop rotation, reduced tillage, and application of microbes for plant growth promotion. (Tilman et al., 2011; Wezel et al., 2014).

1.1 Intercropping for enhanced productivity and fertility of the soil

Intercropping is one of the ancient cropping systems, which involves co-cultivation of two or more crops together in the vicinity on the same land and without necessary sowing or harvesting at identical time points (Vandermeer et al., 1998; Malézieux et al., 2009). It is practiced worldwide in the combinations of different crop species that can be grown either in alternate rows, or strips, or mixed patterns on the same field. It sustainably benefits agriculture by manifesting ecological mechanisms like weed suppression, pest and disease control, efficient usage of light and water, and also conserves soil resources (Martin-Guay et al., 2018). Moreover, it diverges from crop rotation in terms of growth pattern wherein two or more crops are grown one after the other. Intercropping and crop rotation are alternative options applied in multiple cropping systems that add resilience, increase yields, and generate co-benefits for both farmers and ecosystem services (Layek et al., 2018). Both these cropping systems ensure increased farm production and profitability per unit land area in selected crops. On average, the practice of intercropping and crop rotations positively affects ecosystem service delivery (Kleijn et al., 2019). Fig. 1.1 highlights the influence of these practices and suggests that both have a sustainable positive effect however none of these practices consistently improves in an overall manner. When higher crop diversity is aimed from a given piece of land, then intercropping is a suitable option as it increases the land utilization ratio, and also emits significantly lower amounts of greenhouse gases (GHG) as compared to cultivating a single type of crop (monocrops) (Agegnehu et al., 2008; Oelhermann et al, 2009; Naudin et al., 2014). Recently, Yin et al (2018) highlighted one such importance in maize-wheat intercropping with a straw covering surface that reduced the tillage and thereby effectively lowered CO_2 emissions in an arid oasis irrigation region.

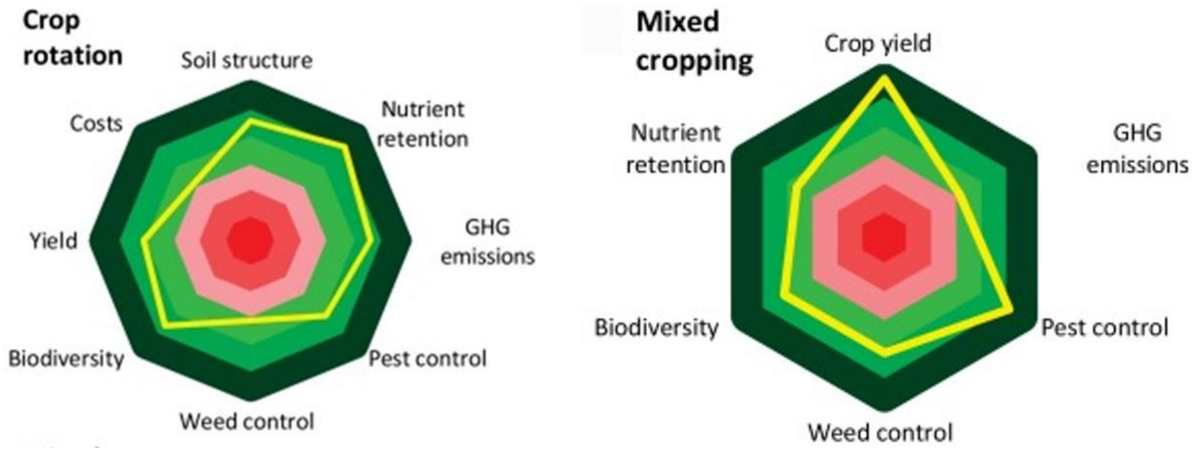

Fig. 1.1 Radar plots summarizing the effects of intercropping (mixed cropping) and crop rotation on different aspects. Here, yellow lines indicate the positive effects of the particular trait (Kleijn et al., 2019).

Intercropping also provides immediate net income in some years for the farmer compared to the sole crops and thus it can be regarded as better security for smallholder farmers (Chimonyo et al., 2019). Furthermore, intercropped plants maintain the stability of most of the soil chemical and enzyme activities compared to the monocropped or rotational cropped plants in the long term (Wang et al., 2015). Similarly, Malobane et al., (2020) also demonstrated that incorporation of intercropping can improve the no-tillage practice with 30% crop residue retention that enhanced soil fertility in marginal soils of South Africa.

1.2 Advantages of intercropping system

The most important role of intercropping is the inland land equivalent ratio (LER). The LER is described as the ratio of the area under mono-crops (sole crop) plants to the area under intercrop required to give the same yields. Meta-analyses data estimates that the LER of intercropping is between 1.22±0.02 and 1.30±0.01 (Li et al., 2020). The prevalence of intercropping is also observed due to other benefits like greater utilization of resources, enhance yield, conservation of the resources, reduction of harmful biotic agents, and more production and sustainable output of the system as shown in Fig. 1.2. Moreover, when small millets are intercropped with legumes it enables the diversity of beneficial soil microorganisms and also facilitates soil fertility enhancement (Maitra et al., 2021). Compared to the monocrops of modern industrialized agriculture, meta-analysis data represented that intercropping was 16% to 29% more efficient in terms of grain per hectare by using 19% to 36% less fertilizer per unit output (Li et al., 2020).

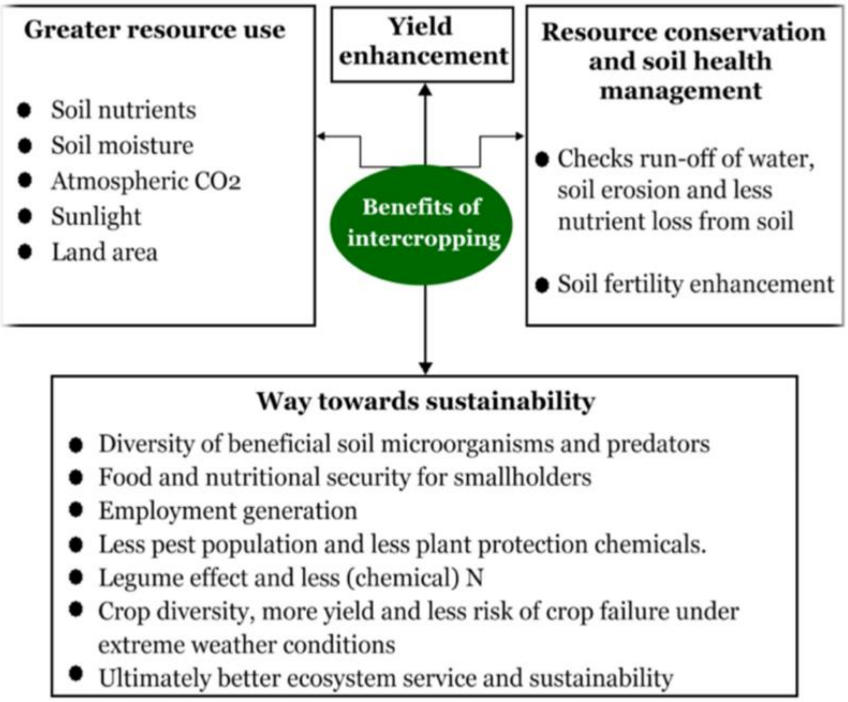

Fig. 1.2 Benefits of the intercropping system (Maitra et al., 2021)

