Common mycorrhizal network as facilitator of bioirrigation for rainfed agriculture tested in legume – millet intercropping system

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Acronyms

AMF – Arbuscular mycorrhizal fungi

CMN – Common mycorrhizal network

FM – Finger millet

HL – Hydraulic lift

HLW – Hydraulically lifted water

LPW – Leaf water potential

NSR – Non-split root

PGPR – Plant growth promoting rhizobacteria

PP – Pigeon pea

SR – Split root

Contents

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Summary

Food security for growing population and achieving the zero hunger target by 2050 is a major challenge for mankind. Sustainable intensification of agriculture, i.e. increased food production without causing environmental damage has been foreseen as the way forward to address this challenge. In this study we tested a sustainable legume – millet intercropping model based on "bioirrigation" and biofertilization to mitigate drought induced yield loss in rainfed areas of arid and semiarid tropics. "Bioirrigation" is based on the principle of hydraulic lift (HL) where transfer of water occurs through roots from wet deep soil layers to dry top soil layers as a consequence of a soil water potential gradient. Specifically, the process of bioirrigation describes the transfer of hydraulically lifted water from a deep-rooted plant to a neighbouring shallow-rooted plant. The main challenge for bioirrigation derives from distance between rhizospheres of the two plants, water released into the rhizosphere of bioirrigator is not available to neighbouring plant since it is tightly held up in to the rhizosphere. In this study, we tested a potential solution to facilitate bioirrigation between rhizosphere of deep-rooted pigeon pea and shallow-rooted finger millet by connecting the rhizosphere through a common mycorrhizal network (CMN) using arbuscular mycorrhizal fungi (AMF).

In this study, we conducted several pot experiments under controlled conditions inside the greenhouse at University of Basel to test the hypothesis of CMN mediated bioirrigation between pigeon pea and finger millet. The results of pot experiments clearly showed that pigeon pea does perform HL, and when roots of pigeon pea and finger millet are connected through AMF network water relations of finger millet are supported by pigeon pea through bioirrigation. In our experimental set up, after testing the role of CMN in pot experiments, we scaled up (approx. 3 times) the pot size to mimic the field like conditions and test if bioirrigation facilitated through CMN can help shallow-rooted to survive a long drought period of 10 to 11 weeks. The results from scaled up pot experiment did not show significant effect of CMN on water-relations (stomatal conductance) of finger millet in intercropping treatments, but finger millet in treatments with CMN had significantly lower foliar damage percentage and mortality than treatments without CMN. The results from pot experiments show the importance of bioirrigation for rainfed agriculture i.e. if bioirrigation based intercropping is practiced, shallow-rooted plants would be able to tolerate the drought period.

To test the efficacy of bioirrigation driven intercropping system, we conducted field trials at two experimental sites (GKVK, Bengaluru and Kolli Hills, Tamil Nadu) in southern India to optimize the spatial arrangement of pigeon pea and finger millet and test its effect on yield and water-relations of finger millet. The field trial results demonstrated that, planting two rows of pigeon pea and flanking eight rows of finger millet showed improved yield of finger millet compared to pigeon pea plants planted in between eight rows of finger millet plants in a mosaic fashion. However, the effect of spatial arrangement varied with change in experimental site. At Kolli Hills site, within row plantation of pigeon pea and finger millet performed similarly to row wise (2 pigeon pea : 8 finger millet). However, the intercropping effect was not driven by the CMN facilitated bioirrigation because finger millet in intercropping treatments had lower leaf water potential than monoculture treatments due to interspecific competition between pigeon pea and finger millet. We envision that sustainable intercropping on the basis of our bioirrigation and biofertilization model will help to design appropriate intercropping system especially in rain-fed areas that could provide sustainable food security, particularly for the marginal farmers in arid and semi-arid tropics.

Introduction

According to the United Nations, the global human population is projected to reach 9 billion by middle of the 21st century, and it will continue to grow. Achieving the food security for the growing population whilst limiting natural resources (e.g. arable land and water) offers an unprecedented challenge to mankind. To achieve food security for growing population, with growing impact of climate change, sustainable intensification of agriculture has been suggested as the only option since the goal is not only to maximize the productivity but to optimize utilization of land and natural resources required for agriculture (Pretty et al. 2010). Sustainable intensification is required for agriculture sector in general, but rainfed areas require a special attention because of its total dependency on monsoon. Due to increasing impact of climate change, farmers in rainfed areas are facing high variability in rainfall (timing and amount of rainfall) that creates intermittent drought condition and ultimately ends up in reduced yield (Sidibe et al. 2018). To stabilize the agricultural productivity in rainfed areas, sustainable intercropping models are required that would allow plants to use soil water conservatively and exploit all available soil water sources such as deep soil moisture which is mostly not accessible to shallow-rooted plants such as cereals. In this thesis, an intercropping model based on the concept of "bioirrigation" for sustainable farming under rainfed system has been proposed.

Bioirrigation and its ecological significance

"Bioirrigation", as defined and tested in this study, is transfer of hydraulically lifted water (HLW) from a deep-rooted plant, conducting hydraulic lift (HL), to a neighbouring shallow-rooted plant which is not able to access deep soil moisture. The bioirrigation process is driven by HL, which is a passive movement of water from deep (wet) soil layer to top (dry) soil layer via root system along the soil water potential gradient (Richard and Caldwell, 1987). HL could enhance plant performance, particularly in drought-prone areas, by maintaining the activity and extending the lifespan of fine roots in dry soil layers (Bauerle et al., 2008; Scholz et al., 2008). Emerman (1996) proposed two theories to explain the existence of HL, (i) first theory states that HL promotes the uptake of nutrients therefore it provides a competitive advantage to the plant. While, (ii) the second theory (known as stress-response theory), states HL as an unwanted side effect of water flow. If the root membranes are permeable to water in both directions, and water-saturated root passes through a dry soil layer, the root has to lower its osmotic potential in order to prevent

leakage. According to the stress-response theory, HL would happen if water-filled roots are not able to lower its osmotic potential, thus it is a passive process. The amount of water lifted through HL could vary between 17% and 80% of the water transpired (Domec et al. 2010).

Bioirrigation process have potential benefits for HL conducting plant and its neighbouring plant. The water supplied through HL, moisten the dry topsoil layer that helps neighbouring plant to maintain transpiration rates and nutrient gain (Meinzer et al. 2004). Water released as HL efflux benefits the rhizosphere of the lifting plant in topsoil layer because roots in dry topsoil layer are prone to hazardous effect of soil drying therefore redistribution of HL water could increase the survival of fine root system in topsoil layer (Bauerle et al. 2008). As the process of HL maintains the root hydrated in dry topsoil layer, some fraction of the water can be absorbed by root symbionts such arbuscular mycorrhizae fungi (AMF) thereby increasing their survival in dry topsoil condition (Warren et al. 2008). Furthermore, bioirrigation enables root system of both plants (HL conducting and neighbouring plant) to uptake nutrients, because plants take up nutrients from the soil via mass flow or diffusion, and both of these process are dependent of soil moisture (Amras et al. 2012). In general, the process of bioirrigation has potential to maintain fine root growth and its function during drought condition, and if the bioirrigation based intercropping model could be established it might provide a solution to mitigate drought induced yield loss in shallow-rooted crops.

Can bioirrigation mitigate drought induced yield loss in shallow-rooted crops?

A number of research studies have been conducted, mostly inside greenhouse or in agroforestry experiments, to demonstrate transfer of HLW from lifting plant to neighbouring plant (Dawson 1993; Moreira et al. 2003; Armas et al. 2010). Sekiya and Yano (2004) through a split-root experiment showed that pigeon pea (a leguminous plant) were able to lift water from bottom layer of the pot, and lifted water was absorbed by neighbouring maize plant. In their study under field conditions, supply of HLW from pigeon pea to maize was further enhanced by reducing transpiration of pigeon pea through shading. In an another study conducted by Sekiya et al. (2011), the effect of interspecific competition between intercropped plant was reduced by removing the shoot of HL conducting (donor) plant. They observed significant difference in yield of *Brasicca rapa*

in the presence or absence of root system of donor plant. The importance of bioirrigation process in supporting shallow-rooted plant can be further seen in agroforestry experiment by Dawson (1993), who showed that few species growing in the understory of sugar maple (*Acer saccharum*) were able to uptake HLW by tree which was further reflected through an improved water-relations (stomatal conductance) and growth. Another study by Ludwig et al. (2003) shows that facilitation through bioirrigation and competition between plants are concurrent. They reported that grasses growing near to the tree (*Acacia tortilis*) used HLW, however grasses had more negative predawn leaf water potential. These studies indicate challenges that needs to be addressed in order to develop a bioirrigation based intercropping model for drought prone rainfed areas.

The bioirrigation process could offer a potential solution to support a neighbouring shallow-rooted crop which are not able to access deep soil moisture during drought period. Nevertheless, facilitation of bioirrigation between two intercrop plants require further research to address two major challenges: (i) interspecific competition between two plants that might lead to negative effect on total yield (Burgess 2011), and (ii) the distance between rhizosphere of two plants, since water released through HL efflux usually tightly held up into the rhizosphere of the plant and water transfer through diffusion would not be optimal (Prieto et al. 2012). Interspecific competition between two crops could be reduced through optimal plant density and spatial arrangement of plant. In an intercropping system plants are usually exposed to limit of light and soil moisture under field conditions (Li et al. 2009), and in bioirrigation based intercropping model where we would allow roots of two plants to interact which could lead to negative impact on plant growth. Research studies have shown common mycorrhizal network (CMN) constitute a pathway for transfer of resources among plants, thus allowing a degree of freedom to keep plants at certain distance to avoid direct root interaction (Simard et al. 1997; Querejeta et al. 2003; He et al. 2004). Furthermore, CMN formed by arbuscular mycorrhizal fungi (AMF) can connect the rhizosphere of two plants to facilitate the transfer of HLW (or bioirrigated water) from deep-rooted to shallow-rooted plant. Yet, research studies using CMN to facilitate bioirrigation in intercropping system with crop plants under field conditions have not yet been reported.

Can CMN facilitate bioirrigation in an intercropping system?

Plant roots are often linked through CMN which constitute a pathway for sharing of soil resources among plant (Perry et al. 1989). Mycorrhizal fungi plays a key role in transport of water from soil bulk to host plant, and this process have been demonstrated through research studies in ectomycorrhizal (Brownlee et al. 1983) and AMF (Read & Boyd, 1986; Auge, 2001). Water transport from bulk soil to host plant is regulated by soil water potential gradient. Similarly, a CMN between a HL conducting plant and neighbouring plant could facilitate transfer of HLW along the water potential gradient. Egerton-Warburton et al. (2007), inside greenhouse set up, used a fluorescent tracer dye to trace the pathway of water transfer from HL conducing oak seedling (*Quercus agrifolia*) into the water stressed seedlings connected through AMF. They observed a significant amount of water was transferred through hyphae network, and facilitation of water transfer by CMN is a potentially important to plant survival during drought period. The source-sink relationship that drives water transfer through CMN among plants, also drives the transfer of nutrients (such as phosphorus, nitrogen) and carbon fluxes between plants (Sun et al. 1999).

Mycorrhizal hyphae usually increases the absorbing surface of the root system (Beniwal 2010), and most of the research studies on water relations comparisons between mycorrhizal and nonmycorrhizal have reported that mycorrhizal networks improve water relations in tree seedlings (Egerton-Warburton et al. 2003; Allen et al. 2009). Mycorrhizas also enhances plant nutrient uptake rate which then leads to higher root/shoot ratio (Davies et al. 1996). Since, mycorrhiza provide a number of benefits to plants, it has been widely applied in agriculture from simple monoculture to complex intercropping system (Arihara and Karasawa 2000; Karasawa et al. 2002). Furthermore, plant growth promoting rhizobacteria (PGPR) and rhizobium interact with AMF synergistically to promote plant growth (Barea 1997). These interaction may be of crucial importance to facilitate bioirrigation through CMN in an intercropping system. Few research studies have demonstrated that PGPR have a strong stimulatory effect on the growth of AMF such as increased mycelial growth (Linderman 1997). Some reports have shown that inoculation with PGPR also increased root colonization by the AMF, thus PGPR promote both AMF development and functioning (Hodge 2000). These reports suggest that co-inoculation of PGPR and AMF into an intercropping system could optimize and enhance the growth of CMN between plant and thus facilitation of bioirrigation. . Yet, research studies to test the

potential of CMN (together with PGPR as biofertilizer) as facilitator of bioirrigation in intercropping system has not been done. This thesis is aimed to address two major challenges (as mentioned in previous section) of bioirrigation based intercropping using CMN in legume – millet intercropping system.

Legume – millet intercropping

In dry semiarid areas legumes are one of the most favoured plants for intercropping practices because they possess two key characteristics: (i) deep-rooting system (> 1 m) which can access deep soil moisture to avoid drought stress, and (ii) the symbiotic N_2-fixation that does not only benefit the legume plant itself but neighbouring plants as well (Vanaja et al., 2010). Cereals such as millets with shallow-root system are often combined with legumes to produced higher crop yield (Dida et al. 2008). In this thesis, pigeon pea (*Cajanus cajan*) and finger millet (*Eleusine coracana*) was selected as a model legume and millet to develop bioirrigation based intercropping system. Pigeon pea (PP) is a member of the Fabaceae family and one of the rich source of protein and young seeds are consumed fresh as vegetable or can be allowed to mature before drying and eating as a pulse (Fu et al. 2008). Finger millet (FM) is an annual member of the family Poaceae and it is grown for the grains that are used in food or brewing. FM is high in nutrients and some varieties have high level of methionine, an essential amino acid (Subbarao & Murlikrishna, 2001).

Aims of the thesis

In this thesis, greenhouse experiments were performed to evaluate potential of CMN as facilitator of bioirrigation in PP – FM intercropping system to reduce impact of drought on growth and survival of shallow-rooted FM. And field trials of PP – FM intercropping system were performed at two different locations in southern India to optimize the spatial arrangement of component crops in PP – FM intercropping to reduce interspecific competition and promote facilitation of bioirrigation. This study, specifically, addressed following research questions:

(i) Does PP perform HL?

(ii) Does PP support water-relations of neighbouring shallow-rooted FM during drought by bioirrigation?

(iii) Does presence of PP as bioirrigator result in interspecific competition for water with FM during drought condition?

(iv) Can CMN in PP – FM intercropping system facilitate bioirrigation of shallow-rooted FM by a deep-rooted PP, and if CMN-facilitated bioirrigation can ameliorate the water-relations of shallow-rooted FM during drought?

(v) Does spatial arrangement of intercropping partners affect straw and grain yield in a FM – PP intercropping system compared to monoculture of the same crops?

(vi) Does the application of biofertilizers have an influence on the intercropping effect in spatially differently arranged intercropping systems?

(vii) Can intercropping effect driven by CMN be explained by bioirrigation?

The chapters of this thesis specifically address above mentioned questions. These questions have been grouped into three independent manuscript. Reference from the introduction section are mentioned at the end of this thesis after concluding discussion. Co-authors of each chapter are explicitly named on the title pages.

Chapter 1

Bioirrigation: a common mycorrhizal network facilitates the water transfer from deep-rooted legume to shallow-rooted finger millet under drought

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Bioirrigation: a common mycorrhizal network facilitates the water transfer from deep-rooted pigeon pea to shallow-rooted finger millet under drought

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Abstract
Background and aim Hydraulically lifted water can be redistributed to a neighbouring plant, a process referred to as "bioirrigation". Facilitation of bioirrigation by beneficial microbes such as arbuscular mycorrhizal (AM) fungi that form a common mycorrhizal network (CMN) between neighbouring plants has often been suggested but is not yet well explored. In this study, we tested if the presence of a CMN can facilitate the transfer of hydraulically lifted water from pigeon pea (PP) to finger millet (FM) and ameliorate thereby the water relations of the shallow-rooted FM during drought.
Methods In a compartmented microcosm set up, PP roots were grown up to the bottom layer of the pot to access the soil moisture. Whereas FM roots were restricted into a shallow compartment, separated through a 21 μm nylon mesh, without access to the moist bottom layer. We applied deuterium labelled water to the bottom layer of the pot to test if PP can perform hydraulic lift (HL) and if hydraulically lifted water is transferred to FM via a CMN. During the drought period we also assessed the water relations of FM to determine if bioirrigation mediated through a CMN can support the water relations of FM.
Results Application of deuterium-enriched water to the moist bottom layer of the microcosms demonstrated the capability of PP to hydraulically lift water to the drier topsoil through an insulation layer of coarse gravel. Only FM plants that were connected to PP via a CMN were able to utilize HL water. As a consequence, FM bioirrigated by PP in the presence of a CMN was able to maintain its water relations during drought conditions and showed higher rates of survival than FM plants in monoculture.
Conclusions Connecting the rhizosphere of two intercropping partners with a CMN can improve the water relations of shallow-rooted crops by bioirrigation. This finding has great potential for reducing drought induced crop yield loss in arid and semi-arid tropics.