Furthermore, there are many problems associated with monocropping which starts from nutrient imbalance to a high risk of occurrence of diseases and pests. To alleviate this problem, cereals and legumes can also be grown as intercrops (Jat et al., 2020). The main advantage of cereal-legume intercropping is that legumes can fix nitrogen from the atmosphere, release soil high-quality organic matter, and facilitate soil nutrients' circulation and water retention. Based upon these multiple functions, legume crops have a high potential for conservation agriculture, like intercropping (Stagnari et al., 2017). Among the other combinations, the *Cajanus cajan - Zea mays* intercropping system is a classic example of legume-cereal intercrop, wherein the drought-tolerant late-maturing *C. cajan* increases nitrogen through leaf litter and biological nitrogen fixation and thereby enhances soil fertility (Lithourgidis et al., 2011; Layek et al., 2018). *Z. mays* are dominant as the cereal components in legume–cereal intercrops, mature early, and utilizes nitrogen from the legume plant (Adu-Gyamfi et al., 2007). It can be sown in strips of several rows or with an alternate several rows of a C3 species (for instance, wheat (*Triticum aestivum*) and soybean (*Glycine max*). Maize-based intercropping has been shown to raise the average total yield of harvested seed by ~1.7–2.1 tones ha^{-1} relative to sole plantation (Li et al., 2020). The increase in the yields of intercropping means that more food is produced per unit of fertilizer in less space of land (16–29%). This outcome in the future will decrease land clearing as well as greenhouse gases emissions and species extinctions (Tilman, 2020). In addition, the cereal-legume combination offers a natural defense against the failure of a crop and thus provides food and nutritional security to smallholders of dryland (Maitra et al., 2019) and paddy fields (Hei et al., 2021).

1.3. Resource use efficiency in cereal-legume intercropping system

Due to the diversification of plants, there was about a 1.7 times increase in biomass production and other ecosystem functions than in monoculture. In the field, plant traits for resource acquisition and underlying mechanisms are identical in intercropping and monocropping systems, however, to optimize overall plant performance the intercropping systems should include the best combination of different plant traits (Brooker et al., 2015). There exist three main types of plant-plant interactions (Bedoussac et al., 2015) that are illustrated in fig. 1.3.

1) Competition occurs when one of the plant species (Fig. 1.3 A) alters the environment and contribute negatively on to another species, e.g. shading for light or by creating nutrient limiting condition for the other plant.

2) Complementarity among the species cultivated (Fig. 1.3 B) is very important for increasing crop yields and leads to a decrease in interspecific competition and competitive exclusion through resource partitioning in a given time or space between intercropped species. One of the notable examples of the complementarity is between cereals- legumes, wherein both the species require soil N from the available pool of resources however only the legume can substantially access the additional pool of atmospheric N through symbiotic fixation.

3) Facilitation occurs when one species (Fig. 1.3 C) enhances the growth or survival of another and is achieved by combining plants that increase the phytoavailability of water, phosphorus (P), the nitrogen (N) available to the system (through N2 fixation), or micronutrients (iron (Fe), zinc (Zn), copper (Cu)). For example, legumes root exudates release bound P, while maize root exudates enhance the iron nutrition of plants (Orrell and Bennett, 2013; Brooker et al., 2015). Besides, the transfer of N and phosphorus solubility by legumes towards cereal plants makes accessible to these nutrients or allopathic effect by either of the plant species reduces the disease spread and attack by pathogens.

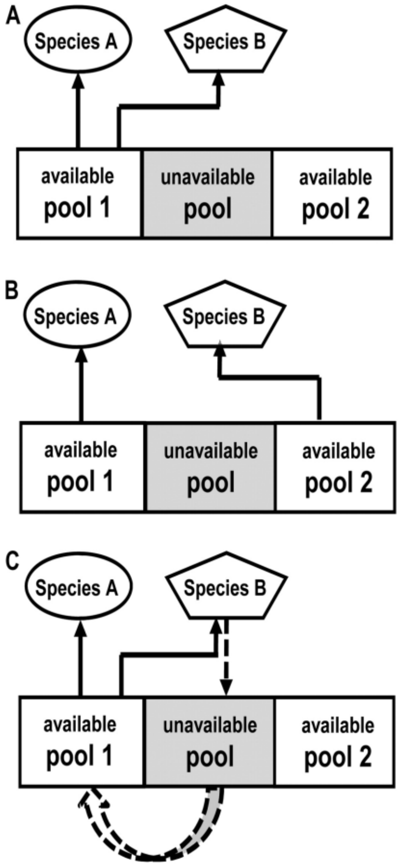

Fig. 1.3 Possible below-ground interactions in the rhizosphere of the monocropping and intercropping (A) Competition, (B) Complementarity/ partition of the resources, and (C) Facilitation between two intercropped species. Nutrient resources are reserved in pools. Solid arrows indicate uptake of the resource by the intercropped species, while dotted arrows show mechanisms by which species B modifies unavailable resources (indicated by the curved arrow),

can enhance the uptake of the resource by the intercropped species A (facilitation) (Hinsinger et al., 2011).

Moreover, facilitation can be achieved by either direct positive mechanisms, such as favorable alteration of light, temperature, soil moisture, soil nutrients, etc., or by indirect mechanisms, that is by attracting beneficial organisms, such as natural enemies and pollinators, the deterrence of pests and pathogens, and the suppression of weeds leading to favorable changes in soil mycorrhizal or microbial communities. In contrast to resource partitioning, resource sharing in facilitation can be affected by common mycorrhizal fungal networks or recycling of nutrients through leaf senescence and root turnover in the rhizosphere (Hinsinger et al., 2011; Brooker et al., 2015). Therefore, the facilitation mechanism between legumes and cereals plants can get better off resource requirements, limitations for each plant, and the optimization of the resource cycling during the growing season (Brooker et al., 2015).

1.4. N and P utilization from the rhizosphere of cereal - legume intercropping system

The diversification of the crops achieved via intercropping can be used for simultaneous production of both portions of cereal and grain legumes. This increases the chance using of N-sources, reduces external inputs of N fertilizers, and thereby enhances the sustainability of agriculture shown in Fig. 1.4 (Rodriguez et al., 2020). Also, the intercropping system reduces N loss with legumes, Hei et al., (2021) proved it in rice/water mimosa intercropping. According to the survey, the global input of N fertilizer by 26% can be lessened in the legume-cereal intercropping system, this leads to a potential saving for cereal sole crop. Besides, less N would be lost as greenhouse gas N_2O or as nitrate leaching to aquatic environments and improve the sustainability of agricultural systems (Jensen, 2020). Therefore, in the future farmers can opt for intercropping to avoid the greater dependence on industrial N-fertilizers.

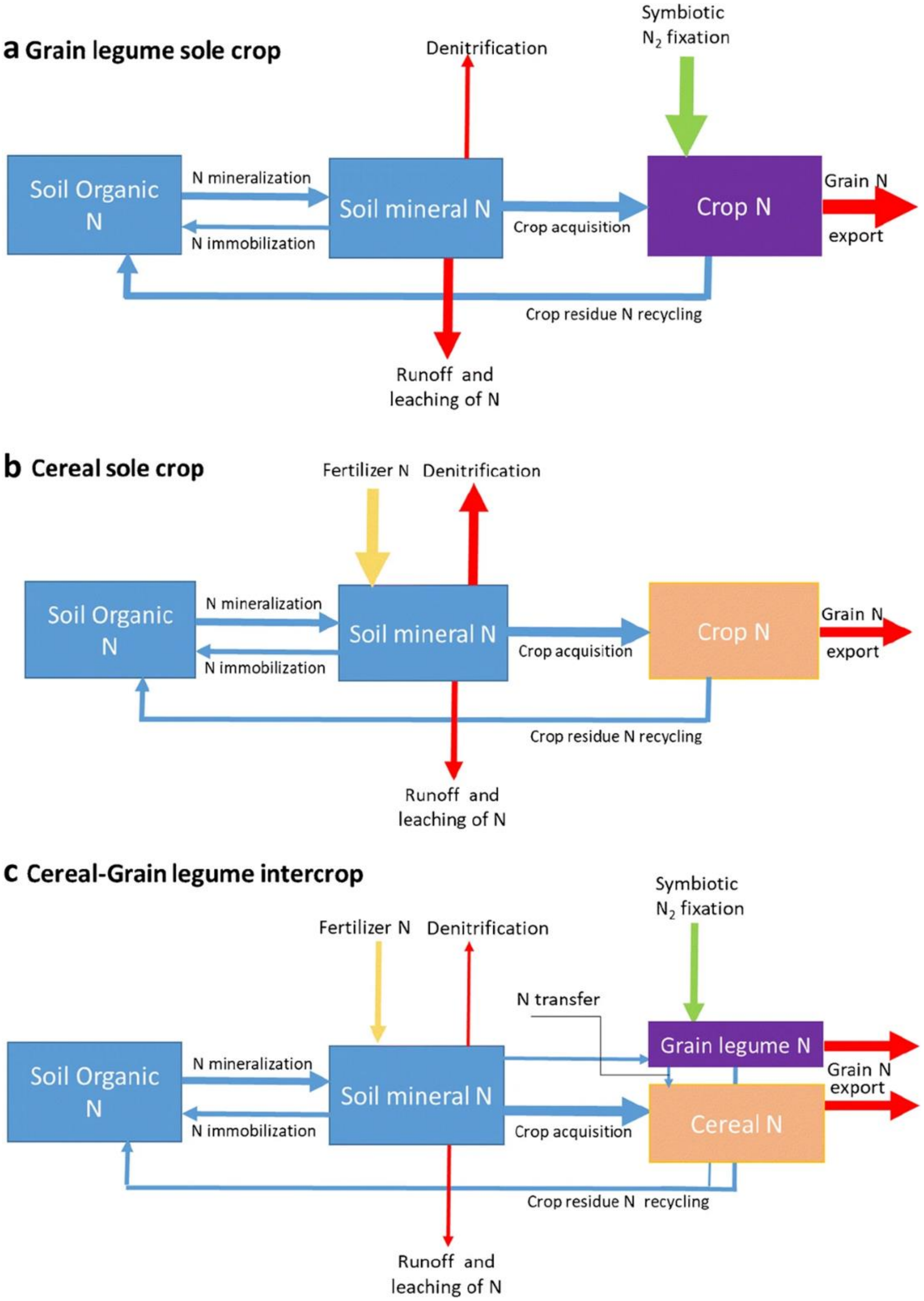

Fig. 1.4 Representation of the important N cycle processes in the rhizosphere of cereal-legume intercropping system (a) Cereal monocrops, (b) grain legume monocrops, and (c) crops of cereals and grain legumes. The width of arrows indicates the relative importance of processes but might vary significantly between cropping systems (Jensen, 2020).

In addition to N uptake, the positive interaction between cereal- legume cropping systems, helps in facilitation that plays a significant role in P acquisition (Mommer et al., 2016a). It is previously known that plants respond to this nutrient limitation by the biological interactions mediated via root exudates released in the rhizosphere and thus change the chemical environment of the rhizosphere, alters the root morphology~~,~~ and recruits microbes (McNear Jr., D. H. 2013). Researchers showed various reports for their enhanced P uptake in a legume-based intercropping system, for example, pigeon pea- sorghum intercropping (Ae et al., 1990) and lupin- wheat intercropping (Cu et al., 2005). Moreover, the biological and chemical process ensures the availability of some micro-nutrients like iron and zinc (Xue et al., 2016). The following are the examples that necessitate the interspecific mobilization and uptake facilitation of sparingly soluble Fe (Dai et al., 2019), while P and Zn from the soil benefits during cereal/ legume intercropping system (Xue et al., 2016), within the rhizosphere by acidifying or changing the redox conditions or directly chelating with the nutrient (Fig. 1.5). One of the recent meta-analysis reports on the P uptake in cereal-legume intercrop observed similar yields and 21% less P fertilizer was required in intercrops than in sole crops (Tang et al.,2021).

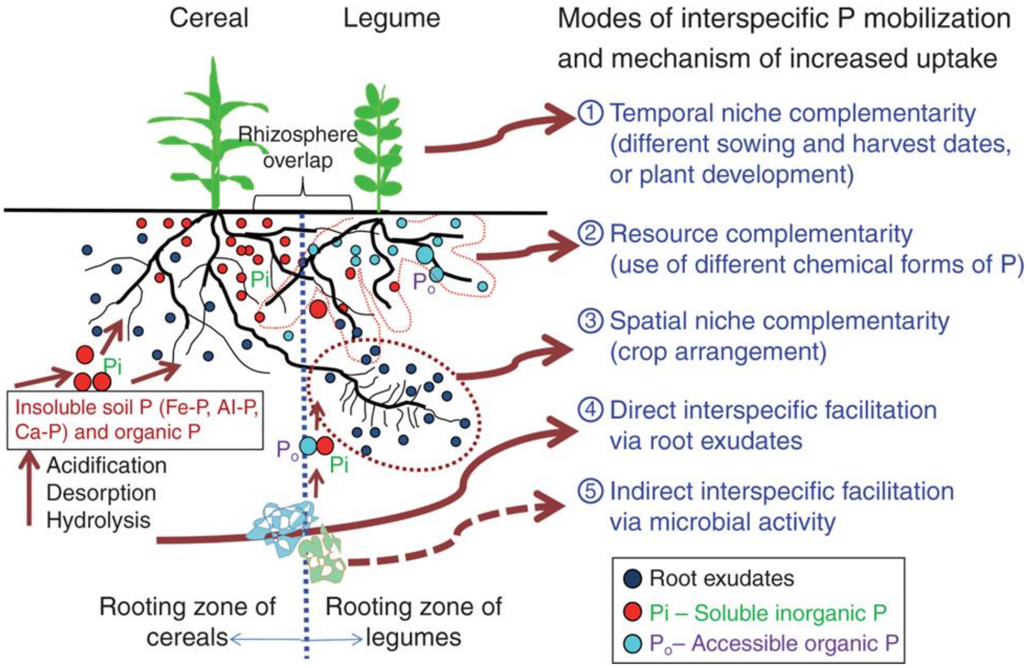

Fig. 1.5 Representation of possible direct and indirect modes of increased phosphorus (P) uptake in cereal- legume intercropping Temporal, spatial, and resource complementarity, and direct and indirect mobilization are indicated (Xue et al., 2016).