Keywords AM fungi · Bioirrigation · Drought · Finger millet · Intercropping · PGPR · Pigeon pea · Water relations

Introduction

Water is a fundamental resource that is required by plants for their growth and thus affects agricultural production in arid and semi-arid areas (Schenk 2006). Stabilizing and improving yields in water-limited areas could come from designing sustainable agroecosystems that allow plants to use soil moisture more conservatively and exploit all available water sources such as deep soil moisture, which is often not accessible to shallow-rooted crops (Peñuelas et al. 2000; Meinzer et al. 2004). In a cereal-legume intercropping system, a possible way for shallow-rooted cereal crops to get access to deep soil

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moisture is through bioirrigation, where deep-rooted legume plants can bring moisture from deeper soil layers to shallow soil layers via hydraulic lift (Burgess 2011).

Hydraulic lift (HL) describes the passive flow of water from deep moist soil layers to drier shallow soil layers through roots of plants, driven by a water potential gradient (Caldwell et al. 1998; Carminati et al. 2010; Dawson 1993; Zarebanadkouki et al. 2013). The process of HL can play a key role in the water dynamics of ecosystems, especially in arid and semi-arid ecosystems (Horton and Hart 1998; Lee et al. 2005). Richards and Caldwell (1987) reported the first evidence for HL in the field by observations of diel fluctuations in soil water potential of shallow soil layers associated with *Artemisia tridentata*. Since then, HL has caught the attention of researchers and there are many reports today showing that HL is a wide-spread phenomenon (Bleby et al. 2010; Brooks et al. 2002, 2005; Burgess et al. 1998; Smith et al. 1999). The ability to perform HL has significant positive implications for a plant as it can improve the plant water status, enhance the ability of a plant to tolerate drought and enhance the plant's nutrient availability from the upper soil (Armas et al. 2012; Caldwell and Manwaring 1994).

Hirota et al. (2004) reported that a markhamia tree (*Markhamia lutea*) and upland rice (*Oryza sativa)* that grew in an experimental split-root system were competing for water during a first drying period. During later periods, rice plants whose roots were intermingled with markhamia tree roots appeared more green and viable than roots in a rice monoculture. Sekiya and Yano (2004) have demonstrated in an agricultural field trial that water hydraulically lifted by deep-rooted pigeon pea (*Cajanus cajan*) plants was used by neighbouring shallow-rooted crops (*Zea mays*) that had no direct access to the deep water. Recent studies by Bogie et al. (2018a, b) have shown that under extreme drought conditions growth of pearl millet (*Penisetum glaucum*) and water relations of groundnut (*Arachis hypogea*) were supported via bioirrigation by *G. senegalensis* and *Piliostigma reticulatum,* respectively, in an intercropping system.

Despite these promising studies on bioirrigation, practical application of HL in intercropping systems remains challenging (Burgess 2011). Partly this is because the redistribution of hydraulically lifted water (HLW) from a deep-rooted to a shallow-rooted plant critically depends on an efficient transfer from one plant to another in their common rhizosphere and fails when the intercropped plants are not sufficiently connected. The main challenge for bioirrigation-based crop production is therefore to establish an efficient pathway for water transfer between two plants so that the water relations and eventually the yield of shallow-rooted crops can indeed be improved by the presence of a deep-rooted bioirrigator.

A potential solution to facilitate an effective pathway of water transfer between the rhizospheres of two plants would be the connection of the rhizospheres through a common mycorrhizal network (CMN) using arbuscular mycorrhizal fungi (AMF). AMF represent a key interface between a plant and the soil. Beneficial effects of AMF on plants are well studied for many crop plants (Augé 2001; Parniske 2008; Schütz et al. 2018; Wu and Xia 2006). Among others, the amelioration of the water status in AMF colonized plants under drought condition has been ascribed to enhanced water uptake and transfer via external hyphae (Kothari et al. 1990). AMF hyphae, which are 2–5 μm in diameter, can penetrate into soil pores not accessible to root hairs (10–20 μm in diameter) and thereby provide access to water that is not available to the plant itself (Khalvati et al. 2005). The CMN between intercropped plants has also been shown to facilitate the transfer of water and nutrients between plants (Mikkelsen et al. 2008; Saharan et al. 2018; Simard et al. 2012; Walder et al. 2012). Egerton-Warburton et al. (2007) showed with a fluorescent dye that CMNs linking the roots of two plants can provide a pathway for the transfer of HLW between plants. Other studies (Warren et al. 2008; Prieto et al. 2016) showed that transfer of HLW through ectomycorrhizal fungi and AMF network enhanced the survival of seedlings during drought. Although, these reports indicate that CMN could facilitate bioirrigation, there is yet no evidence for CMNs (formed by AMF) improving the water relations of crops during drought in intercropping systems.

Using a microcosm system with pigeon pea (PP) and finger millet (FM), the main goal of our study was to test if a CMN in a legume-cereal intercropping system (PP (*Cajanus cajan*) and FM (*Eleusine coracana*)) can facilitate the bioirrigation of a shallow-rooted crop by a deep-rooted crop, and if CMN-facilitated bioirrigation can improve the water relations of the shallow-rooted crop during drought. Additionally, we also tested if the facilitative effect of the CMN depends on mycorrhizal strain and performed two experiments with a similar set up but different strains of AMF. PP is a perennial member of the Fabaceae family and one of the most

commonly grown legume crops in rain-fed areas of the tropics and subtropics (Vanaja et al. 2010). PP is a deep-rooted, hardy and drought tolerant crop and these traits allow its cultivation in a wide range of environments and cropping systems (Fu et al. 2008). FM is a shallow-rooted annual member of the family Poaceae. It is grown for the grains that are used for food or brewing and has a high mineral nutrient content, particularly calcium and iron (Subbarao and Muralikrishna 2001).

Material and methods

Experimental set up

To identify the potential of a CMN for facilitating bioirrigation and supporting the water relations of shallow-rooted FM during drought, a microcosm experiment was performed in a greenhouse under controlled climatic conditions: 14 h of day light with photosynthetic photon flux density (PPFD) 350 to 400 μmol/s at 26 ± 5 °C and 10 h of dark (night) duration at 20 ± 5 °C and 60 ± 10% relative humidity. To test for the effect of different mycorrhizal strains on facilitation of bioirrigation and consistency of results, the experiment was conducted twice with near identical conditions in 2015 and in 2017.

PP and FM plants were grown in compartmented microcosms in a similar way as previously described by Saharan et al. (2018). In brief, each pot (21 cm height and 12.8 cm diameter) was filled with layers of different materials terragreen (Maagtechnic AG, Dübendorf, Switzerland), sand and gravel (Quratz d dÀlsac LA, France) as shown in Fig. 1. The layer of gravel (6 cm) above the bottom layer (3 cm) prevented the capillary rise of water from the bottom layer to the upper soil layers. Above the gravel layer we installed two layers of medium fine sand (1–2 mm) and fine sand (0.1–0.4 mm). Above this sand layer the FM compartment was installed into a 6 cm deep layer filled with mixture (1:1) of fine sand and terragreen similar to the bottom layer. The FM compartment in our study was made out of a 7 cm wide and 6 cm deep nylon mesh (21 μm pore diameter, Anliker AG, Basel, Switzerland) and placed in the centre of the pot. The nylon mesh prevented FM roots from growing into deeper soil layers but allowed AMF to grow through the mesh. All sand and terragreen material used in this experiment was sterilized by heating to 100 °C for 12 h in drying ovens.

The pots were fertilized with 20 ml of Hoagland solution every second week till beginning of the drought period. As high P content is detrimental for AMF proliferation, the Hoagland solution (Gamborg and Wetter 1975) was modified to contain 75% less P content than the standard solution.

Plant material

The deep-rooted PP plants used in this study were variety BRG-2. The shallow-rooted FM were variety GPU-28. Seeds were surface-sterilized by shaking the seeds for 2 min in a 1% Sodium Hypochlorite (NaOCl) solution and later rinsing the seeds with tap water for two times (Sauer and Burroughs 1986). PP was pre-grown and 15 days old seedlings were transferred into the respective treatments. FM seeds were directly sown into the pots on same day when PP seedlings were transplanted. The day when PP seedlings and FM seeds were put into the pots was counted as first day of the experiment. All pots had one FM plant and two PP plants in intercropping treatments, while monoculture treatment (control) contained only one FM or two PP plants.

Treatments

The two microcosm experiments were designed to have six or eight different treatments, as illustrated in Fig. 2. The treatments included monocultures of FM and PP (PP monoculture only in the experiment 2) as controls, FM and PP intercropped either with a split-root (SR) treatment or non-split-root (NSR) treatment. In the split-root treatment lateral roots of PP were allowed to grow into the FM compartment so that the rhizosphere of PP and FM are in close vicinity. In addition, all monoculture and intercropping treatments were established with and without AMF inoculation for the establishment of a CMN. All treatments were established in five replicates.

CMN and bioinoculants

To establish a CMN, we used biofertilizers containing AMF and rhizobia strains. AMF *Rhizophagus irregularis* strain BEG-75 (500 spores per 5 g) was used in experiment 1. AMF strains *Glomus fasciculatum* (63 spores per 10 g) and *Glomus leptotichum* (67 spores per 10 g) cultured in Rhodes grass (*Chloris gayana*) roots

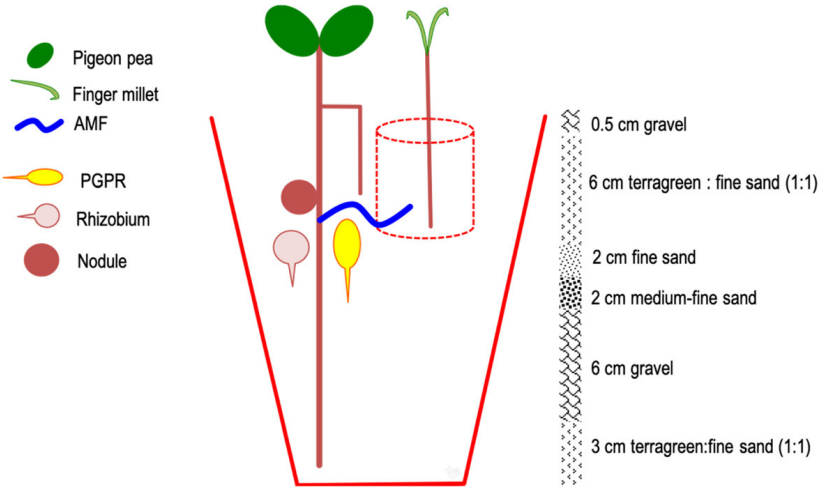

Fig. 1 Pot (21.0 × 12.8 cm) filled with different layers of sand and gravel used for the split-root experiment. The bottom layer of 3 cm consisted of a mixture of terragreen and fine sand (1:1), followed by 6 cm of gravel, 2 cm of medium fine and fine sand were used to separate the gravel layer from top layer. The top layer of 6 cm consisted of the same mixture of terragreen and fine sand as the bottom layer. A nylon mesh with a pore size of 21 μm was used to form a central compartment for FM. The pore size of nylon mesh allow mycorrhizal hyphae but it restricted roots to pass through it

were used in experiment 2. As previous work (Artursson et al. 2006; Nadeem et al. 2014) has shown that AMF inoculation of crops is most effective in combination with plant-growth promoting rhizobia (PGPRs), these were also added. For this, all treatments with CMN were inoculated with *Bradyrhizobium* sp. (DSMZ-5969, Leibniz Institute DSMZ-German Collection of Microorganism and Cell Cultures, Germany) and *Pseudomonas fluorescens* strain R62 and R81 (Mathimaran et al. 2012). All treatments with CMN (including monoculture) were given 5 g AMF culture per plant and 2 ml of each bacterial inoculum containing 1×10^6 cfu ml^{-1} were added. The AMF culture was placed next to each plant and the bacterial inoculum was added to the topsoil layer surrounding the plants. Treatments without CMN were given AMF wash and cell free broth of similar volume were added (see below).

Experiment 1 contained microorganisms that were added through AMF and bacterial culture in CMN treatments. In experiment 2 all pots (with and without CMN) were given 2 ml of soil wash (soil collected from field sites used for PP and FM intercropping at the University of Agricultural Sciences, Bengaluru, India) to provide the natural microbiome in all treatments. Soil and AMF wash was prepared separately by dissolving 10 g of each component in 200 ml tap water and solution was filtered three times using Whatmann No. 1 filter paper. All treatments were checked for presence of mycorrhizal colonization through root colonization analysis at the end of the study.

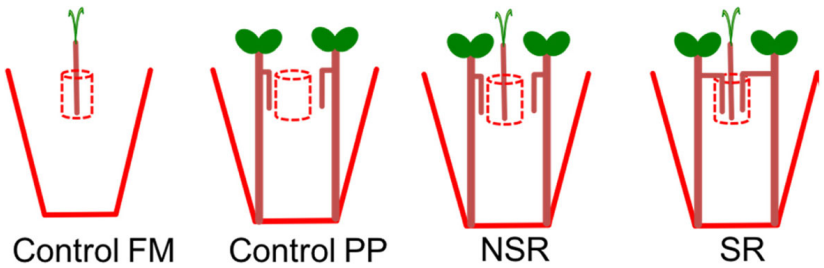

Fig. 2 The study consisted of eight different treatments: FM monoculture without and with biofertilizer, PP monoculture without and with biofertilizer, non-split-root (NSR) treatment without and with biofertilizer, and split-root (SR) treatment without and with biofertilizer. In the split-root treatment, lateral roots of PP plant were connected to the FM compartment. Monoculture treatments had two PP plants in PP control and one FM plant in FM control. Experiment 1 did not have a treatment control for PP

Watering strategy and drought period

From day of experiment (DOE) one, the pots were watered every fourth day with 300 ml of water that was gently poured from the top into the pots to saturate the entire pot. Pots were watered from the top until PP roots reached the bottom layer of the pot 35 days after transplanting. A growth period of 30 days was then added to allow the development of a well-established root network and CMN. After that, we started a drought treatment that was aimed to imitate the end of the monsoon (a seasonal rainfall pattern known to occur in southern Asia) and that PP and FM typically experience in the field. To avoid a sudden drought shock for the plants, the amount of watering was reduced gradually from 300 to 200, 100, 50, and 10 ml every four days. We defined the start of the drought period from the week when 10 ml of water were given from top layer. In experiment 1, the drought period was started on DOE 102 and continued until DOE 118. In experiment 2, the drought period began on DOE 93 and continued until DOE 109. From there on, pots were watered only from the bottom, every fourth day, to simulate moist deep soil layers. This was done by immersing the pots up to 5 cm into a bucket with tap water for 5 min.

Deuterium labelling to identify bioirrigation

To test (i) if PP performs HL and (ii) if the uptake of HLW by FM is facilitated by a CMN, deuterium enriched water with a δ^2H value of 418‰ and 1280‰ in experiment 1 and 2, respectively, was applied to the bottom layer of each pot at DOE 118 in experiment 1 and DOE 109 in experiment 2. Deuterium enrichment in FM monoculture where roots were restricted to the topsoil layer is considered as reference point of no HL and any significant differences in deuterium enrichment from this value in the intercropping treatments are attributed to bioirrigation. This is, because the bottom layers of the pots were hydrologically decoupled from the upper layers through a layer of gravel so that any occurrence of deuterium enriched water in the top soil layer of the pots and in the root crowns of FM must have originated from bioirrigation. In experiment 1, pots were immersed into the deuterium labelled water up to 5 cm once for 15 min and soil and plant samples (stem from PP and root crown from FM) were collected after 24 h. In experiment 2 pots were immersed into the deuterium labelled water up to 5 cm for 15 min two times at an interval of 48 h, and plant samples and soil samples from the PP and FM compartments were collected 24 h after the second treatment. Plant samples consisted of root crown of FM and PP as root crowns have been shown to isotopically reflect the source water of a plant (Barnard et al. 2006). Soil samples were collected from the FM and PP compartments separately. For a sample, the soil from a compartment was mixed using a spoon and then a sub-sample (ca. 5 g) was collected. Soil and plant samples were placed into 10 ml Labco® exetainers, sealed airtight and were kept frozen at −18 °C upon water extraction. For the extraction of water, the soil and plant samples were put on a cryogenic water extraction line for 3 h to extract the water as described by Newberry et al. (2017). Extracted water was used to analyse the hydrogen isotope composition (δ^2H) on a TC/EA (Thermal Conversion / elemental analyser) coupled to a Delta V Plus continuous-flow isotope ratio mass spectrometer (IRMS) via a Conflo IV interface (Thermo Fisher Scientific, Bremen, Germany).

FM water relations

To monitor the water relations of shallow-rooted FM, stomatal conductance (g_s) of FM was measured 48 h after watering during the drought period at midday between 12:30 to 14:30 h using a SC-1 leaf Porometer (Decagon Devices, USA). To select the leaf surface for stomatal conductance measurement, FM leaves were measured on both (upper and lower) surface, the lower leaf surface had very low stomatal conductance therefore only upper leaf surface was selected. Central leaves of FM were selected for measurements and two leaves per plant were measured on the upper leaf surface.

Biomass harvest

Fresh and dry biomass of shoots and roots were measured at the end of the experiment. For this, shoot and root parts were separately harvested. Firstly, the FM compartment was removed and shoot and root parts were separated. Later both PP plants were removed. The roots were washed with tap water in a bucket and dried at 80 °C in a hot air oven (model UF260, Memmert GmbH + Co. KG, Germany) for 48 h.