1.5 Biological interactions existing at an eco-physiological level can minimize the input of chemical fertilizer in intercropping system

Despite the high financial costs and potentially harmful effects on the environment, agricultural ecosystems are highly dependent on the input of N fertilizers such as urea, nitrate, and ammonia to obtain greater yield. The major disadvantage of N fertilizer is that it contributes 70% of the total N_2O emission and 77% of the total NO^{3-} (nitrous oxide) emission from agricultural soils (Pathak et al., 2010). The high input of N rates increases the activity of ammonia-oxidizing and denitrification bacteria which results in a decrease in nitrogen-use-efficiency in maize plants. Also, this leads to a change in both root exudation and the abundance of soil bacteria, resulting in N loss from the system at higher N rates (Zhu et al., 2016). Therefore, its usage in excess might turn into an ecosystem hazard where nitrifying microbes convert them to nitrate (Pereg et al., 2020).

To mitigate the above problem, the beneficial microbes are exhaustively used either as inoculants, biofertilizers, or biostimulants to assist in plant growth and to act as biological control agents (Bargaz et al., 2018). There are several nitrogen-fixing bacteria non-symbiotic bacteria of cereals that are used to increase plant vegetative growth and grain yield (Malik et al., 2002; Kennedy et al., 2004). Moreover, there exists a hub of biological interactions like that of plant-microbe interactions that promotes beneficial interactions and root-root interactions. In this context, intercropping practices provide favorable below-ground interactions that influence the soil rhizosphere through their root activities, exudation, and rhizodeposition, and attract particular communities of bacteria and fungi (Duchene et al., 2017; Wang et al., 2020).

1.5.1 Importance of interspecific facilitation in cereal- legume intercropping system

Ze-qiang et al., (2021) recently suggested that its role in maize/alfalfa intercropping systems nitrogen fixation, transfer, and acquisition significantly improved due to root contacts between the two plants. This has led to an increase in maize growth with a smaller decrease in the biomass of alfalfa. Likewise, there are several other reports for the same (Table 1.1) that enabled the usage of the low amount of fertilizer into the soil. Hence, the intercropping system facilitates root-root interactions and plays an advantageous role to sustainably increase agricultural production.

Table 1-1 Root-Root interactions involved in the growth of the legume –cereal intercropped plants with minimal application of chemical fertilizer

Intercropped plants	Patterns of root-root interactions	Remarks	References
Alfalfa- Triticale (Hybrid of Wheat & Rye)	Closer root interaction	There was enhanced nodulation and N fixation ability due to the interspecific facilitation leading to a higher yield and N accumulation.	(Zhao et al., 2020b)
Pigeon Pea - Maize	Primarily caused by physical root contact	Pigeon pea- maize intercropping showed a positive effect on soil structure and also on nutrient storage in the high P-sorbing soils.	(Garland et al., 2017)
Soybean- Maize	N transfer during relay intercropping	Intercropping of maize and soybean enabled major increases in land productivity with less fertilizer N use.	(Xu et al., 2020)
	The closer distances of 15–30 cm between the intercropped plants was a better choice in maize-soybean intercropping	There was an increased N fixation and N use efficiency compared to that of conventional application without decreasing grain yield in intercropped plants.	(Yong et al., 2018)
Pea - Maize	Occurrence of interspecific facilitation between pea- maize intercropping system	There was a lower rate of N fertilizer required to get a higher density of maize and an increased grain production.	(Yang et al., 2018)
Cowpea/ Soyabean/ Groundnut- Maize	Intense benefits were achieved due to the ability of legumes' to fix N2 and because of minimal competition for light in this type of cropping system	The economic patterns and land equivalent ratio in all of these intercrop patterns were greater than unity. This overall implied intercrops had greater efficiency and productive use of environmental resources.	(Kermah et al., 2017)

1.5.2 Impact of rhizospheric microbes in intercropping systems

The rhizosphere is best described as a zone of 1-3 mm round roots which is influenced by roots exudates. The rhizosphere microbiome consists of up to 10^6-10^9 bacteria, 10^4 protozoa, 10^1-10^2 nematodes, and 10^5-10^6 fungi per gram of rhizosphere soil (Hinsinger et al., 2009; Mendes et al., 2013). It has a compact area wherein roots of neighboring plant species participate in space, water, and mineral nutrients, and also interacts with soil microbes (Ryan and Delhaize, 2001). The rhizospheric soil is distinguished by its close connections with microbial populations and provides an ideal living environment for bacteria, fungi, and innumerable other organisms. Hence, the study of plants with their microbial populations residing in the rhizosphere can be harnessed for the development of environmental sustainability and efficient agricultural products such as bio-fertilizers and bio-pesticides (Huang et al., 2014; Nwachukwu et al., 2021).

In recent years, the positive effect of rhizospheric microbes on intercropping has been closely examined. Cropping systems like cereal-legume intercropping enhance microbial interactions and profit from the stimulated living rhizosphere. As the root systems are not separate but intermingled, this enables legume plants to modify the chemical properties of the entire rhizosphere and, with their specific genera of bacteria, stimulate the rhizosphere for the potential benefit of both the legume and the cereal (Duchene et al., 2017). Such improvement was observed in the maize-bean intercropping system, wherein there was an increase in rhizobial symbiosis in intercropping over monocropping treatments (Latati et al., 2016). Below table 1.2, also highlights the importance of microbes in the intercropping system.

Table 1-2 Role of root-microbe/microbiome interactions involved in cereal- legume intercropping systems

Intercropped plants	Involvement of rhizospheric microbes	Remarks	References
Cassava- peanut	The dominance of bacteria like *Pilimelia*, and *Ramlibacter*	Intercropped plants enhanced the available soil nitrogen content	(Tang et al., 2020)
Pigeon Pea- Finger millet	Co-inoculation of AMF and PGPR	Nitrogen and phosphorus uptake was strongly improved by both plants, under well-watered and drought conditions The application of combined biofertilizer in finger millet- pigeon pea intercropping increased yield up to +128%	(Saharan et al., 2018) (Mathimaran and Jegan, 2020)
Peanut- Maize	An abundance of bacteria belonging to P-solubilization, pathogen suppression, and N-cycling (*Rhizobium hainanense*, *R. leguminosarum* and *Frankia).*	Enhanced soil nutrient (N and P) supply capacity and promoted nitrogen-fixing organisms in the rhizospheric soil.	(Chen et al., 2017; Li et al., 2018)
Faba bean- maize	Relative abundance of rhizobia	Suppression of the negative effect of pathogens due to maize plantation and increased beneficial microbes.	(Wang et al., 2021a)
Sugarcane- legume	Higher diazotrophic populations (γ-Proteobacteria-28%) in the rhizosphere and plant endosphere	Increased soil organic matter and urease activity improved soil fertility	(Solanki et al., 2019; Solanki et al., 2020)
Soybean-maize	The higher proportion of phylum Proteobacteria	This correlated positively with maize soil organic matter and soybean soil total nitrogen content	(Zhi-dan et al., 2019)
Medicago sativa- Dactylis glomerata	The soil-root environment possessed different groups of bacteria involved in N-cycling processes. Like ammonia oxidizers intricated in nitrification, were abundant in the soil while those related to N fixation and gaseous N losses (denitrification) were	Legume impacted the root-associated N-cycling communities on the grassroots	(Zhao et al., 2017a)

	more profoundly occupied on the roots.		
Kura clover-prairie cordgrass	The richness of arbuscular mycorrhizal fungi	Yielded high desirable soil microbial community structure and enzymatic activities compared to the N fertilization	(Sekaran et al., 2019)
Mung bean-proso millet	The bacterial taxa (like Proteobacteria) and fungal taxa (like Ascomycota) were significantly changed	Showed definite improvement in N assimilation	(Dang et al., 2020)
Pea-Wheat	Intercropping promoted a synergetic interaction of Actinobacteria	Actinobacteria phylum possesses dominant plant growth-promoting bacteria in pea- wheat intercropped plants that were grown without the fertilizer.	(Taschen et al., 2017)
Fenugreek-Barley	Dual inoculation of *Sinorhizobium meliloti* F42 and *Variovorax paradoxus* F310	Bio-inoculants like Rhizobial and PGPR promoted the growth in the cereal–legume intercropping system.	(Toukabri et al., 2021)

From the above table, it can be concluded that root- microbe association also plays a magnificent role in intercropping system. Root exudates the major contributors ensures in plant-micro-organism interactions and significantly shapes the structure of the microbial communities (Berg and Smalla, 2009; Wieland et al., 2001). During the initial growth of legume plants, root exudates act as a mediator for N transfer. Eventually, roots also release organic forms of N, primarily through root nodules and root tips (Thilakarathna et al., 2016), which can be advantageous in N nutrition, growth, and yield of maize (Coskun et al., 2017). In general, below-ground nitrogen transfer is delineated as either “direct” or “indirect”. The direct N transfer is the transfer of N from a donor plant to a receiver plant without undergoing mineralization, whereas the indirect N transfer implies mineralization followed by the uptake of N compounds by the receiver as shown in Fig. 1.6.

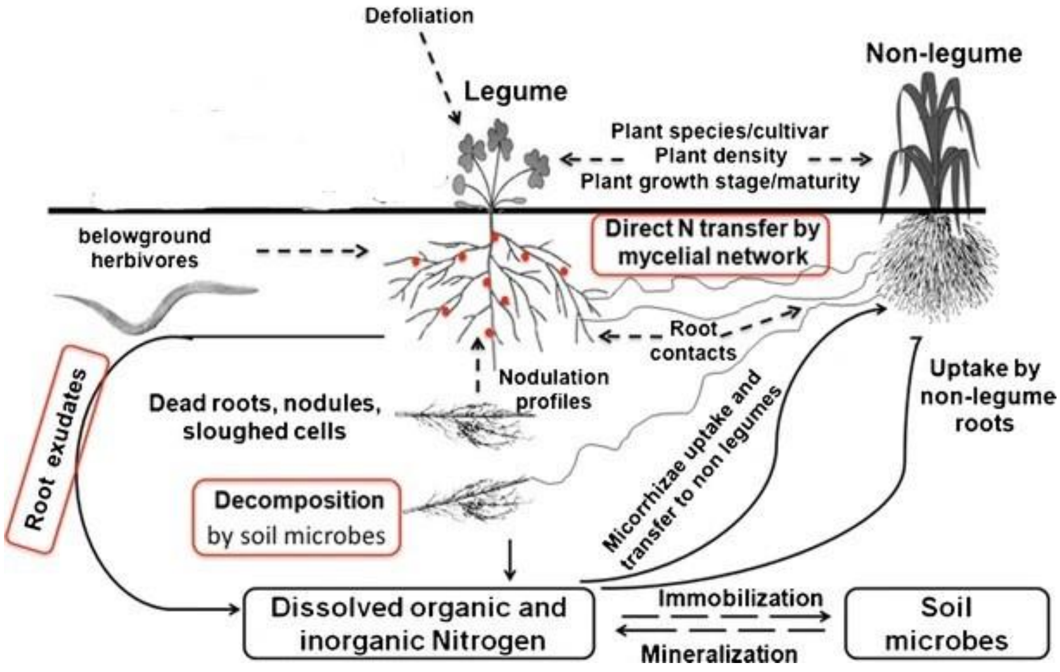

Fig. 1.6 Role of microbes in nitrogen transfer mechanisms from legumes to cereals Various activities including the decomposition of roots and nodules, root exudates, and mycorrhizal mediated N transfer) and the wide range of biotic and abiotic factors that affect N transfer is depicted (shown with dashed arrows) *(Thilakarathna et al., 2016).*

The soil organic matter (SOM) decomposition was observed to be increased in intercropping possibly by lower SOM recalcitrant, higher root/ leaf litter, and better soil N retention (Cong et al., 2015). This leads to the-net enhancement of organic N mineralization and nitrification, also in gross mineral N immobilization rates~~,~~ and increased yields. A similar type of soil conservation in soils via intensification of the mineralization-immobilization turnover was as reported in wheat- faba bean intercropping (Xu et al., 2018). Recently, rice intercropping with water mimosa plants also facilitated lower apparent N loss and mineralization and increased the total N, NH_4^+-N, and NO_3^--N contents of soil (Hei et al., 2021).

1.6. Rhizospheric interactions attributed to root exudates

The 'rhizosphere effect' results primarily from the release of carbon-containing compounds from roots, collectively known as Rhizo-deposition. There are a wide variety of substances in rhizodeposition that are originated from sloughed-off root cells and tissues of damaged cells while mucilages, volatiles, soluble lysates, and exudates are released from intact cells (Dennis et al., 2010). The carbon released from root epidermal and cortical cells provides a niche for the multiplication of microbes within the endorhizosphere as endophytes or on the root surface (rhizoplane) as an epiphyte, and outside the roots (ectorhizosphere) (Jones et al., 2004).

Plant roots secrete around 5% - 21% of total photosynthetically fixed carbon being transferred through root exudates (Marschner, 1995; Derrien et al., 2004). Root exudates are the major source of soil organic carbon released by plant roots (Hütsch et al., 2002), that passively diffuses into the soil (Bertin et al., 2003; Bais et al., 2006). They are mainly classified into main two classes of compounds:

i) low-molecular-weight primary metabolites such as organic acids, amino acids, sugars (Canarini et al., 2019) and also constitutes a diverse array of secondary metabolites (Bais et al., 2004), and

ii) high-molecular-weight exudates in terms of molecular mass, such as mucilage (polysaccharides) and proteins, account for a large proportion of root exudates (Badri and Vivanco, 2009).

Based on the mechanisms of release shown in Fig.1.7 (Oburger et al., 2013), and the biochemical nature or functions of rhizodeposits in the rhizosphere, different rhizodeposits nomenclatures have been proposed.