Root colonization

In order to analyse the percentage colonization of roots by AMF in PP and FM, fresh roots were sampled and stored in 50% ethanol (ethanol:H_2O, *v*/v). For the assessment of root colonization by AMF, root segments were cleared in KOH (10%, *w*/*v*; at 4 °C, 1 week) and stained with trypan blue (0.05% w/v, at room temperature, 6 h). Root segments were destained and randomly selected segments were observed for the presence or absence of functional structures (hyphae, vesicles and arbuscules) of AMF. Percent root colonization was calculated after examining 100 intersections on 25 randomly selected root fragments for each root sample (Brundrett 1994).

Statistical analysis

Data are expressed as mean ± one standard error of the mean (SEM). GraphPad Prism software (version 7.0 for Mac OS X, GraphPad Software, La Jolla California USA) was used to perform statistical analysis. Tukey's test was used for post-hoc multiple treatment comparison following one-way ANOVA. The criterion for significance was $p < 0.05$.

Results

AMF colonization in PP and FM

In experiment 1, FM and PP without AMF inoculation showed low colonization rates that ranged from 1.4% to 4.4%. In contrast, FM and PP with AMF inoculation showed significantly higher root colonization rates than treatments without AMF inoculation. This was, however, only in the intercropping treatments but not in the monoculture treatments (Fig. 3a). FM and PP in intercropping treatments with and without split roots had root colonization rates of 20% and 17% in FM and 25% and 34% in PP, respectively.

In experiment 2 we observed similar patterns, where FM and PP without AMF inoculation had very low colonization rates that ranged from 0.2% to 0.4% for FM, while 1% to 3% for PP. FM in treatments with AMF inoculation showed significantly higher root colonization rates in the intercropping treatments than in the monoculture (Fig. 3b), while PP had similar root colonization rates in intercropping and monoculture treatments with AMF inoculation. Intercropping treatments SR and NSR had root colonization rates of 11% and 17% in FM and 29% and 36% in PP, respectively. PP roots in control treatment with AMF inoculation had 51% colonization. Root colonization rates by AMF hyphae were similar for FM in experiment 1 and experiment 2 but generally higher for PP in experiment 2 compared to experiment 1.

Deuterium enrichment to trace bioirrigation

In experiment 1, water in the top soil layer of the FM compartments showed significantly higher δ^2H values in the intercropping treatments with AMF inoculation as compared to the FM monocultures either with or without AMF inoculation or compared to the intercropping treatments without AMF inoculation (Fig. 4a). Likewise, water in the top soil layer surrounding PP showed significantly higher δ^2H values with AMF inoculation than without AMF inoculation in both, the SR and NSR treatment. The δ^2H values of root crown water of FM showed no significant differences among treatments. We did observe, however, a non-significant trend to higher δ^2H values in the root crown of FM in the split-root and AMF treatment (Fig. 4b).

Similar to experiment 1, water in the top soil layer in the FM compartments of experiment 2 also showed significantly higher δ^2H values in the intercropping treatments with AMF present (independently of split or non-split-roots) compared to all other treatments (Fig. 4c). The lowest δ^2H values, −10.40‰ and −12.63‰, were found in the top soil layer of FM controls, independently of AMF treatment. Water in the top soil layer of the PP compartments showed highest δ^2H values in the control treatments with (164.98‰) and without (99.97‰) AMF inoculation (Fig. 4c). Water in the top soil layer of the PP compartments in the intercropping treatments showed the lowest δ^2H values 25‰ in SR treatment without AMF and highest δ^2H values 85.31‰ with AMF inoculation. The δ^2H values of FM root crowns showed generally no sign of δ^2H enrichment. Yet, we found a significant effect in the SR treatment with AMF inoculation (Fig. 4d). This confirms the trend already observed in experiment 1 that close association of PP and FM roots along with presence of a CMN plays a key role in facilitating bioirrigation.

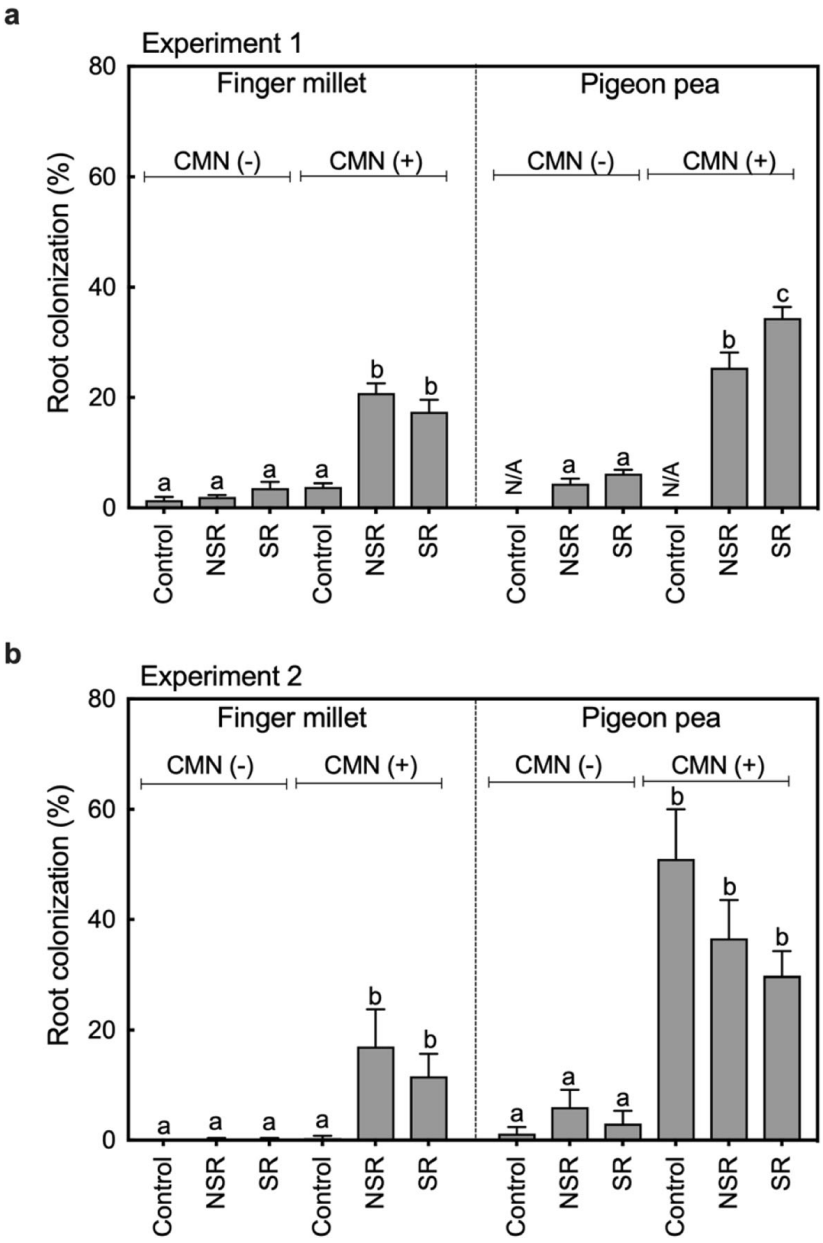

Fig. 3 Percent root colonization of AM fungi in FM and PP roots in experiment 1 (Fig. 3a) and experiment 2 (Fig. 3b). Bar represents the average of five replicates with one standard error of the mean. Tukey's test (one-way ANOVA) was used for multiple comparison (PP and FM separately). Values with the same letters are not significantly different at $p > 0.05$

Water relations and mortality of FM during drought

We observed in experiment 1, that stomatal conductance (g_s) of FM was similar in all treatments at the onset of the drought and ranged among treatments between 20.8 and 40.3 mmol m^{-2} s^{-1} (Fig. 5a). With progressive drought, g_s declined and reached values of zero in most treatments at DOE 110 of the experiment, partly because plants had died in response to water limitation. This was in all treatments except for FM that were intercropped, inoculated with AMF and had a SR treatment. Here g_s was maintained at 26.5 mmol m^{-2} s^{-1} until the end of the experiment at DOE 118.

In experiment 2, we observed similar overall trends in stomatal conductance as in experiment 1 (Fig. 5b). At the onset of drought FM had similar stomatal conductance in all treatments, irrespective of AMF inoculation. As drought progressed g_s of FM in all treatments declined except for FM that was intercropped, inoculated with AMF and had a SR treatment, which maintained stomatal conductance starting from 53.74 mmol m^{-2} s^{-1} at DOE 93 to 26.86 mmol m^{-2} s^{-1} at DOE 109. As in experiment 2, we observed a high mortality of FM in response to the drought treatment. This was particularly pronounced in all control treatments, where we observed 100% mortality (desiccated leaves with no g_s)

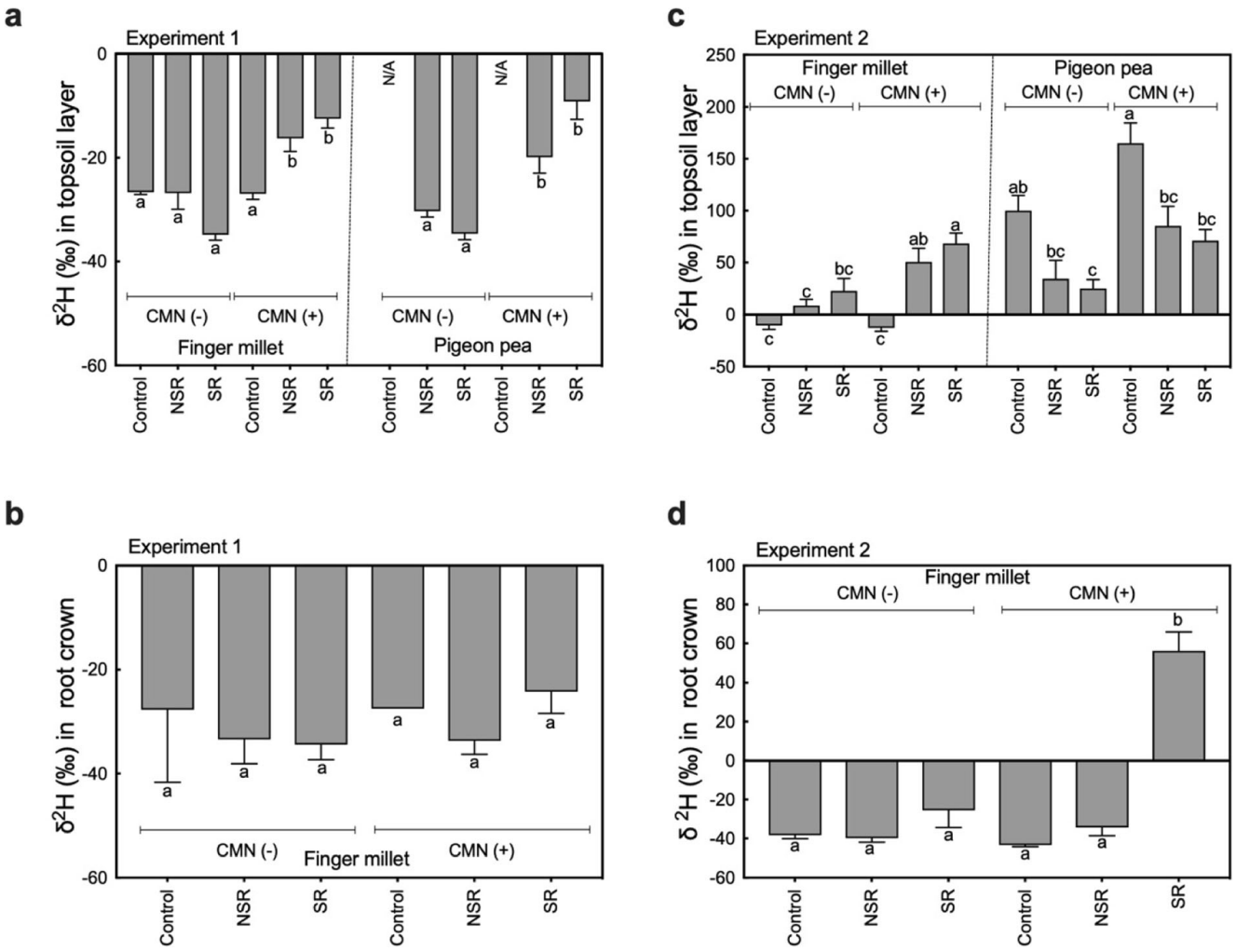

Fig. 4 Deuterium enrichment in the topsoil layer and root crowns of FM from experiment 1 (Fig. 4a and b) and experiment 2 (Fig. 4c and d). Experiment 1 did not have a PP control (monoculture), so that no data are available for this treatment (N/A). Bars show the mean of five replicates with one standard error of mean. Tukey's test (one-way ANOVA) was used for multiple comparison (PP and FM separately) and values with the same letters are not significantly different at $p > 0.05$

at DOE 109. In the SR treatment without CMN, four out of five replicates were alive at DOE 105 and three at DOE 109 (Fig. 5b). In the SR treatment with CMN all five replicates were alive.

Growth of FM and PP in intercropping system

In experiment 1, FM produced generally more shoot and root biomass per plant than PP (Fig. 6a and b). FM did not show any significant effect of AMF inoculation on shoot or root biomass production, while PP produced significantly higher shoot and root biomass per plant when inoculated with AMF (Fig. 6a and b). Intercropping treatments had no significant effect on shoot biomass of FM, however, NSR treatment with CMN had significantly lower shoot biomass than FM in control treatment with CMN. The root biomass in FM was significantly reduced in all intercropping treatments.

In experiment 2, FM also produced higher shoot and root biomass per plant than PP (Fig. 6c and d). FM did not show any significant effect of AMF inoculation while PP did show a significant effect of AMF inoculation on shoot and root biomass (Fig. 6c). The per plant biomass in FM was significantly reduced in intercropping treatments as compared to monoculture.

Discussion

In this study, we tested if the presence of a CMN can facilitate the transfer of HLW from PP to FM and

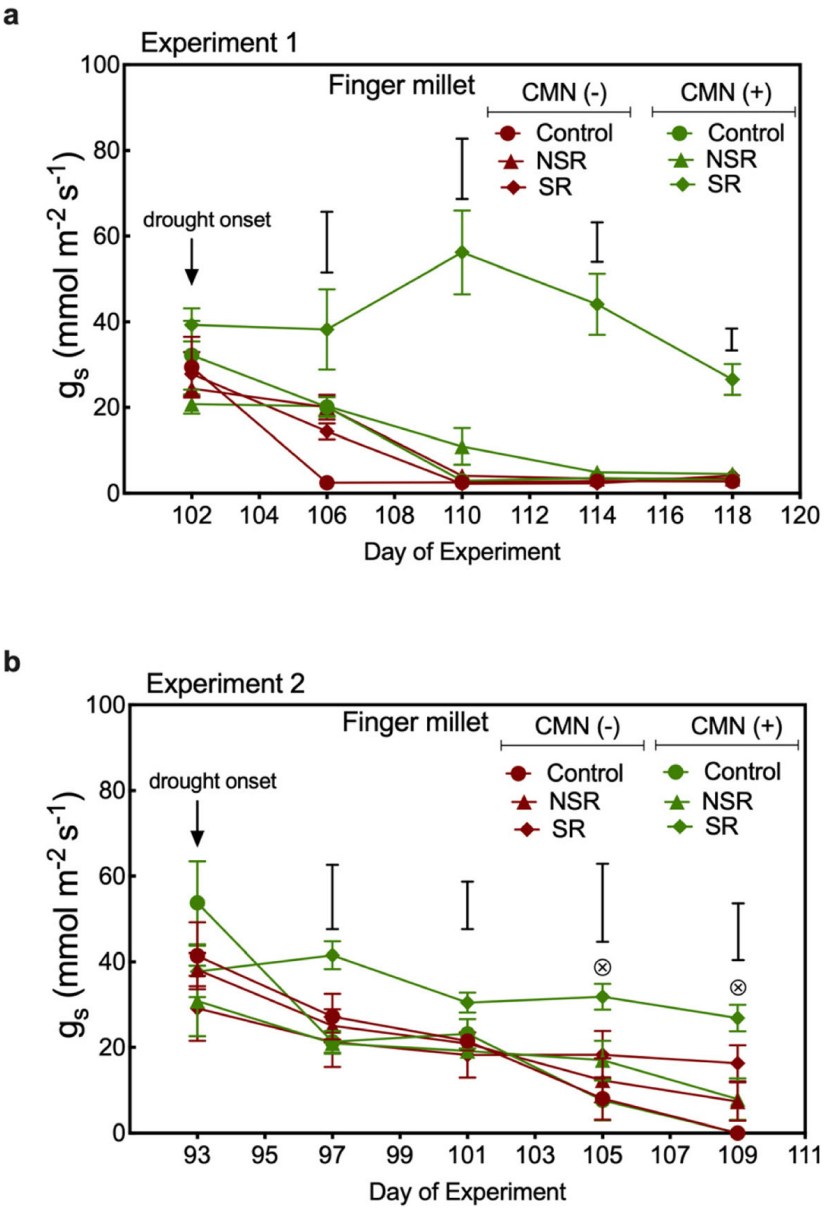

Fig. 5 Stomatal conductance (g_s) of FM during the drought period in experiment 1 (Fig. 5a) and 2 (Fig. 5b). Values shown here are average of five replicates with one standard error of mean. The bar over day of experiment (DOE) shows $HSD_{0.05}$ values when significant difference occurred among treatments by Tukey's test at $p > 0.05$. Symbol (ⓧ) over DOE 105 and 109 in experiment 2 indicates that at DOE 105 only 2, 2, 3, 4 and 4 replicates of control (−), control (+), NSR (−), NSR (+) and SR (−), respectively were alive respectively, while at DOE 109 all replicates in control A and B were dead, and only 2, 2, and 3 replicates were alive in treatments NSR (−), NSR (+) and SR (−), respectively. In SR(+) treatment all 5 replicates were alive till end of experiment

ameliorate thereby the water relations of the shallow-rooted FM during drought. The results of our study indicate that a CMN plays a key role in transferring HLW between two plants but that a close association of roots of FM and PP is necessary for the transfer of water. If these circumstances are provided the water relations of FM can be improved by bioirrigation under drought. Based on these findings, we argue for the importance of connecting the rhizosphere of two intercropping partners with a CMN in order to improve the water relations of shallow-rooted crops with bioirrigation.