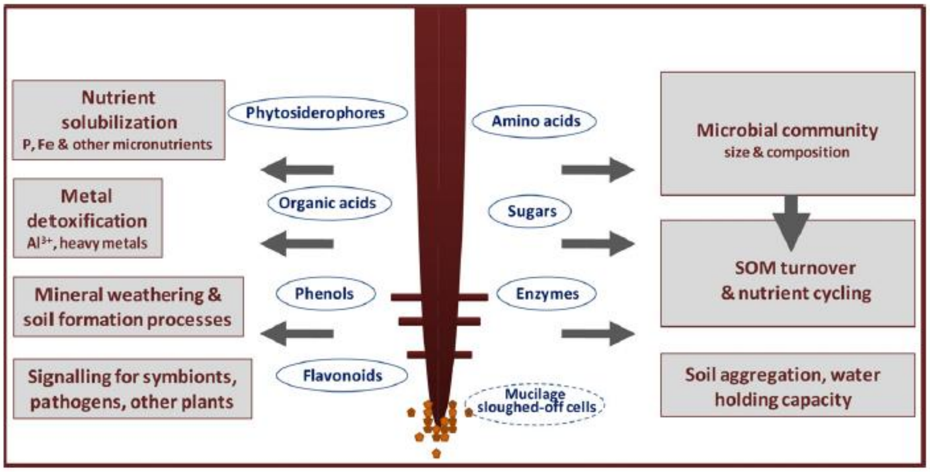

Fig. 1.7 Schematic overview of biochemical processes driven by low and high molecular weight organic compounds released by plant roots (Oburger et al., 2013)

Apart from the conventional view of resource competition (Fig. 1.8A), there exists a mutual interaction between root exudates and the rhizobacteria that affect root–root interactions (Mommer et al., 2016b). The below-ground communications like root-root and root-microbe interactions (Fig. 1.8B) can be either/or positive (symbiotic) or negative to the plant. Positive interactions help in the association of epiphytes, mycorrhizal fungi, and nitrogen-fixing bacteria with roots; while negative includes interactions with parasitic plants, pathogenic soil microbes. Hence, the rhizosphere comprises of these biological active connections occurring in a continuum process (Walker et al., 2003). However, detailed information regarding these interactions is largely missing (Mommer et al., 2016b).

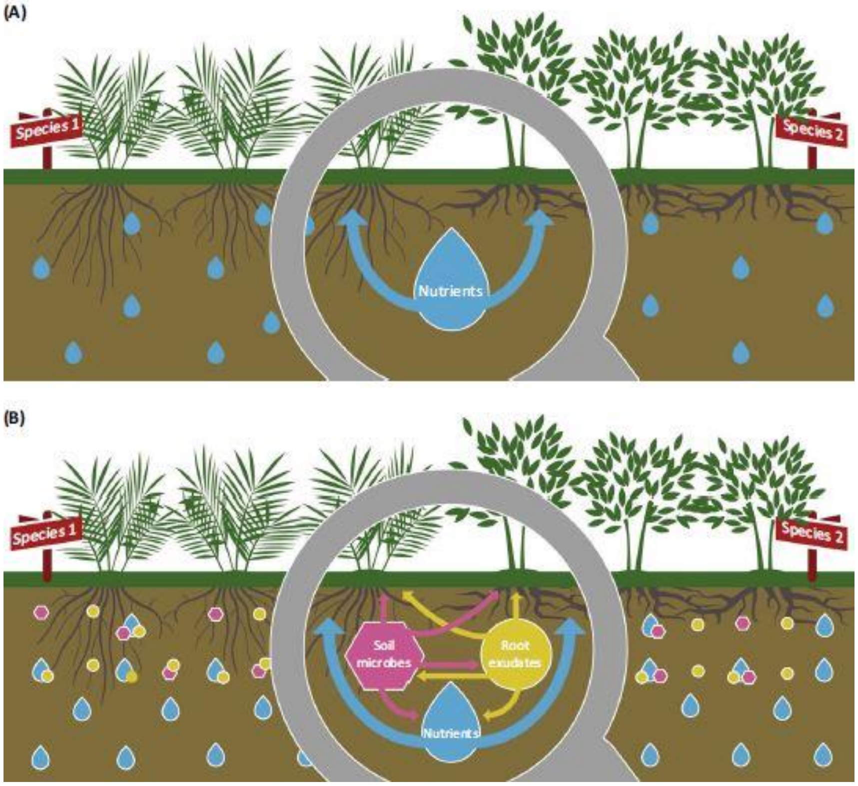

Fig. 1.8 Ecological drivers of plant-plant and plant-microbe interactions existing in an intercropping system (Mommer et al., 2016b)

1.6.1 Role of root exudates in plant-plant signaling

Identity recognition is also of great importance for the outcome of below-ground interactions (Depuydt, 2014). Root exudates have the potential to provide intricate signals to the neighboring plants (Mommer et al., 2016b). The most prominent signaling mechanism is allelopathy demonstrated through the release of secondary metabolites from the roots and harming the adjacent plants (Bais et al., 2004).

Moreover, root exudates act as facilitators for positive intraspecific and interspecific interactions that exist between the two plants (Fig. 1.9). Like in intraspecific interactions, a similar type of plant differentiates kin from non-kin at the root level by root exudates. Such type of kin recognition exists in rice plants is mediated by root exudates and occurs in a cultivar-dependent manner (Wang et al., 2021b). In the case of interspecific facilitation, the best example is of legume-cereal intercropping systems wherein legumes enhance nitrogen availability for the cereal plants while root exudates from maize improve the nodulation, symbiotic nitrogen fixation, and gene expression in an intercropped faba beans by exuding signaling chemicals like flavonoids that attract Rhizobia (Li et al., 2016). Similarly, in the wheat/faba bean intercropping system increased release of flavonoids affect positively on nodulation and nitrogen availability (Liu et al., 2017). During the peanut-cassava intercropping system, cyanide released by cassava plants triggers ethylene emission from peanut roots. In exchange, root ethylene allows recognition of cassava plants grown adjacently and thereby influences the rhizosphere microbial community (Chen et al., 2020). Also in the case of interspecific facilitation, positive interactions lead to root attractions and negative interactions lead to root avoidance between the two plants (Wang et al., 2021b). Thus, there exists an underpinned mechanism of facilitation in intercropping systems, however little is known for the role of the root exudates in interspecific facilitation. Therefore, effort must be directed in the identification and understanding of Spatio-temporal dynamics exhibited by root exudates in the rhizospheric soils of the intercropped plants.

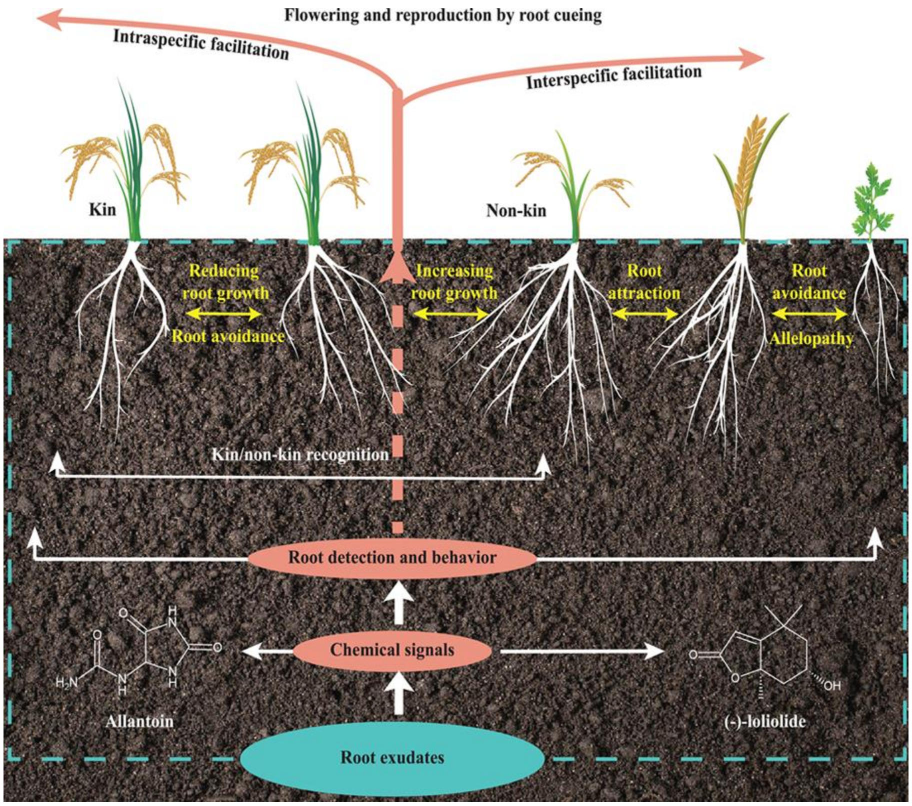

Fig. 1.9 Importance of root exudates in root-root signaling (Wang et al., 2021b)

1.6.2 Role of root exudates in plant-microbe interactions

Root exudates comprise a broad range of substrates and signaling molecules that are produced by the plant. They act as a key messenger for below the ground interactions existing with rhizosphere-inhabiting microbes. The microbes respond to these signaling molecules via chemotaxis and thereby supports the growth of plants by serving either as facilitators or regulator (Haichar et al., 2014).

The free-living bacteria then can apply advantageous effects to the plant by adapting to the favorable environment provided by plant roots (Glick, 2012; Lugtenberg & Kamilova, 2009). Among these beneficial bacteria, plant growth-promoting rhizobacteria (PGPR) exhibit many positive effects on the plant that are diverse. However, PGPR must first be established as a robust colonizer on the root to exert their favorable actions through the process named as bacterial colonization process that includes several steps (Fig. 1.10), modulated by the host plant (Beauregard, 2015).

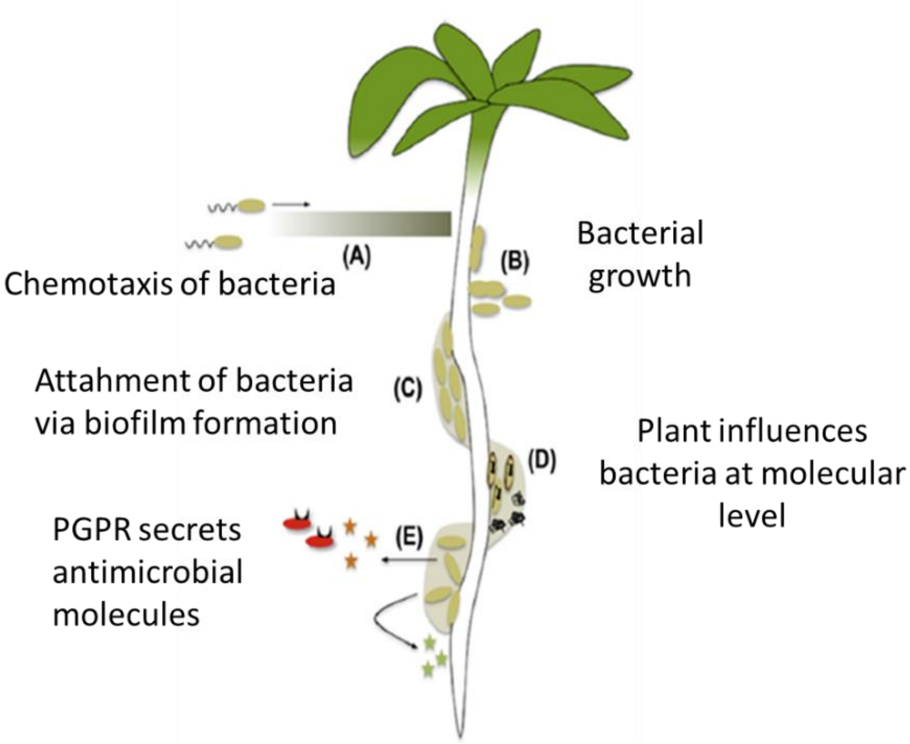

Fig. 1.10 Representation of the different steps involved in root colonization by PGPR and the response of the plant towards them (Beauregard, 2015).

Various molecules are released from the root that can behave as regulatory signals that decide the colonization efficacy of the plant-beneficial microbes (Fig. 1.11). Many PGPR needs to secrete exo-polysaccharides to colonize on roots while certain microbial species secrets out other extracellular polymeric substances. Like that observed in the case of *Pseudomonas putida* wherein secretion of large adhesion F protein extracellular matrix is required during its attachment on plant surface (Martínez-Gil, Yousef-Coronado, & Espinosa-Urgel, 2010). Bacterial secretion of these polymeric substances helps during biofilm formation, sessile bacteria are embedded in a self-secreted matrix (Flemming & Wingender, 2010).

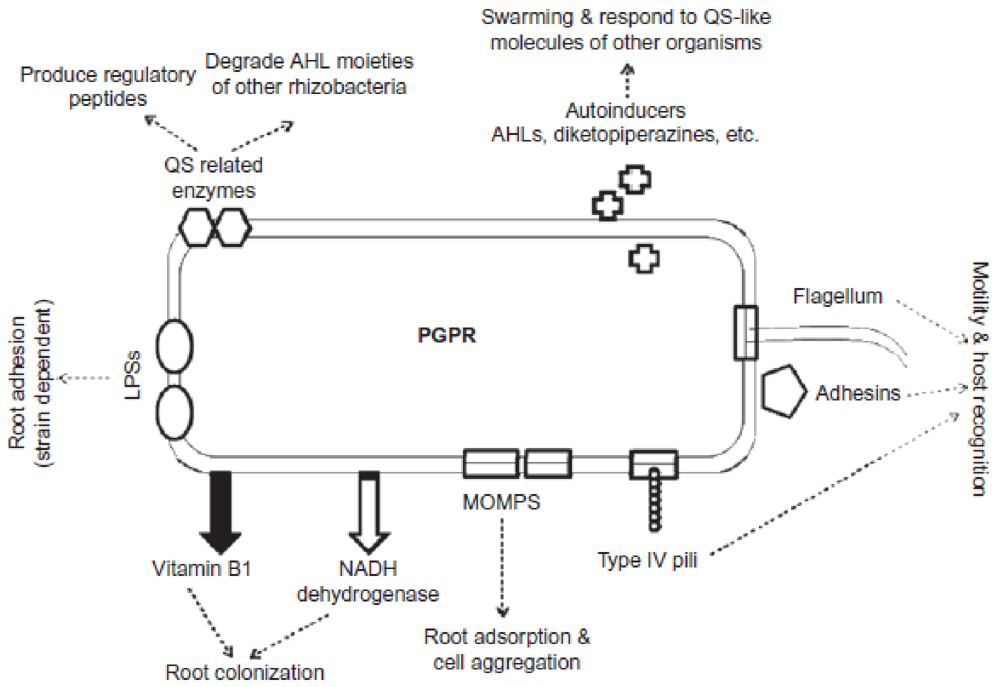

Fig. 1.11 Host root recognition and colonization by PGPR strain Bacterial system utilize flagella and pilli for motility and attachment to plants, autoinducers like N- acyl-homoserine lactone and quorum sensing mechanisms for communication and lipopolysaccharides helps in facilitation to adhere on roots and Vitamin B1 and NADH dehydrogenase help in root colonization (Dutta & Podile, 2010).