AMF root colonization in FM and PP

FM plants in intercropping treatments with AMF inoculation were colonized only in the presence of PP. We used a combination of AMF strains, PGPRs and rhizobia in the treatments to develop a CMN in our experiments. It has been reported in previous studies that AMF colonization is more effective in legumes when they are nodulated by N_2-fixing rhizobia (Barea et al. 1991; Schenck and Smith 1982).

PP and FM roots have been reported to show colonization up to 65% and 75% in other studies (Saharan et al. 2018). In our study, we observed AMF

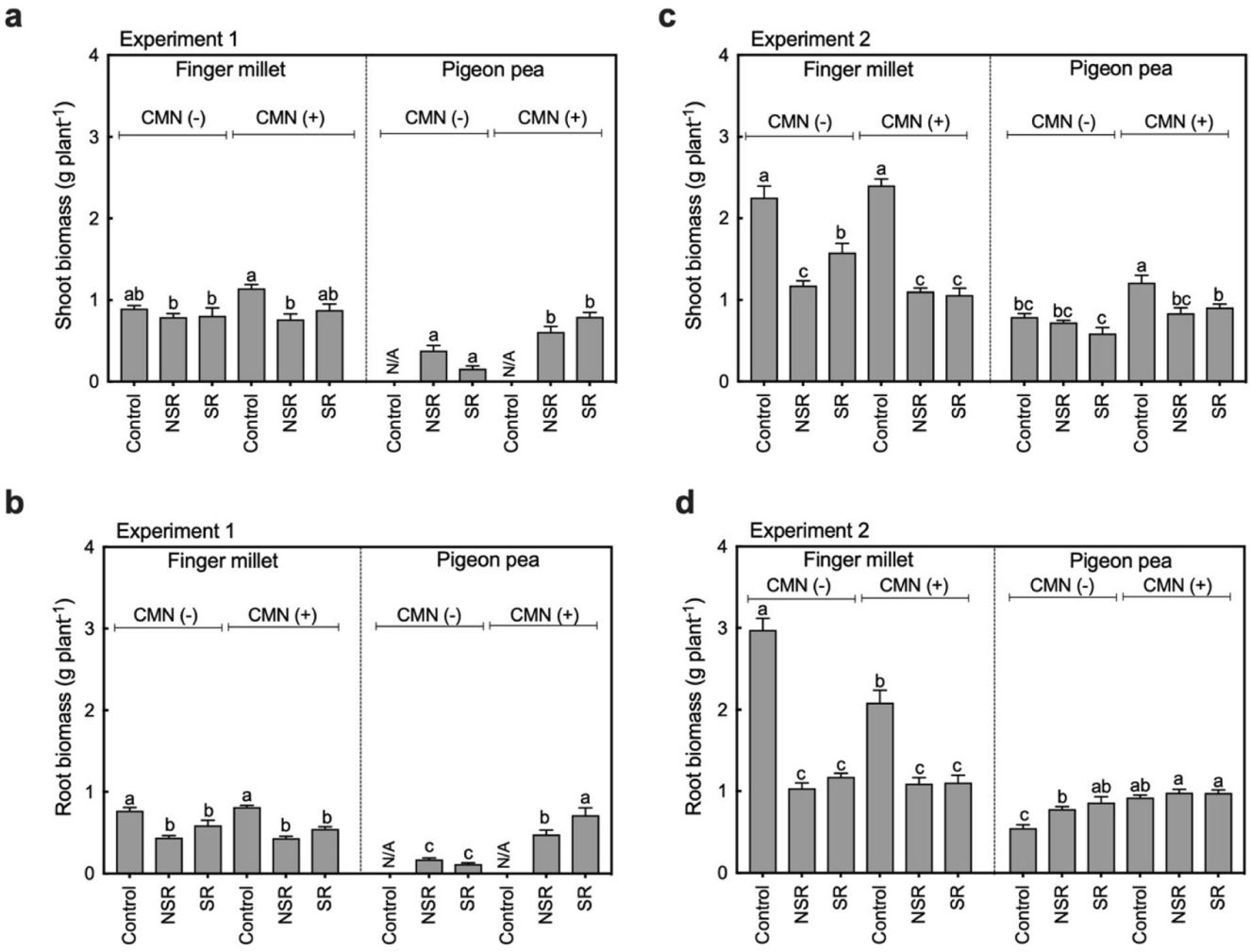

Fig. 6 Shoot and root dry biomass of FM and PP in the different intercropping and CMN treatments in experiment 1 (Fig. 6a and b) and experiment 2 (Fig. 6c and d). Bars represent the mean of five replicates with one standard error of mean. Since experiment 1 did not have a PP control, data for PP control are not available (N/A) in Fig. 6a and b. Tukey's test (one-way ANOVA) was used for multiple comparison (PP and FM separately) and values with same letters are not significantly different at $p > 0.05$

colonization up to 51% in PP roots. For FM roots, AMF colonization rates varied from 11% to 20% in intercropping treatments. Similar to this, Beggi et al. (2016) has also reported low colonization rates in FM that varied from 12% to 30%. Enhanced P uptake is generally considered the main benefit that AMF provides to its host plant (Zhu et al. 2003). If soil P levels are high, AMF colonization is typically low (Hetrick et al. 1996; Sorensen et al. 2005). Low AMF colonization in FM and PP roots in our study could possibly be due to high soil P. The source of high soil P could be the terragreen substrate that contains up to 0.1% of P_2O_5 (technical data from manufacturer). Despite a low AMF colonization in FM roots, the general findings of our study yet indicate that a functional CMN was established between FM and PP roots in intercropping treatments of our experiment.

Hydraulic lift and bioirrigation in PP – FM intercropping

The deuterium enrichment in the topsoil layer of the NSR and SR treatment (with CMN) showed similar patterns in both experiments, suggesting that PP can perform HL. Also, the topsoil layer in the FM compartments had significantly higher deuterium enrichment in the intercropping treatments with a CMN present as compared to the intercropping treatments without a CMN in both experiments. This indicates that CMN helped in facilitating transfer of HLW from PP to FM compartment. Yet, the deuterium label shows that FM was able to absorb HLW in the SR treatment only with a CMN present. With this, our data suggest that PP performs HL and that a CMN plays a key role in facilitating bioirrigation between PP and FM (Fig. 4), but FM and

PP roots need to be in close proximity to allow the transfer of HLW from PP to FM via a CMN.

The application of AMF and PGPR showed a positive impact on HL by PP, as we observed higher deuterium enrichment in the topsoil layer of the PP compartment when PP was inoculated with AMF and PGPR (Fig. 4a and c). Rhizobia colonize legumes and promote growth through nitrogen fixation and PGPR have been reported to promote formation of fine roots and root hairs of leguminous plants (Espeleta et al. 2004; Höflich et al. 1994; Liste 1993). Enhanced root growth due to rhizobia and PGPR may have increased water efflux rates from PP roots into the top soil explaining a significant deuterium enrichment in the topsoil layer in the SR and NSR treatments only with CMN present (Fig. 4a and c). Similarly, significantly higher δ^2H values in the topsoil of the FM compartment with CMN than without a CMN suggests that the application of AMF played a key role in facilitating the transfer of HLW into the FM compartment. There are three explanations for this: (i) with AMF inoculation of PP more HL water was released into the PP compartment through PP roots and AMF hyphal tips (Egerton-Warburton et al. 2008). This water can then diffused into the FM compartment. In addition (ii) the CMN actively facilitates the transfer of water into the FM compartment. Finally (iii) the presence of a CMN and PGPR may have improved the water-retention capacity of the soil (Querejeta et al. 2012; Querejeta 2017).

Interestingly, HL water was only taken up by FM in the presence of a CMN and when roots were intermingling in the SR treatments (Fig. 4b and d). This was, although HLW was isotopically detected in the topsoil layer of both the SR and the NSR treatments. The pathway that could allow FM to access HLW from the topsoil could be either direct absorption from the soil after efflux from PP roots or uptake via a CMN formed between PP and FM. Since we did not detect a significant label in FM root crown in the NSR treatment with CMN, our data suggest that placing the roots of PP and FM into close proximity was necessary for FM to absorb HLW released from PP roots and AMF hyphal tips. In the NSR treatment where PP and FM roots were separated FM could not absorb HLW effectively and deuterium label in topsoil layer was mainly caused by moisture retained by the fungal hyphae in the topsoil layer under drought.

Previous studies (Bingham and Simard 2012; Plamboeck et al. 2007; Schoonmaker et al. 2007) have demonstrated that CMNs formed by ectomycorrhizal fungi can facilitate transfer of HLW from a tree and help to establish understory seedlings under drought conditions. The role of a CMN in transferring water between plants has also been shown by Egerton-Warburton et al. (2007), who used a fluorescent tracer dye to show that water released by coastal live oak seedlings is transported via a common mycorrhizal network (ectomycorrhizal fungi and AMF) to a neighbouring plant. Our findings indicate that, under drought conditions, CMN formed by AMF between two plants can effectively facilitate the transfer of HLW but that this is effective only when roots of both plants are intermingled. The close association of roots probably helps with the establishment of a functional CMN between plants.

Effect of bioirrigation on water relations of FM during drought

In our study, we observed a higher stomatal conductance of FM under drought in the SR treatment with AMF inoculation compared to all other treatments (in both experiment 1 and 2). This suggests that the transfer of HLW that we observed for the same treatments resulted in improved water relations of the shallow-rooted FM under drought. This is corroborated by the fact that in experiment 2 FM in SR with CMN survived drought in all replicates until DOE 109, while FM in monoculture and intercropping treatments without CMN showed substantial drought-induced mortality.

Few studies have shown that shallow-rooted plants growing in proximity of deep-rooted plants conducting HL benefit from this process and can improve their water relations (Caldwell 1990; Ludwig et al. 2003; Prieto et al. 2011; Querejeta et al. 2012). Dawson (1993) reported that shallow-rooted plants growing in close vicinity of maple trees conducting HL were able meet their water demands by up to 60% through the uptake of HLW resulting in higher stomatal conductance. Similarly work of Hirota et al. (2004) reports that intermingling the roots of a markhamia tree (*Markhamia lutea* (Benth.) Schumnann) and upland rice (*Oryza sativa* (L.)) in a split-root apparatus resulted in rice staying green and viable for longer duration than when grown alone. However, in our experimental setup intertwining of PP and FM roots was effective with application of AMF with PGPR and rhizobia, only. This suggests that when PP and FM root were put in

close proximity in the SR treatment, the CMN between PP and FM roots facilitated transfer of HLW more efficiently than in NSR treatment. AMF has been reported to support stomatal conductance of plants under drought condition (Augé et al. 2015). Our study now indicate an additional functional role of CMN in facilitating bioirrigation.

Effect of bioirrigation on plant growth in intercropping system

This study shows that bioirrigation of FM by PP facilitated through a CMN could help FM to maintain its water-relation and survive a short drought period. However, our study does not indicate that bioirrigation directly translates into biomass improvements in the SR treatment with CMN, where bioirrigation was most effective (Fig. 6). In our study, most of the biomass in PP and FM accumulated in well-watered conditions since the applied drought period was only relatively short. We did find, however, a reduction of FM biomass due to intercropping treatments suggesting that competition rather than facilitation of soil resources is driving productivity at the community level in the studied system. Competition for resources (such as water and nutrients) is a key factor that can counter affect the facilitative effects in intercropping systems. This is clearly illustrated in our results where the biomass of FM plants was significantly more reduced in intercropping treatments than its monoculture, while PP did not show any reduction in per plant biomass in intercropping (Fig. 6c and d). As reported in earlier studies, a plant may suppress the growth of a neighbouring plant through direct competition for resources (light, water and nutrients) due to overlapping of roots (Dohn et al. 2013; Ludwig et al. 2004a; Scholes and Archer 1997). Ludwig et al. (2004b) showed, for example, that grasses – although using hydraulically lifted water of Acacia trees – had reduced biomass under the trees due to competition for soil water and nutrients in the topsoil layer. This illustrates that although facilitative interactions such as bioirrigation might occur between co-existing plant species competitive interactions yet determine the overall productivity of the system. However, a recent study by Bogie et al. (2018b) reported that pearl millet grown as intercrop with the shrub *Guiera senegalensis* had 900% greater biomass than pearl millet crops as monocrop under extreme drought condition. They assign this effect to bioirrigation of pearl millet by *Guiera senegalensis* and illustrate as such that the facilitative effect of bioirrigation can – other than in our experiment dominate community productivity. Although our study was not designed to test if bioirrigation promotes plant biomass under extreme drought conditions, the results we present here are yet important from an agronomic view point, where loss of shallow-rooted crops could be avoided if CMN based bioirrigation model is implemented into field. Future studies with the aim to implement the bioirrigation of FM by PP in more field-like settings need to specifically address how intercropping systems can be designed so that the facilitative effects of bioirrigation dominate over the competitive effects between the intercropped species.

Effect of single and mixed AMF strain on water relations of FM during drought

In our study, the application of different strains of AMF in experiments 1 and 2 showed similar overall trends for facilitating bioirrigation: FM in the SR treatment maintained higher water relations than in all other treatments. However, in experiment 1 FM showed a rapid decrease in stomatal conductance in all intercropping treatments except in treatment SR with CMN after onset of drought. In experiment 2 in contrast, FM showed a gradual decrease in stomatal conductance in all treatments except in SR with CMN. In experiment 2, we had introduced two AMF strains to form a CMN between the rhizospheres of PP and FM. Possibly, this helped to hold the moisture in topsoil layer surrounding the FM (Augé et al. 2001, 2004), and explain the more gradual decrease in stomatal conductance during the drought period. The diversity of AMF species in the rhizosphere has been shown to correlate with increased functionality and nutrient uptake by plants (Johnson et al. 2004). The results from the study presented here suggest that increasing the diversity of AMF in the soil may alleviate the sudden impact of drought to some extent. Moreover, AMF strains *Glomus leptotichum* and *Glomus fasciculatum,* used in experiment 2, have been found to have more symbiotic efficiency for PP and FM, respectively, than other AMF strains (Byra and Bagyaraj 1991; Govinda et al. 1983). Further research focusing on total hyphal density with inoculation of single or mixed strains of AMF and its effect on water relations will provide a better understanding if a mixed

inoculation of AMF strain is beneficial for the facilitation of bioirrigation during a drought period.

Furthermore, in this study, we observed a higher enrichment of the deuterium label in the topsoil layer and in the FM root crowns in experiment 2 as compared to experiment 1. This could be the result of differences in the duration of deuterium application during labelling. In experiment 1, only a one-time application of the deuterium solution ($\delta\ ^{2}H = 418$ ‰) during the last week of drought might not be enough for the deuterium label to appear in the root crown of FM (Fig. 4b). Additionally, since pots were being watered with tap water from the bottom during the drought period, FM could have already taken up significant amounts of bioirrigated tap water. This could have resulted in a dilution of the deuterium signal due to which FM from SR treatment (with CMN) did not show any significant ^{2}H enrichment in root crowns (Fig. 4b).

Conclusions

Our study provides proof-of-concept for a bioirrigation based intercropping system where deep-rooted PP supports the water relations of shallow-rooted FM during drought period. The split-root set up with CMN application showed that the presence of a CMN as well as a close spatial association of roots are key factors to facilitate bioirrigation in an intercropping system. These circumstances provided, bioirrigation can effectively support the water relations of shallow-rooted crops during drought. While bioirrigation promotes also the survival of shallow-rooted crops, it does not necessarily translate into the biomass increase of the drought stressed crop. Further studies, ideally in the field, should test if PP can indeed improve the water relations of shallow-rooted FM during drought period and mitigate as such the yield loss due to drought.