In certain gram-negative microbes like *Pseudomonas* and *Rhizobium*, biofilm formation is triggered by quorum sensing (QS), a process through which bacteria can sense population density by secreting specific molecules called autoinducers. Mainly, the predominant autoinducers which belong to the family of N-acyl-homoserine lactones are involved in biofilm formation (Danhorn & Fuqua, 2007). Thus, root colonization, occurred via QS signaling by beneficial or pathogenic gram-negative bacteria found in the rhizosphere is modulated by the plants (Beauregard, 2015).

Moreover, there exist bi-trophic plant-microbe associations in an intercropping system like in maize/faba bean (Fig. 1.12). Mainly root exudates from maize (e.g., flavonoids such as genistein) induce rhizobial Nod factors, as well as nodulation and biological nitrogen fixation in faba bean roots, resulting in enhanced N nutrition, biomass, and yield (Li et al., 2016). In return, faba bean root exudates comprising of fixed N in form of NH4+, amino acids, etc. are available to maize,-also provides N nutrition, growth, and improve yield to the maize plants. Additionally, both the plants' species release root exudates like strigolactones, flavonoids that can induce Myc factors from arbuscular mycorrhizal fungi (AMF), which initiates AMF symbiosis, and colonizes diazotrophic plant growth-promoting rhizobacteria on roots and overall improves plant N nutrition (Coskun et al., 2017). Therefore, Li et al., (2016) provided an important framework to the well-established phenomenon seen in legume-cereal intercropping systems for improved productivity in the ecosystem and high yield. To understand its full potential, the role of bacteria in cereal-legume intercropping systems needs to be studied in detail.

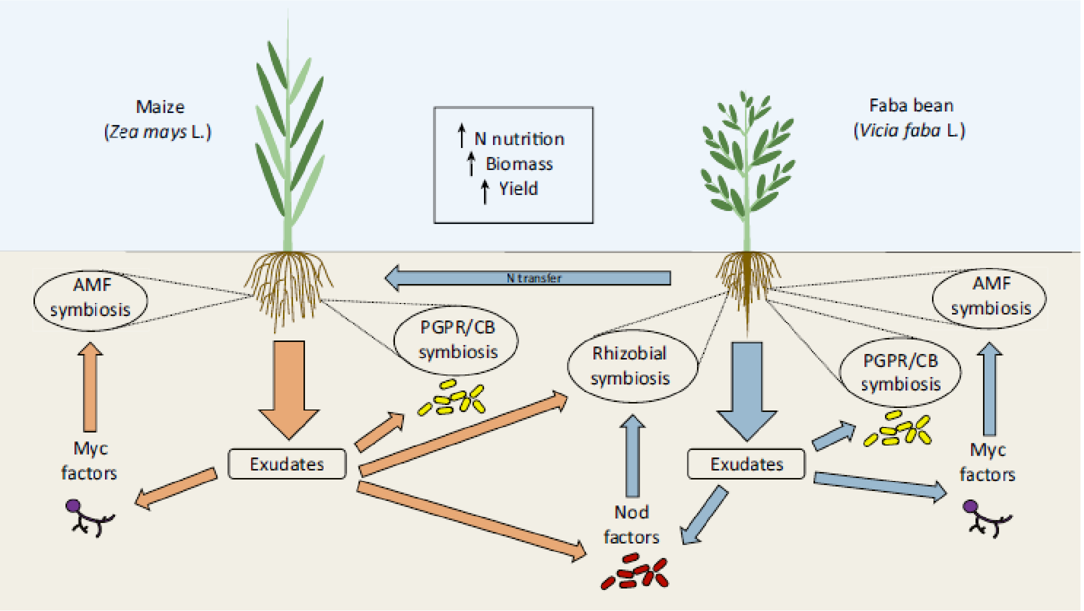

Fig. 1.12. Impact of root exudates on symbiotic relationships in maize - faba bean intercropping system (Coskun et al., 2017)

Scope of the thesis

The rhizosphere microbes help in crop production and minimize the dependence on chemical fertilizers to attain sustainable yields. Root exudates being the key players for the below-ground interactions makes them an important aspect of the study. To date, root exudates of legume-cereal intercropping systems have been studied for plant-plant interactions and plant-pathogen interaction, however, their role in plant-microbe interactions has been less explored. Primary metabolites like sugars, organic acids, and amino acids are the main components of root exudates that attract the bacteria and help them to sustain onto the roots by colonization. However, very few studies have been carried out to know their implication on microbial colonization in a legume-cereal intercropping system.

When the external inoculants of specific plant growth-promoting bacteria are applied to one of the plants of the intercropping system, the temporal dynamics of these bacteria onto the intercropped plants is also one of the important mechanisms to be explored. Moreover, studies suggest that inoculation of rhizobia, a natural symbiont of legume plants, benefits in an intercropping system, however, their molecular interactions with the cereal plants are not clear. It has been envisaged that understanding the rhizosphere interactions of the intercropped plants will take us a step further for the development of suitable biofertilizers and in turn, will minimize the input of chemical fertilizer.

In the above context, the current study sheds light on knowing tripartite interactions of plant roots of intercropped plants, microbes, and root exudates in the rhizosphere of a legume-cereal intercropping system. To this end, the scope of the study involved the identification of primary metabolites of *C. cajan –Z. mays* intercropping system by targeted and non-targeted approach and its physiological effect on plant growth-promoting strains. This study was also limited to the model *C. cajan- Z. mays* intercropped plant root exudates grown on sand and collected 28 days after sowing. Further, the study also included the protein profile of legume symbiont *Ensifer fredii* NGR234 in response to the monocropped and intercropped *Z. mays* root exudates. Three laboratory strains of PGPR belonging to different genera (*Enterobacter* sp. C1D, *Pseudomonas* sp. G22, *Rhizobium* sp. IC3109) and the well-studied nodule symbiont *Ensifer fredii* NGR234 are considered in the present study.

Objectives

1. Influence of root exudates on chemotaxis and colonization of diverse plant growth promoting rhizobacteria in *Cajanus cajan – Zea mays* intercropping system
    a. Study the effects of plant growth-promoting strains on monocropped plants *C. cajan* and *Z. mays* plants
    b. Study the cross colonization of PGPR in *C. cajan*- *Z. mays* intercropped plants
    c. Colonization of NGR234 on *C. cajan* and *Z. mays* plants by Confocal laser scanning microscopy
    d. Assessment of the chemotactic response and biofilm formation of PGPR towards root exudates
    e. Analysis of organic acids in the root exudates of monocropped and intercropped plants by a targeted approach
    f. Study the effect of organic acids on the chemotaxis and biofilm formation of PGPR
2. Identification of primary metabolites from the root exudates *Cajanus cajan – Zea mays* intercropping system and their influence on *Ensifer fredii* NGR234
    a. Study the variation in the root exudates profile of monocropped and intercropped plants
    b. Effect of root exudates on the chemotaxis of NGR234
    c. Effect of root exudates on the growth and biofilm formation of NGR234
3. Molecular effects of *C. cajan* & *Z. mays* root exudates on NGR234 and its interactions with monocropped and intercropped maize plants
    a. Differentially expressed genes in the presence of *C. cajan* and *Z. mays* monocrop plants root exudates
    b. Visualization of P*nodA*::*gfp* expression on *C. cajan* and *Z. mays* roots
    c. Differentially expressed proteins in presence of monocrop and intercrop *Z. mays* root exudates
    d. Quantitative expression of proteins in presence of monocrop and intercrop *Z. mays* root exudates