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Chapter 2

Facilitation or competition? Bioirrigation and availability of water in the top soil layer of a model intercropping system

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Abstract

Drought stress is one of the main abiotic factors which impacts the productivity of crop plants, especially shallow-rooted plants which cannot access deep soil moisture. In this study, we tested the concept of bioirrigation in an intercropping system of deep-rooted pigeon pea (PP) with shallow-rooted finger millet (FM). Bioirrigation is defined as the transfer of hydraulically lifted water by a deep-rooted plant to a neighbouring shallow-rooted plant which cannot access deep soil moisture. We tested if bioirrigation can support water-relations and longevity of shallow-rooted FM during a drought period. Additionally, we tested how the presence of a common mycorrhizal network (CMN) affects the balance between facilitative (i.e. bioirrigation) and competitive interactions between two intercropping species. Our results indicate that in a PP-FM intercropping system, bioirrigation supports the water-relations of FM during drought and helps FM to tolerate (or survive) the drought period. However, our data also show that FM in intercropping treatments tends to face drought conditions earlier than in monoculture due apparent competition between PP and FM for water in the topsoil layer. The competition for water explains the lower total biomass of FM in the intercropping treatments as compared to monocrops despite bioirrigation during drought. In contrast to our expectations, the establishment of a CMN did not influence the competitive or facilitative interactions between PP and FM with respect to water in the experiment. Yet, the drought-induced foliar damage was significantly lower in treatments with CMN.

Keyword: hydraulic lift; bioirrigation; intercropping; drought; biofertilizer; common mycorrhizal network

Introduction

Deep-rooted plants can re-charge the topsoil layer through hydraulic lift (HL). HL is a process where transfer of water takes place through roots of a plant from deep moist soil layers to top dry soil layers as a consequence of a soil water potential gradient [1–4]. HL performing deep-rooted plants could be used as a tool to recharge the topsoil layer in agricultural fields and possibly also to facilitate HL water to neighbouring shallow-rooted crops through "bioirrigation" [5]. Thus, bioirrigation could provide a simple and effective way to improve the water-relations of shallow-rooted crops during drought in water-limited areas.

An early and strong-evidence of bioirrigation comes from work of Corak et al. [6], where tritium-labelled water lifted by alfalfa plants was transferred to neighbouring maize plants resulting in prolonged survival of maize plants during drought. Sekiya and Yano [7] conducted a field study to demonstrate that maize plants grown near pigeon pea was able to utilize water that was hydraulically lifted by pigeon pea. In a recent study Bogie et al. [8] showed that during experimentally imposed drought shallow-rooted pearl millet (*Pennisetum glacum*) was able to uptake hydraulically lifted water by a deep-rooted shrub (*Guiera senegalensis*), and millet biomass production when intercropped with shrubs was over 900% greater than millets in monoculture. Similarly, in an agroforestry set up, Hirota et al. [9] showed that upland rice (*Oryza Sativa*) plants grown in split-root system with a markhamia tree (*Markhamia lutea*) were viable and green during drought period, while rice plants alone could not survive. These studies indicate the potential of bioirrigation to provide water to shallow-rooted crops when these are intercropped with deep-rooted plants.

While facilitative effects of bioirrigation might support the water-relations and survival of shallow-rooted crops, two plant species placed in close vicinity in intercropping systems can also compete with each other for resources such as light, nutrients and particularly soil moisture with impacts on growth and yield of the individual plants [10–12]. Ludwig et al. [13] reported that grasses interspersed with *Acacia tortilis* were able to take up water hydraulically lifted by *Acacia*. However, the biomass production of grasses was higher in trenched plots (grass-tree root systems separated) than in grasses that had their roots interspersed with *Acacia.* Reduced growth was thus the result of below-ground competition

for water that overwhelmed the facilitative effects of bioirrigation during drought periods. Similarly, Zegada-Lizarau et al. [14] showed that pearl millet (*Pennisetum glaucum*) in intercropping with cow pea (*Vigna unguiculate*) had lower leaf water potential (under drought) and biomass than in monoculture due to competition. To make bioirrigation effective for the promotion of yield in intercropping system, it is thus important to assess how facilitative and competitive effects between the two co-occurring plants interact in the overall determination of yield [15].

A further important limitation for the facilitative aspects of bioirrigation is the distance between the rhizosphere of two plants. Efflux of HL water from one plant is usually tightly held up into the rhizosphere of the same plant [16] and an effective transfer of water between two plants is hindered by the distance of their rhizospheres. Arbuscular mycorrhizal fungi (AMF) could provide a pathway for the transfer of water between two plants via a common mycorrhizal network (CMN) and facilitate as such bioirrigation [17]. In a recent study, Saharan et al. [18] showed that the presence of a CMN between pigeon pea (PP) and finger millet (FM) alleviates the negative effect of drought on finger millet, indicating that a CMN can connect the rhizospheres of two plants and facilitate as such bioirrigation. Furthermore, Egerton-Warburton et al. [19] showed that AMF facilitated transfer of water (released as HL efflux) from coastal live oak seedlings to water-stressed oak seedlings. Similarly, in a recent study, we [20] reported that presence of a CMN facilitated transfer of HL water from deep-rooted PP to shallow-rooted FM, and FM was able to maintain its stomatal conductance under drought. These studies present concrete evidence that AMF could indeed be used as a facilitator of bioirrigation and promote the facilitative effects of bioirrigation.

Importantly, however, the presence of a CMN can significantly affect the competitive interaction between different plant species [21,22]. Weremijewicz and Jonas [23] reported that, in the absence of root system overlap, CMN promotes asymmetric below ground competition and CMN may benefit large individuals at the expense of the small plant. Previous studies testing the effects of CMN on bioirrigation have largely focussed on identifying facilitative aspects of CMN-mediated plant-plant interaction. To determine the balance between positive (facilitative) and negative (competitive) effects in intercropping system, it is however, not only necessary to identify bioirrigation as a process but to also quantify its effect on yield. The balance between positive and negative effects in CMN-

facilitated intercropping systems has previously not been quantified, possibly because most previous studies have tested the effects of CMN-facilitated bioirrigation utilizing rather smaller pot sizes [18,24,25]. However, in small pots reduced plant growth [26] cannot fully inform on the quantitative facilitative and competitive interactions between two plants as they would occur in the field. In addition, drought periods simulated in small pots are often not realistic because of the rapid pre-emption of the soil water reservoir in small pots preventing the possibility to maintain moderate but realistic drought situations for extended period of time.

In the current study, we tested how the presence of a CMN affects the balance between facilitative (i.e. bioirrigation) and competitive interactions between two intercropping species. We used an established intercropping system of a deep-rooted PP and shallow-rooted FM to quantify CMN-affected competitive and facilitative interactions with respect to water-relations and bioirrigation. We aimed to conduct this study under controlled conditions and designed an intercropping system in a cylindrical large pot of 50 L to address following specific research questions: (i) Does the presence of PP as bioirrigator result in interspecific competition for water with FM before and during drought conditions? (ii) How are the competitive interactions between PP and FM influenced by a CMN network? (iii) Does PP support the water-relations and longevity of neighbouring shallow-rooted FM during drought? (iv) Can CMN promote the efficiency of bioirrigation? (v) Does the balance between competitive and facilitative effects lead to an increase or reduction of yield in CMN-facilitated intercropping?

Material and methods

Experiment set up

To test the potential of bioirrigation in intercropping systems of deep-rooted PP and shallow-rooted FM under drought, a pot experiment was performed inside the greenhouse under controlled conditions (14 hrs of day light with PPFD 350 to 400 μMol/S at 26±5°C and 10 hrs of dark (night) duration at 20±5°C and 60±10% relative humidity) at the University of Basel, Switzerland.

PP and FM plants were grown in pots of 70 cm height and 30 cm diameter. The pot design was modified after Saharan et al. [18]. In brief, each pot was filled in layers with different materials (sand, gravels and terragreen) as shown in Fig 1. The bottom layer of each pot (40 cm) contained a mix (1:1:2) of sorbix (0.6 – 3.0 mm), terragreen and fine sand (0.1 – 0.4 mm) which has the capacity to hold water in order to serve as a source of water for the deep-rooted PP. Above the bottom layer a gravel (2 – 4 mm) layer of 5 cm was installed with the purpose to prevent the capillary rise of water from the bottom layer to the top layer of the pot. Above the gravel layer, there was a 2 cm layer of medium fine sand (1 – 2 mm). The top layer of 15 cm was filled with mix (1:1) of terragreen and fine sand. The top layer was divided into two compartments: A central compartment for FM, which was 12 cm wide and 15 cm deep. The compartment was made of a nylon mesh (21 µm pore diameter, Anliker AG, Basel, Switzerland) to restrict roots of FM to grow outside the compartment. In addition, pots contained an outer compartment for PP, where roots were allowed to reach the bottom layer of the pot. All sand (purchased from Quratz dÀlsac LA France) and terragreen (Maagtechnic AG Dübendorf, Switzerland) material used in this experiment were sterilized by heating at 80°C for 12 hours.

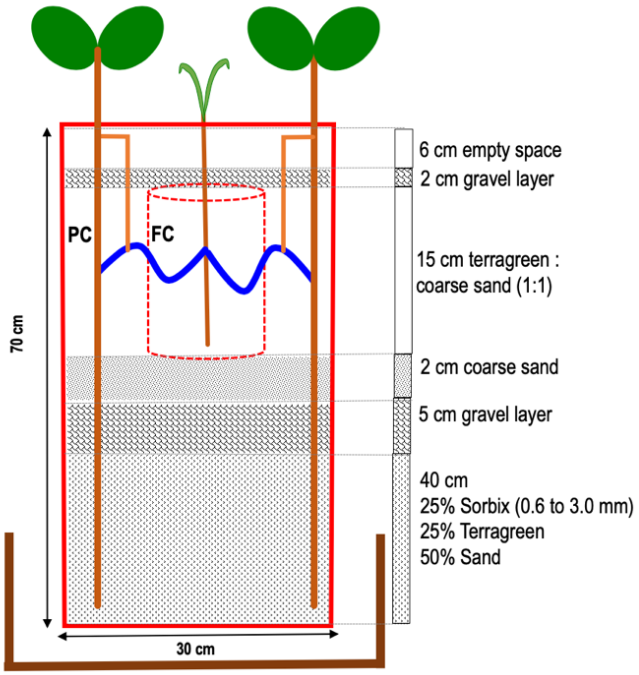

Fig 1. Pot set up. Pot with dimension of 70 x 30 cm (h x d) filled with different layers of sand and gravel. A finger millet compartment (FC) was made with nylon mesh (21 µm pore size) to restrict the growth of FM roots. FC had two FM plants per pot. Pigeon pea

compartment (PC) had two PP plants per pot. The pot was put into a wider pot of size 31 x 45 cm (h x d) to water the pot from bottom.

Plants were fertilized with 50 ml of modified Hoagland solution (with P content 75% reduced) every third week until beginning of the drought period. The Hoagland solution was reduced in P because a low P content is required for AMF to be actively involved in nutrient mobilisation and colonization of plant roots [27].

Treatments

We installed eight different treatments in our experiment: monoculture of FM and PP with and without biofertilizer as control, non-split-root (NSR) treatment and split-root (SR) treatment with and without CMN (Fig 2). In the SR treatment, lateral roots of PP were inserted into the FM compartment to allow intermingling of roots and facilitate direct transfer of water efflux from PP root. NSR and SR treatments were established to study if intermingling of two roots could enhance the transfer of hydraulically lifted water (HLW) from PP to FM.

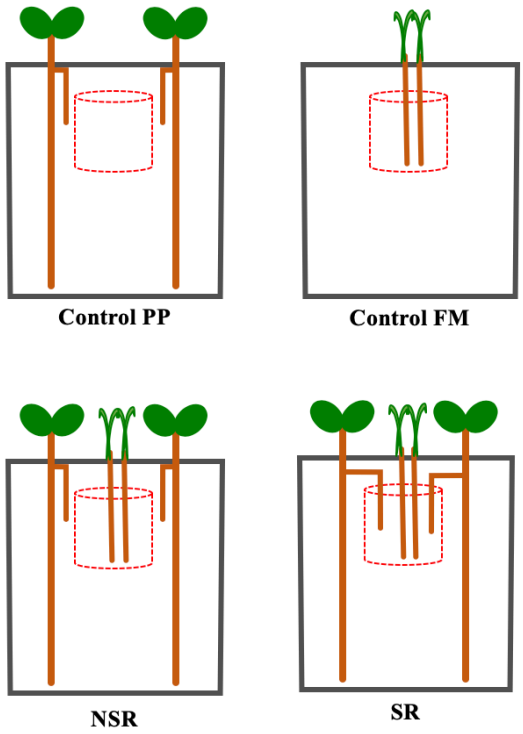

Fig 2. Experiment set up with different treatments. This study includes eight different treatments: FM monoculture without and with biofertilizer, PP mono culture without and with biofertilizer, Non-split-root (NSR) treatment without and with biofertilizer, and split-

root (SR) treatment without and with biofertilizer. In split-root (SR)treatment, lateral roots of PP plant were inserted into the FM compartment. Monoculture treatments had two PP plants in PP control and one FM plant in FM control.

Plant material

The deep rooting plant used in this study was PP (*Cajanus cajan* cv. BRG2) and the shallow-rooted plant was FM (*Elusine coracana* cv. GPU28). Seeds were sterilized by shaking seeds for 2 minutes in a 1% sodium hypochlorite (NaOCl) solution [28]. PP seeds were pre-grown into a 50 cm long tube (with 5 cm diameter) filled with four different layers: bottom layer of 10 cm with a mix (1:1) of fine sand (0.1 – 0.4) and terragreen. Above this was a gravel (2 – 4 mm) layer of 15 cm and layer of medium fine sand (1 – 2 mm). The top layer of 15 cm was filled with a mix (1:1) of fine sand and terragreen. The pre-germination of seedlings was done to ensure PP roots to reach the bottom layer of the big pots. This was necessary as PP seeds directly germinating in the pots did not grow to the bottom of the pot, possibly due to the high physical resistance of the gravel layer at 17 cm depth. Pre-grown PP were then carefully taken out of the tube and transplanted into the pots after 45 days, when roots were about 50 cm long. The day when PP seeds were sown for germination was counted as day one of the experiment. In order to keep the age difference between PP and FM not more than 30 days, FM seeds were germinated, at day 30 of the experiment, in a tray filled with a mix (1:1) of fine sand (0.1 – 0.4) and terragreen, and both 15 days FM and 45 days PP seedlings were transferred into the pots on day of experiment (DOE) 45. Monoculture treatments had two plants of FM or PP, while all intercropping treatments had two plants of FM in the central FM compartment and two plants of PP in the outer compartment.

Bioinoculants

To establish a CMN between PP and FM, AMF strains of *Rhizophagus fasciculatum* (63 spores per 10 g substrate) and *Rhizophagus leptotichum* (67 spores per 10 g) cultured in Rhodes grass roots were used as inoculants. To ensure nodulation of PP, we also used the Rhizobia strain *Bradyrhizobium* sp. (DSMZ-5969, Leibniz Institute DSMZ-German Collection of Microorganism and Cell Cultures, Germany). In addition, two PGPR strains (*Pseudomonas fluorescens* strains R62 and R81) were used [29]. PGPRs are known to have beneficial effects on plant growth especially in the development of fine root growth [30].

Treatments with CMN were inoculated with 5 g AMF culture per plant, and 2ml of bacterial inoculum containing $1x10^6$ cfu/ml were added. In all treatments with CMN, the *Rhizophagus fasciculatum* culture was placed in the FM compartment, while the *Rhizophagus leptotichum* cultured was placed in the PP compartment. 2 ml of the bacterial inoculum was added into FM and PP compartment in all treatments with CMN. Treatments without CMN were given AMF wash and cell free broth. In order to provide a natural microbiome in all treatments soil wash (soil collected from field site used for pigeon pea and finger millet intercropping at University of Agricultural Sciences, Bengaluru, India) was added into all pots. Soil and AMF wash was prepared separately by dissolving 50 g of each component in 1000 ml of tap water and the solution was filtered three times using Whatmann No. 1 filter paper.

Watering and drought treatment

Pots were watered once a week with 3 litres of tap water from the top to saturate the entire pot. Watering from the top was done until PP roots established a good network at the bottom layer of the pot. This was checked by inspecting destructively additional pots that were established for this purpose. In order to start the drought period, watering was gradually reduced to 1.5 litre, 1.0 litre and 500 ml on DOE 147, 154 and 161, respectively. The full drought period then started from DOE 168. During this drought period pots were watered by submersing only the bottom part of the pot up to 25 cm in tap water (Fig1) for two minutes, once a week. The drought period continued till FM in control treatments died (DOE 245).

AMF root colonization

To analyse the percentage root colonization by AMF, aliquots of fresh root material from PP and FM were stored in 50% ethanol. For the assessment of root colonization by AMF, root segments were cleared in KOH (10%, w/v; at 4°C, 1 week) and stained with trypan blue (0.05% w/v, at room temperature, 6 h). Root segments were destained and randomly selected segments were observed for the presence or absence of functional structures (hyphae, vesicles and arbuscules) of AMF. The percentage root colonization was calculated according to Brundrett [31] by examining 100 intersections on 25 randomly selected root fragments for each root sample.

Physiological and growth parameters

To monitor the water-relations of FM, stomatal conductance (gs) was measured, at mid-day between 12:30 to 14:30 hours, using an SC-1 leaf Porometer (Decagon Devices, USA). Measurements were done 48 hours after watering from DOE 147 to 245 of the experiment. Central leaves of FM were selected for measurements and two leaves per plant were measured on the upper surface. Soil moisture in the top layer of the pot was also measured on the same day, before measurement of stomatal conductance, using a ML3 theta probe (Delta-T Devices, Cambridge, UK) every week from DOE 182 onwards (drought started on DOE 168) till DOE 245. To observe growth during the experimentally induced drought period, plant height of FM was measured every week from DOE 161 onward till end of the experiment i.e. DOE 245.

Assessment of total foliar damage and biomass at harvest

Foliar damage of FM plants was measured at end of experiment on DOE 245. Foliar damage was assessed by counting the number of dead leaves. A leaf was defined as dead when less than one third of its length was green/yellowish green, and the remainder was desiccated. Foliar damage percentage was calculated (number of dead leaves/total number of leaves). At the end of the drought period, shoot and root parts of each plant were harvested separately. First the FM compartment was removed and shoot and root parts were separated. After this the PP plants were harvested. The roots were washed with tap water to remove sand particles. For determining dry biomass, shoot and root samples were kept in paper bags at 80°C in a hot air oven (model UF260, Memmert GmbH + Co. KG, Germany) for 48 hours.

Statistical Analysis

Data are expressed as mean ± standard error of mean (SEM). GraphPad Prism software (version 7.0 for Mac OS X, GraphPad Software, La Jolla California USA) was used to perform statistical analysis. Tukey`s test was used for post hoc multiple treatment comparison following one-way ANOVA. The criterion for significance was $p<0.05$.

Results

AMF colonization

Root colonization data show that the treatments without CMN had very low root colonization that ranged from 1.4% to 3.0% in FM and 1.4% to 2.8% in PP (Fig 3). Also, FM roots in the monoculture treatment with added AMF had a similarly low colonization (Fig 3), but in the intercropping treatment, FM was significantly better colonized ranging from 13.2 % to 18.2% in SR and NSR treatments, respectively. PP plants had an about ten-fold higher colonization compared to non-inoculated controls, both in monoculture and in intercropping treatments.

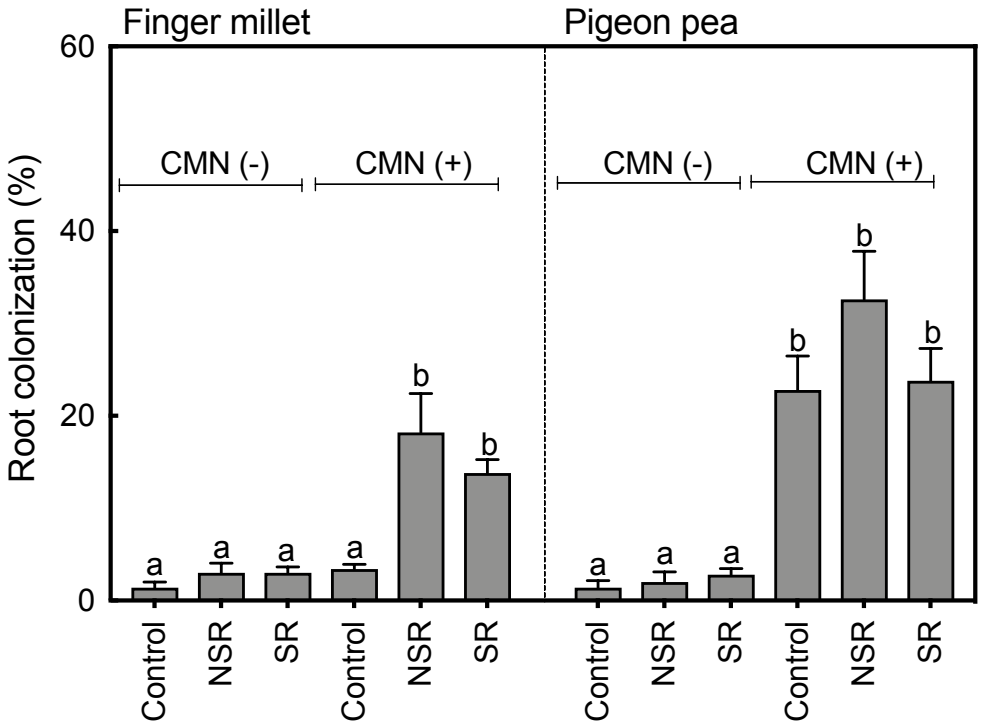

Fig 3. Percentage root colonization of AM fungi in FM and PP roots. Bars represent the average of five replicates with standard error of mean. Tukey`s test (one-way ANOVA) was used for multiple comparison (PP and FM separately) and values with same letters are not significantly different at $p>0.05$. Treatments with biofertilizer application are represented with CMN (+), and without biofertilizer application are represented with CMN (-). Control represents monoculture, while NSR and SR represent non-split root and split root treatments of intercropping, respectively.

Water-relations of finger millet during drought

Two weeks after the onset of the drought (DOE 182), soil moisture in the topsoil layer in all intercropping treatments had already declined to a value of ca. 0.06 m^3/m^3 (Fig 4a). This was independent of the presence of a CMN. In contrast, soil moisture in the monoculture (control) treatments of FM was 0.19 m^3/m^3, which was significantly higher than in the intercropping treatments two weeks after the onset of drought. Soil moisture in the topsoil layers in the control treatments gradually decreased as the drought period prolonged, reaching 0.05 m^3/m^3 at DOE 217, which was similar to the values observed in the intercropping treatments.

The response of stomatal conductance (g_s) during the drought in the different treatments can be separated into three phases. Phase 1 (before and at the onset of drought DOE 154 – 168): all treatments had similar values for g_s, ranging from 147.2 to 199.5 mmol $m^{-2}s^{-1}$ at DOE 154 before the drought started. With the onset of drought we observed that g_s declined in all treatments (DOE 175). This decline was more rapid in the intercropping treatments than in the monocropping controls and independent of the CMN. Phase 2 (progression of drought DOE 175 – 224): FM in the control and intercropping treatments maintained a low yet stable g_s for seven weeks. In general, g_s was higher in the monocropping treatments than gs in the intercropping treatments, but this effect was mostly independent of CMN. Phase 3 (end of drought DOE 224 – 245): at DOE 231, g_s in the controls dropped to low values ranging from 27.1 to 33.6 mmol $m^{-2}s^{-1}$, while g_s in the intercropping treatments was maintained at values around 60 mmol $m^{-2}s^{-1}$ (Fig 4b). As such, FM in intercropping treatments maintained a low but consistent gas-exchange until the end of the drought treatment, while g_s of FM in monoculture treatments (with and without CMN) dropped to very low values ranging from 9.4 to 20.3 mmol $m^{-2}s^{-1}$ at DOE 245. Presence of a CMN did not show any effect on stomatal conductance of FM in intercropping treatments (NSR and SR) or controls during phase 3.

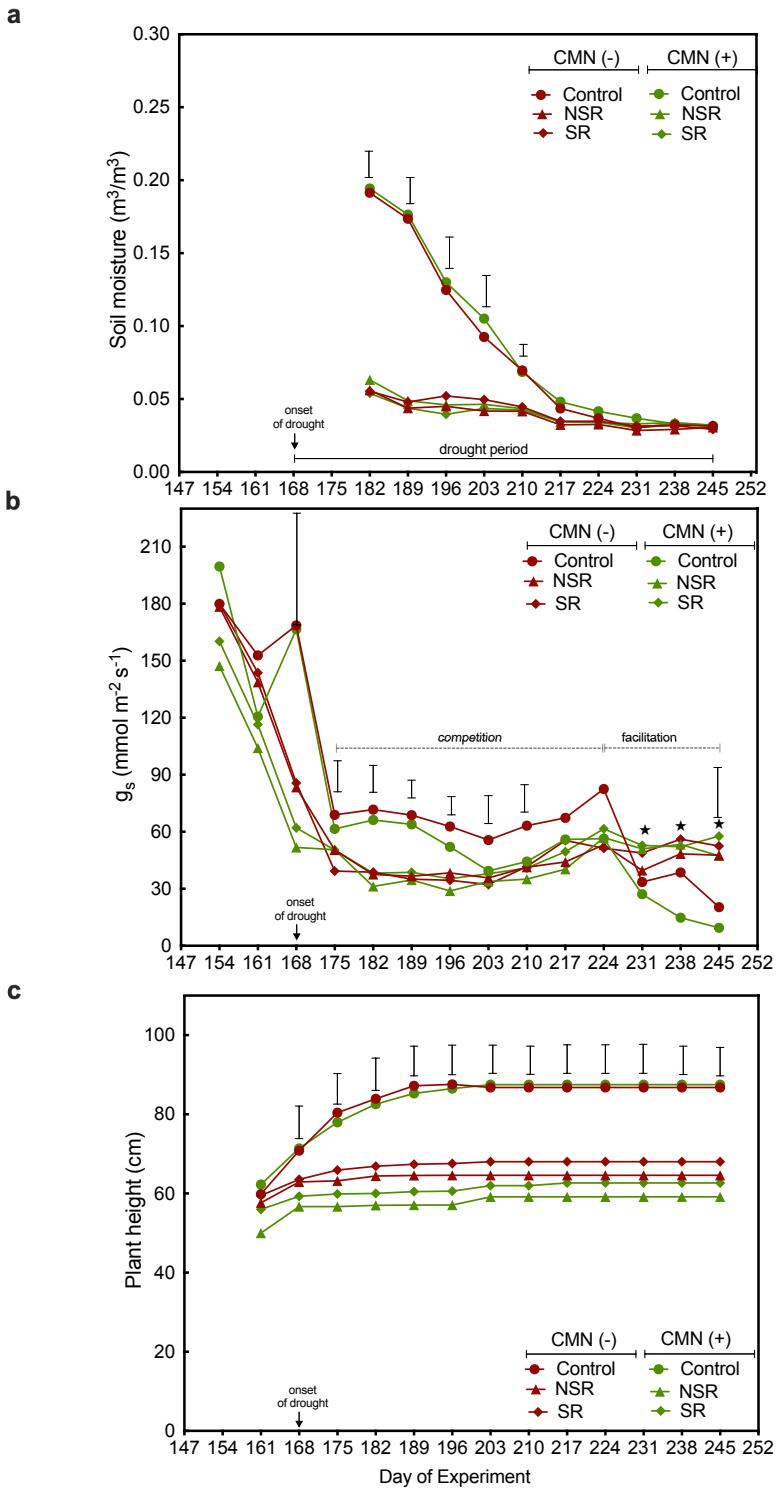

Fig 4. Soil moisture of the topsoil layer in all treatments (Fig 4a), stomatal conductance (g_s, Fig 4b) and plant height of FM during drought (Fig 4c). Treatments with biofertilizer application are represented with CMN (+), and without biofertilizer application with CMN (-). Control represents monoculture, while NSR and SR represent non-split root and split root treatments of intercropping, respectively. Values shown in the graph are the average of five replicates, and bars represent Tukey`s $HSD_{0.05}$ value above weeks at which

significant difference occurred among treatments. Drought period started at day 168 of experiment. Star (□) symbols in Fig. 4b represent data points for control treatments where some replicate plants did not survive; the surviving plants were used to measured stomatal conductance (see Table 1).

FM growth

Before the onset of drought, at DOE 161, plant height of FM was similar in all treatments (Fig 4c). In the first weeks after the onset of drought (DOE 168), plant height increased more in the control (monoculture) treatments than in intercropping treatments. This trend continued until 4 weeks after the onset of drought. From DOE 196 onwards, there was no increase in plant height in monoculture. While, FM in all intercropping treatments did not grow in height one week after the onset of drought. FM plants in monoculture with or without CMN had significantly higher plant height than FM in all intercropping treatments. CMN did not have any positive effect on plant height of FM in the controls but FM plants in the intercrop were slightly higher, when a CMN was present.

Total foliar damage of FM in response to drought

FM in control treatments with and without CMN showed 100% total foliar damage at the end of the experiment, while FM in intercropping treatments with CMN (NSR and SR) showed lower damage rates of 78.5% and 76.5%, respectively, than FM in monoculture (Fig 5). FM plants in intercropping treatments with lower total foliar damage percentage had 100% survival rate (Table 1), as at the end of drought period (DOE 245), only 1 and 2 replicates of FM were alive in monoculture with and without CMN, respectively, while FM in all replicates of intercropping treatments were alive.

Table 1. Number of surviving replicates of FM from day of experiment (DOE) 224 to 245 when all treatments had similar level of drought. FM in all five replicates of intercropping treatments were alive and maintained a low but consistent stomatal conductance, while in FM control treatments started to die from DOE 231 to 245.

Treatment	CMN	DOE 224	DOE 231	DOE 238	DOE 245
Control	Yes	5	3	2	1
Control	No	5	3	3	2
NSR	Yes	5	5	5	5
NSR	No	5	5	5	5
SR	Yes	5	5	5	5
SR	No	5	5	5	5

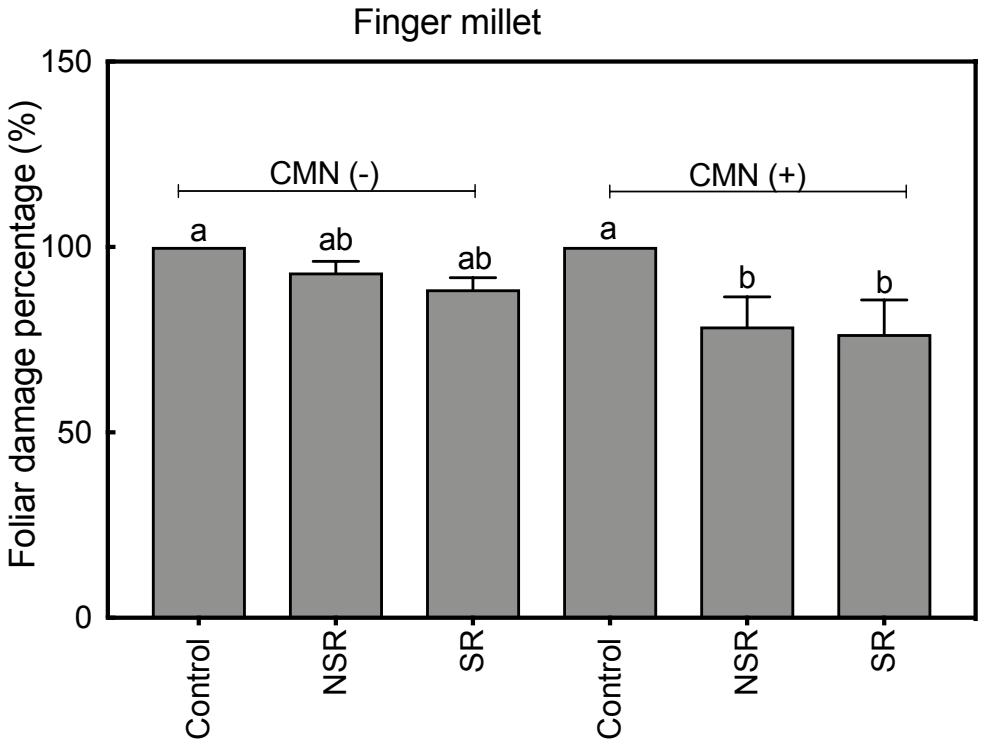

Fig 5. Foliar damage percentage of FM at day 245 of experiment. Treatments without or with biofertilizer application are shown as CMN (-) and CMN (+), respectively. Control represents monoculture, while NSR and SR represent non-split root and split root treatments of intercropping, respectively. Bars show average of five replicates with standard error of mean, and Tukey`s test (one-way ANOVA) was used for multiple comparison and values with same letters are not significantly different at $p>0.05$.

Biomass of FM and PP

Shoot and root biomass of FM was significantly lower in intercropping treatments as compared to monocropping treatments. While we did not observe a significant effect of intercropping on PP root and shoot biomass (Fig 6), there was yet a trend of reduced PP biomass in the intercropping treatments. We did not observe any significant effects of CMN presence on FM biomass (both shoot and root biomass), while PP produced significantly more shoot biomass with CMN than without CMN in monoculture.

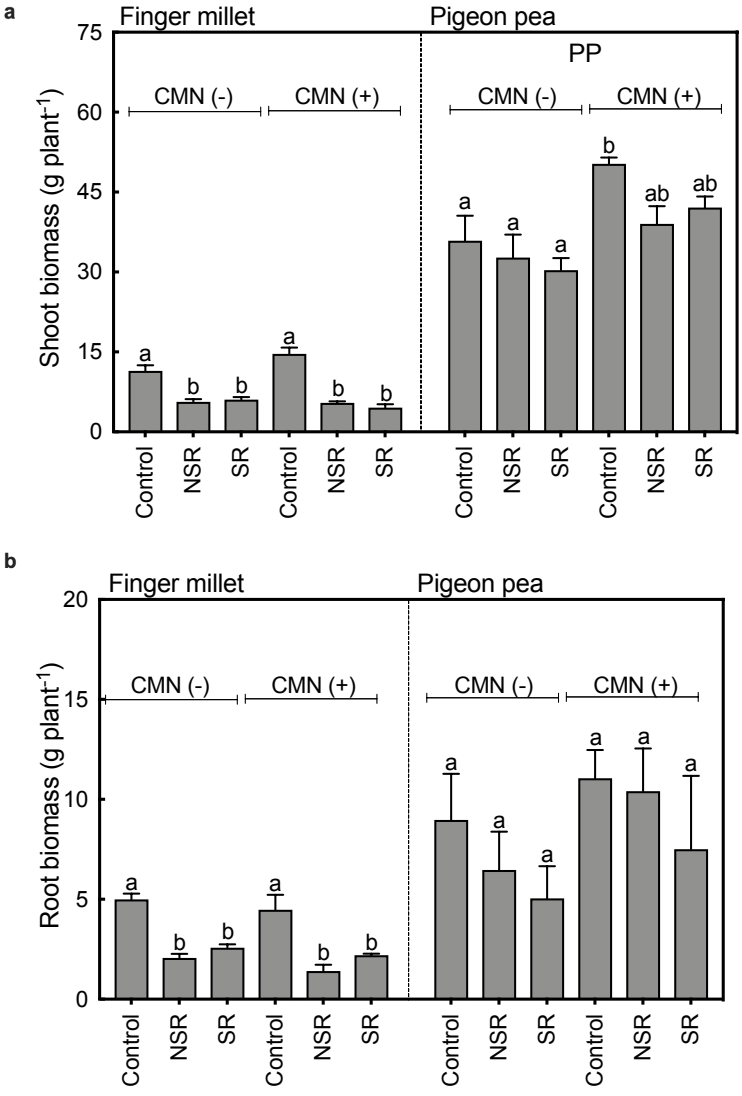

Fig 6. Shoot and root dry biomass of FM and PP in different intercropping treatment. Treatments without or with biofertilizer application are shown as CMN (-) and CMN (+), respectively. Control represents monoculture, while NSR and SR represent non-split root and split root treatments of intercropping, respectively. Bars represent average of five

replicates with standard error of mean. Tukey`s test (one-way ANOVA) was used for multiple comparison (PP and FM separately) and values with same letters are not significantly different at $p>0.05$.

Discussion

Our results indicate that in a PP-FM intercropping system, bioirrigation supports the water-relations of FM during drought and helps FM to tolerate (or survive) the drought period. However, our data also show that FM in intercropping treatments tends to face drought conditions earlier than in monoculture due to apparent competition between FM and PP for water in the topsoil layer. This can explain the lower total biomass of FM in the intercropping treatments as compared to the monocrops despite bioirrigation during drought. In contrast to our expectations, the establishment of a CMN did not influence the competitive or facilitative interactions between FM and PP with respect to water in the experiment. Yet, the drought-induced foliar damage was significantly lower in treatments with CMN.

Water-relations, foliar damage and growth of FM during drought

Our results suggest the facilitation of water to FM by PP during prolonged drought periods so that FM plants in all intercropping treatments maintained their stomatal conductance and showed lower foliar damage than FM in monoculture during drought. Since FM was not able to access to deep-soil moisture in our experimental set up, we assign the improved water-relations of FM to bioirrigation by PP. These results are in line with previous studies showing that plants growing in close vicinity of a HL performing plant can benefit from the process of HL with respect to its water-relations [32–35]. Sekiya et al. [35] performed a split-root experiment to demonstrate that neighbouring shallow-rooted plants had access to deep soil moisture lifted by deep-rooted donor plant and as a result had higher stomatal conductance. Similar findings were reported by Dawson [2] showing that shallow-rooted plants growing next to Maple tree conducting HL maintained high stomatal conductance and were able to utilize hydraulically lifted water.

In contrast to the observed facilitation during drought, we also detected strong competition between FM and PP for soil moisture in the topsoil layer before or just after the onset of

drought. This is indicated by soil moisture in intercropping treatments becoming more rapidly depleted than in FM monoculture with the onset of drought. In fact, FM in the intercropping treatments dropped to the low levels of the monoculture treatments five weeks earlier. The apparent competition for water can also explain why we did not observe any beneficial effects of intercropping on the growth of FM during drought (Fig 4). Plants in the FM monoculture treatments grew well at the beginning of the drought period, most likely due to absence of interspecific competition for water in topsoil layer and a relatively high soil moisture despite the drought treatment (Fig 4c). FM in the intercropping treatments (both with and without CMN) did not continue to grow in height after the onset of drought. Previous studies [34,36] have reported similar results where HL efflux through trees supported the survival of seedlings, but biomass of seedlings were reduced due to a strong interspecific competition for soil resources (water and nutrients).

The presence of a CMN between PP and FM did not improve the water-relations of FM during the drought period through facilitation of bioirrigation. This is in contrast to our previous study [20] that involved the same species but an experimental design with smaller pots, where FM in treatments with CMN had significantly higher stomatal conductance than treatments without CMN during drought. Also Saharan et al. [18] has reported that the presence of a CMN in a PP – FM intercropping system alleviates the negative effect of drought on FM. It is possible, that the missing effect of a CMN on the facilitation of bioirrigation could be because the mycorrhizal hyphae could not connect the rhizosphere of two plants since PP and FM plants were placed at 15 cm apart. Therefore, even after showing that PP and FM roots were colonized by AMF hyphae in treatments with CMN, we cannot show an effective CMN was established to connect the rhizosphere of two plants.

In this study, a combination of AMF strains, rhizobia and PGPR were used to develop a CMN between rhizosphere of PP and FM. Previous studies [37,38] have reported that AMF colonization is more effective in legumes when they are nodulated by N_2-fixing bacteria. PP and FM roots have been reported to show up to 65% and 75% colonization, respectively [18], while in our study, we observed AMF colonization up to 32.6% in PP and 18.2% in FM. Similar to this, Beggi et al. [39] has also reported low colonization rates in millets that varied from 12% to 30%. Low colonization percentage in PP and FM roots in our set up could possibly be due to high soil P, since typically low colonization percentage have been reported under high soil P condition [40,41]. In our experimental set up, the source of high

soil P could be the terragreen substrate that contains up to 0.1% of P_2O_5 (technical data from manufacturer).

The effect of CMN on total foliar damage in FM was significant. Reduced total foliar damage (Fig 5) in intercropping treatments with CMN could be assigned to higher water and nutrient uptake in FM in treatments NSR and SR with CMN than without CMN. We used a combination of PGPR, rhizobia as bioinoculants together with the AMF culture. PGPR and rhizobia have been reported to promote formation of fine roots and root hairs of leguminous plants [42–44].

Effect of bioirrigation on plant biomass in intercropping

The facilitative effect of bioirrigation during the drought period did not translate into an increased FM biomass, nor was there a positive effect of a CMN on FM biomass. Rather, plant biomass (shoot and root) of FM was significantly reduced in intercropping treatments, both with and without CMN, most likely due to interspecific competition for resources with PP (Fig 6a & 6b). Similar results of facilitation and competition between deep-rooted and shallow-rooted plants have been reported, mostly in agroforestry field, by many research studies [15,45,46]. Prieto et al. [34] reported that *Retama sphaerocarpa* L. conducts HL and supports establishment (survival) of seedlings of shrub *Marrubium vulgare* under its canopy but biomass of seedlings decreased significantly due to competition for soil resources, showing that competitive effects were stronger than facilitative effect of HL.

Conclusions

Our results demonstrate that PP does support water-relations and survival of FM during a drought period in a model intercropping system. Our results reveal indirect proof of the "bioirrigation" concept, since FM was not able to access deep soil moisture, and in intercropping treatments PP helped FM to maintain its water-relations and longevity through bioirrigation. However, establishment of a CMN through inoculation with AMF did not show any effect on water-relations of FM during drought. Additionally, PP exerts strong competitive effects on FM during well-watered conditions that hinders growth and biomass production of FM when intercropped with PP. We observed for example earlier and more severe drought symptoms in FM in intercropping treatments than in FM

monoculture, most likely due to competition for water in topsoil layer. The extent by which the antagonistic processes facilitation and competition were expressed in the intercropping system was regulated by the availability of soil water in the topsoil layer.

In summary, the results from our study indicate that in intercropping, deep-rooted PP may potentially act as a "bioirrigator" for shallow-rooted crops such as FM. However, the interspecific competition between PP and FM has to be considered in order to avoid yield loss. Further studies involving different pairs of deep-rooted and shallow-rooted plants, and the optimal number of deep-rooted HL performing plants for each shallow-rooted plant would be useful to explore the potential of bioirrigation based intercropping in water-limited areas.

Acknowledgement

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Chapter 3

Influence of spatial arrangement, biofertilizers and bioirrigation on the performance of legume – millet intercropping system in rainfed areas of southern India

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Abstract

Agricultural productivity in rainfed areas is sensitive to weather, and intermittent drought conditions due to shortage in rainfall causes loss in crop yield. In order to avoid impact of intermittent drought on crop yield, sustainable intercropping system with emphasis on efficient use of soil moisture is required. In this study, we tested a legume – millet intercropping system with objective to enhance total yield by optimizing the spatial arrangement of component plants and reduce the impact of drought through bioirrigation facilitated via common mycorrhizal network. A field study was conducted for two consecutive growing seasons in years 2016/17 and 2017/18 at GKVK, Bengaluru and Kolli Hills, India. The process of bioirrigation is based of ecological process of hydraulic lift where transfer of water occurs from deep soil layer to top soil layer through plant roots as a consequence of water potential gradient. We used pigeon pea (PP) as deep-rooted plant and finger millet (FM) as shallow-rooted plant, and we attempted to establish connection between rhizosphere of both plants through common mycorrhizal network to facilitate bioirrigation. The field trial results clearly showed that spatial arrangement of component plants affect the yield of an intercropping system. The row-wise (2 PP : 8 FM) intercropping was more effective than mosaic treatments, at GVK field site. While, at Kolli Hills both row-wise (2:8) and mosaic treatment performed equally. The biofertilizer application enhanced the yield of intercropping and monoculture treatments, and effect of biofertilization was not influenced by the spatial arrangement of component plants and location of field experiment. Despite yield increase in intercropping treatments, we did not see a positive effect of intercropping or biofertilizer on water relations of FM. FM in intercropping had significantly lower leaf water potential than monoculture due to strong interspecific competition for soil moisture in intercropping treatments. Therefore, spatial arrangement of component plant and optimal of plant density of PP is needs to tested in order to develop an efficient bioirrigation based intercropping system. We envision that, with further optimization of spatial arrangement with different legume varieties, a sustainable intercropping model for rainfed areas could be developed to reduce the yield loss due to intermittent drought.

Keywords: intercropping, bioirrigation, mycorrhiza, rainfed agriculture, drought.

Introduction

Climate change is one of the most crucial factors that affects agricultural productivity (Karimi et al., 2018). Approximately, 1.8 billion people will be living in areas with water scarcity by 2025. Due to re-occurring drought events, crop yields in rain-fed areas of arid and semiarid tropics are particularly unstable and decreasing (Loladze, 2014; Pingali, 2012). Especially in subtropical areas, a decrease of 11% in land suitable for agriculture could thus occur by 2080 if the present trend of climate change continues (FAO 2017). Smallholder farmers in rainfed areas of developing countries are most affected by these changes because of their dependency on rainfall and limited access to capital intensive techniques for irrigation and fertilization. Therefore, sustainable agriculture practices with an emphasis on sustainable use of soil moisture are required to maintain the stability of agriculture and food security for smallholder farmers in rain-fed areas.

Intercropping has been considered a sustainable way to utilize and share natural resources among different crop species and to improve and stabilize crop yield (Brooker et al., 2015). In intercropping systems two or more crop species are grown together (Ngwira et al., 2012; Vandermeer, 1989). Crop yield in intercropping systems are often higher than in sole cropping systems because resources such as soil moisture and nutrients are utilized more efficiently (Dahmardeh et al., 2009; Lithourgidis et al., 2007). This is because interspecific competition between intercropping partners is often lower than the intra-specific competition so that a yield advantage occurs (Davis and Woolley, 1993). In addition, beneficial effects of intercropping can come from resource facilitation. As an example, legume–cereal intercropping systems have been widely used in areas with poor soil quality (L Li et al., 2007), where legumes fix nitrogen (N) and solubilize phosphorus (P), which is then used by both intercropping partners (Hinsinger et al., 2011). In return, cereals can support legumes in two ways, by preventing nitrate-N accumulation in soil which inhibits N fixation by legumes, and by increasing iron availability which enhances N fixation (Schipanski and Drinkwater, 2012; Zuo et al., 2004).

In rain-fed areas of the arid and semiarid tropics, intercropping has also been suggested to enhance the water availability of shallow-rooted crops via the facilitation of water by deep-rooted plants through hydraulic lift (HL) (Mao et al., 2012; Xu et al., 2008). The water released from deep-rooted plants due to HL into topsoil layer could be available to

neighbouring shallow-rooted plants through process of Bioirrigation (Burgess, 2011). The functionality of bioirrigation in intercropping systems has only been tested in a few studies – mainly under controlled conditions in the greenhouse. Sekiya and Yano (2002) showed in a field experiment that pigeon pea (a deep-rooted legume) has the potential to perform HL and could supply deep water to shallow-rooted maize. In another study Sekiya et al. (2011) showed that plants with deep roots are ideal for intercropping with shallow-rooted crops in water limited agriculture fields and that this kind of intercropping system allows shallow-rooted plant to access deep soil moisture without having deep roots. Other studies have also shown the transfer of hydraulically lifted water (HLW) from a deep-rooted plant to neighbouring shallow-rooted plants (Bogie et al., 2018; Brooks et al., 2006; Caldwell and Richards, 1989; Moreira et al., 2003). While these experiments have suggested that bioirrigation could be an important mechanism for drought stress avoidance of intercropped field crops, evidence for the efficiency of this mechanism in the field studies is yet lacking.

The success of an intercropping system in the field depends on the avoidance of competitive growth inhibition among the intercropping partners. This requires appropriate spacing of the intercropping partners so that competitive, complementary and facilitative interactions are well balanced and that yield improvements can be achieved. In particular for bioirrigation to be effective it seems that an ideal spacing between the intercropping partners is essential. On the one side, intercropping partners have to be arranged with sufficient space among each other in order to avoid competition. On the other side, plants need to be spaced in close enough distance to allow the rhizosphere to rhizosphere transfer of bioirrigated water (Burgess, 2011; Prieto et al., 2011).

Next to intercropping approaches, biofertilization such as arbuscular mycorrhizal fungi (AMF) inoculum combined with rhizobia and plant growth promoting rhizobacteria (PGPR), are beginning to become established as an effective and sustainable measure to improve yields (Schütz et al., 2018). The role of AMF for the uptake and transfer of nutrients and water to host plants has been well demonstrated (Augé et al., 2001; Querejeta et al., 2003). Biofertilization might have particular potential to boost the yield of intercropping systems because AMF can form common mycorrhizal network (CMN) that can transfer nutrients between two plants and balance as such belowground competition (Smith and Read, 2008). In addition to nutrients, the establishment of a CMN between the roots of two plants can also constitute a pathway for the transfer of water and might thus

act as effective pathway for the transfer of HL water between two plants and thus for bioirrigation (Perry et al., 1989). In fact, a CMN can bridge substantial distances between the rhizospheres of plants (Schütz et al. unpublished) and Egerton-Warburton et al. (2007) have demonstrated that arbuscular mycorrhizal hyphae provide indeed a potential pathway for the transfer of HLW between two plants. Our own recent work has shown that a CMN plays a key role in facilitating the transfer of water between the rhizospheres of two intercropping partners in a greenhouse and can in turn improve the water relations of shallow rooted crops during soil drying (Singh et al., 2019). However, further experiment with bigger pot size (50 L) than previous experiment did not show the effect of CMN on water-relations but treatments with CMN had lower foliar damage percentage than treatments without CMN (Singh et al. unpublished).

Despite their potential and evidence from greenhouse studies, the effects of biofertilizers on stabilizing and improving the yields in intercropping systems by improving water relations via bioirrigation have not yet been tested under field conditions. In addition, it is unclear to what extend beneficial effects of biofertilizers in intercropping systems depend on an appropriate spacing of the crops and if – given the appropriate spatial arrangement of crops - the establishment of a CMN can indeed facilitate bioirrigation and improve as such the water relations of shallow-rooted crops in intercropping systems in dryland agriculture. In this study, we therefore investigated the effects of biofertilization on the yield of a legume – millet intercropping system, and tested different spatial arrangements of the plants in combination with biofertilizer treatments. We used pigeon pea (PP, *Cajanus cajan*) as deep-rooted plant and finger millet (FM, *Eleusine coracana*) as shallow-rooted plant to investigate following research questions: (i) Does the spatial arrangement of intercropping partners affect straw and grain yield in a FM – PP intercropping system compared to monocultures of the same crops? (ii) Does the application of biofertilizers have an influence on the intercropping effect in spatially differently arranged intercropping systems? (iii) Can intercropping effects driven by CMN be explained by bioirrigation, indicated through the improvement of the shallow-rooted plants water relations?

Material and methods

Selection of field experiment site and crop varieties

To test the influence of the spatial arrangement and biofertilizers on crop yields of PP and FM, field trials were carried out at two different locations during the growing seasons 2016-17 and 2017-18. One experimental site was located at the research farm of the University of Agricultural Sciences, Bengaluru, Karnataka. The second site was located at the research farm of MS Swaminathan Research Foundation (MSSRF), Kolli Hills, Tamil Nadu, India. Both experimental sites were selected because farmers have already adapted to cereal-legume intercropping system there and have been cultivating PP and FM as one of their main crops. Based on farmers practice in the region and recommendations from local agronomists, we selected FM variety GPU-28 and PP variety BRG-2 for the field experiment in Bengaluru (both years). While at the Kolli Hills site PP variety Vamban-3 and SA-1 were selected for the field trial during 2016-17 and 2017-18, respectively, along with FM variety GPU-28 in both years.

Rainfall and climatic conditions

The total annual precipitation at GKVK site was 694.9 mm in 2016 and 1104.5 mm in the year 2017. While at Kolli Hills, the total annual precipitation was 281.7 mm in 2016 and 1690 mm in 2017. Rainfall data recorded during the experimental period indicate that the Kolli Hills area received less rain than GKVK site (Fig. 1). Both sites receive the maximum amount of rain during the months of May, June and July. GKVK received up to 40-60 mm rain during September, October and December, while Kolli Hills site was completely dry after July.

Intercrop field design with different spatial arrangement of PP and FM

The plot size for a treatment was 7.2 x 3.6 m (width x length) with a net plot area of 3.6 x 1.8 m (Fig. 2). The net plot areas defines the central part of each plot as marked in Fig. 2, where all physiological, growth and yield parameters were assessed. The field experiments had six basic treatments: FM monoculture (T1), PP monoculture (T2), 2:8 (PP:FM) row-wise intercropping (T3), 1:4 (PP:FM) row-wise intercropping (T4), 100% mosaic (T5) and 50% mosaic (T6) (Fig. 2). Each treatment was replicated four times.

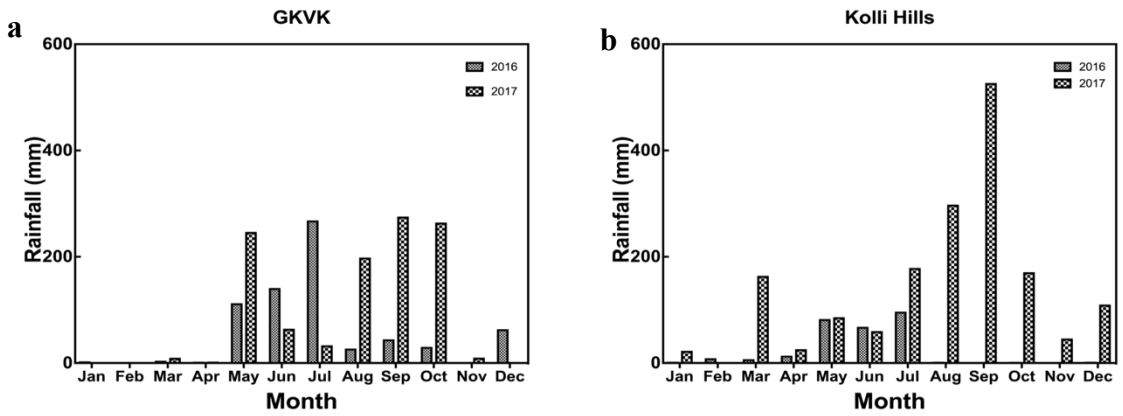


Fig. 1 Rainfall data of GKVK, University of Agricultural Sciences, Bengaluru (Fig. 1a) and Kolli Hills, Tamil Nadu India (Fig. 1b) during 2016 and 2017.

In monocultures, the density of FM was 48 plants per m^2 and the density of PP was 6 plants per m^2. As such, we planted 8 times more individuals of FM than PP per area and the total number of plants for FM in monoculture (T1) was 1152 per plot and 288 plants in the net plot area. While, for PP monocrop (T2), the total number of plants was 144 in the total plot and 36 plants in the net plot area. The spacing between FM rows was 30 cm and the distance between FM plants in a row was 7.5 cm. The spacing between PP rows was 60 cm and the distance between PP plants within a row was 30 cm. In intercropping treatments, spacing between PP and FM rows was 45 cm.

Intercropping systems were based on FM monocultures, where eight FM plants were substituted by one PP plant. Row-wise intercropping systems (treatment T3 and T4) were based on previous investigations under rain-fed conditions in Karnataka, India (Ashok et al. 2010; Padhi et al. 2010). For T3 (2:8 PP:FM row-wise arrangement), each replicate had thus 48 PP (12 plants x 4 rows) and 768 FM (48 plants x12 rows in each total plot area). T4 (1:4 PP:FM row-wise arrangement) had the identical number of PP and FM plants as T3 but it differed in row arrangement where one row of PP was planted after four rows of FM. Treatment T5 (100% mosaic) consisted of identical numbers of PP and FM plants as T3 and T4, but PP and FM plants were planted within the same row in a mosaic design (Fig. 2). In treatment T6 (50% mosaic), the number of PP was reduced by 50% and replaced by FM plants. It consisted of 24 PP plants (2 plants x 12 rows) and 960 FM plants. In the 2017-18 field trial at GKVK, FM plants in T5 were not substituted by PP but PP was accidentally added into mosaic design. Therefore plant density of FM was higher than in the other treatments.

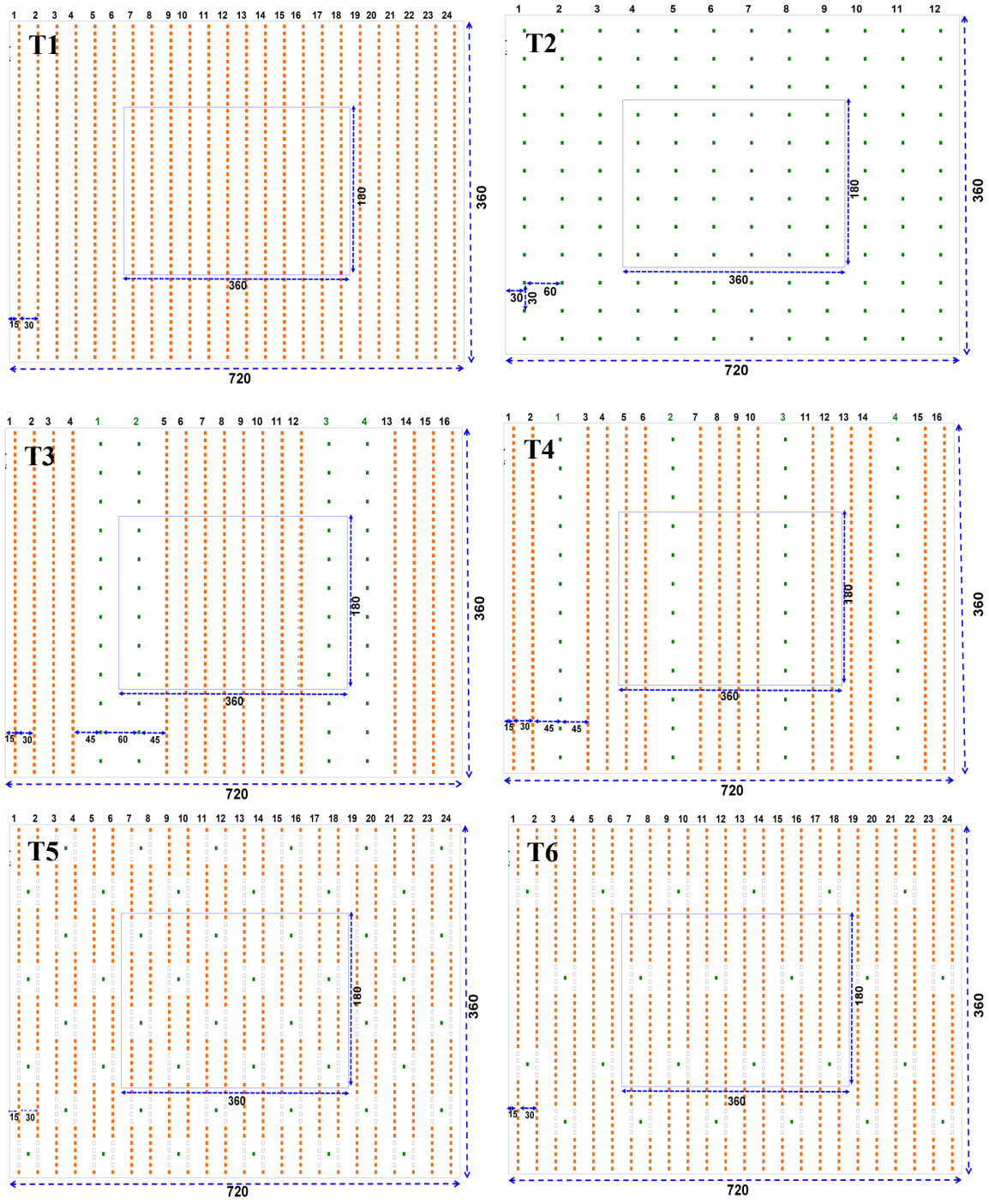


Fig. 2 Schematic diagram of field design consists of monoculture of FM (T1) and PP (T2). T3 represents 2:8 (PP:FM) row-wise intercropping pattern, while T4 represents 1:4 (PP:FM) row-wise intercropping pattern. T5 and T6 shows 100 and 50% mosaic intercrop design, respectively, number of PP in T6 was reduced to 50% (as compared to T3, T4 and T5) and to keep the maintain the planting density similar FM equivalents were transplanted. In this study, we assumed, 8 FM plants are equivalent to 1 PP plant.

We established the same treatments in the years 2016-17 and 2017-18 except for T6, which was not established in 2017-18 based on results from 2016-17 field trial. While field trials during year 2016-17 had only treatments with biofertilizers, field trials during the year 2017- 2018 included treatments with and without biofertilizers (Table 1).

Table 1 Intercropping treatments with (AMF + PGPR) and without (none) biofertilizer application were designed and tested at two experimental sites, GKVK Bengaluru, and Kolli Hills Tamil Nadu, in India. Recommended dose of fertilizer (RDF), and number of FM and PP inside the net plot area are mentioned in the table.

Treatment	**Cropping System**	**PP:FM ratio**	**No. of FM Plant**	**No. of PP Plant**	**Planting system**	**(RDF)**	**Biofertilizer application**
T1+	FM	0:1	288	0	Row	50%	AMF + PGPR
T1-	FM	0:1	288	0	Row	50%	None
T2+	PP	1:0	0	36	Row	50%	AMF + PGPR
T2-	PP	1:0	0	36	Row	50%	None
T3+	FM+PP	2:8	192	12	Row	50%	AMF + PGPR
T3-	FM+PP	2:8	192	12	Row	50%	None
T4+	FM+PP	1:4	192	12	Row	50%	AMF + PGPR
T4-	FM+PP	1:4	192	12	Row	50%	None
T5+	FM+PP	2:8 (100% PP)	192	12	Mosaic	50%	AMF + PGPR
T5-	FM+PP	2:8 (100% PP)	192	12	Mosaic	50%	None
T6+	FM+PP	1:4 (50% PP)	240	6	Mosaic	50%	AMF + PGPR

We applied 50% of the recommended dose of fertilizer (RDF) to all plots during sowing of FM seeds, RDF (100%) for PP is 25:50:25 NPK Kg ha^{-1} and for FM is 50:40:25 NPK kg ha^{-1}. Nitrogen (N) fertilizer was given in the form of Urea (46% N-0P_2O_5- 0K_2O, SPIC India Fertilizer Company), Phosphate (P) fertilizer was given in the form of Single Super Phosphate (SSP, 0N-16%P_2O_5-0K_2O, SPIC India Fertilizer Company), and Potash (K) fertilizer was given in the form of Muriate of Potash (MOP, 0N-0P_2O_5-60%K_2O, SPIC India Fertilizer Company).

Biofertilizers consisted of AMF, rhizobium, and plant growth promoting rhizobacteria (PGPR). Two species of AMF inoculants viz. *Glomus fasciculatum* and *Glomus leptotichum* were selected for FM and PP, respectively, and one PGPR strain (*Pseudomonas sp*. MSSRFD41) was selected for FM were used in this study. The two AMF species were multiplied in a vermiculite based carrier material using Rhodes grass (*Chloris gayana*) as host plant for 40 to 45 days. The harvested dry *Glomus leptotichum* inoculum, consisting of 24 spores g^{-1} of substrate, was applied at the rate of 5 g per PP plant in polybag and ca. 278 Kg ha^{-1}. Similarly, *Glomus fasciculatum*, consisting of 15 spores g^{-1} of substrate was applied at the rate of ca. 444 Kg ha^{-1} for FM. The PGPR strains were multiplied in King`s B medium and the liquid culture consisting 1x 10^9 CFU per ml of *Pseudomonas sp.* MSSRFD41 (Sekar et al., 2018) was applied as seed coating at the rate of 5 ml per kg seed. Additionally, a band application (along the planting rows) was applied at the rate of 49.5 litres (consisting of 1x 10^9 CFU per ml) together with farmyard manure (FYM) 7.5 t ha^{-1}. Rhizobium was applied as seed inoculation at the rate of 10 ml kg^{-1} PP seeds. The AMFs were obtained from Centre for Natural and Biological Resources and Community Development (CNBRCD), Bengaluru and the PGPR strain was obtained from MS Swaminathan Research Foundation (MSSRF), Chennai. Rhizobium was obtained from Agricultural Station, Amaravati, Andhra Pradesh.

Pre-germination, sowing of seeds into field, growth period and harvest

Based on an established practice in the area, PP seeds were pre-germinated before planting in a polybag (15 x 10 cm) filled with 1.6 kg of field soil: FYM: sand mixture ratio of 15:1:1, and a seed hole of 4 x 1 cm was made on the top. The bottom layer of the seed hole was filled with *Glomus leptotichum* in vermiculite, two PP seeds coated with rhizobia and PGPR strains were kept above the vermiculite layer and field soil was filled on the top. The

seeds were allowed to germinate and grow for 45 days, later healthy seedlings from these polybags were transplanted into the field during third week of July 2016 for 2016-17 trial, and on first week of August 2017 during 2017-18 field trial. FM seeds were broadcasted by hand directly into the field immediately after transplanting the PP seedlings, and after germination it was thinned out to maintain the plant density as required in different treatments. FM and PP plants were harvested after 120 and 207 days after sowing, respectively in 2016-17 trial at Kolli Hills, while at GKVK site FM and PP were harvested after 127 and 168 days after sowing, respectively. During 2017-18 field trial, FM and PP were harvested at 133 and 245 days after sowing, respectively at Kolli Hills site, at GKVK site FM and PP were harvested after 124 and 160 days of sowing.

Growth and yield parameters

Plant growth parameters such plant height, number of pods, pod weight per plant, number of panicles, grain weight per panicle, straw and grain biomass (both sun dried and oven dried), weight of 1000 FM seeds and 100 seeds of PP were measured after harvest of plant material in the net plot area. For biomass, plants were harvested row-wise in the net plot area and straw and grains were separated. The sun dried biomass was determined after drying the straw under the sun for 15 days and 20 days for FM and PP, respectively. Grains were dried under sun for 10 days for PP and FM. A subsample of the sun dried straw and grain material was oven dried at 80°C for 24 h for calculating the dry matter yield expressed in t per ha and g per plant. P per plant biomass was calculated by dividing the row biomass by number of plants in each row.

Land equivalent ratio

The facilitative and competitive interactions between PP and FM in response to the different treatments were calculated using the land equivalent ration (LER). The LER indicates the efficacy of an intercropping system for using natural resources compared with monoculture (Willey and Osiru, 1972). The baseline for LER is one. If the LER is greater than one intercropping favours growth and yield of plants, and when it is lower than one intercropping negatively affect the growth and yield of plants. The LER was calculated as

$LER = LER_{FM} + LER_{PP}$

$LER_{FM} = \left(\frac{Y_{FM,PP}}{Y_{FM}}\right)$, $LER_{PP} = \left(\frac{Y_{PP,FM}}{Y_{PP}}\right)$

Where Y_{FM} and Y_{PP} are yield of PP and FM in its monoculture, $Y_{FM,PP}$ is yield of finger millet in intercropping, and $Y_{PP,FM}$ is yield of pigeon pea in intercropping.

Measurement of physiological parameters

One main the goal of this study was to test if different spatial arrangements of FM and PP, and the application of biofertilizers affect the water relations and growth of FM. We therefore determined FM leaf water potential at predawn (04:00 to 05:00) and mid-day (12:30 to 13:30) towards the end of the field trial during first three weeks on November during 2016-17 and 2017-18. For logistical reasons, these measurements were only performed at the GKVK site. Both experimental sites received significant amount of rain till mid of October that's why a dry period during November was chosen for measurement (Fig. 1). Leaf water potential was measured using a pressure chamber (model 1000, Pressure Chamber Instrument Company, USA). For predawn measurements, leaf samples were collected between 04:00 and 05:00 hours and for midday measurements, leaves were sampled between 12:30 and 13:30 hours. After sampling leaves were packed into airtight Ziploc bags to avoid water loss, bags were kept in the dark and leaf water potential was measured within 1 – 2 hours after sampling.

Statistical analysis

Analysis of yield data and LWP from field trials was carried out using GraphPad Prism software (version 7.0 for Mac OS X, GraphPad Software, La Jolla California USA). Data are expressed as mean ± standard error of mean (SEM). Tukey`s test was used for post hoc multiple treatment comparison following one-way ANOVA or multifactor ANOVA using general linear model. The criterion for significance was $p<0.05$

Results

Total biomass, straw and grain yield per hectare and LER

Intercropping and the spatial arrangement of the intercropping partners had a significant effect on the total biomass yield per hectare at the GKVK site in 2016/17 (Fig. 3, Table 2). In particular the treatment T3+ produced significantly more biomass per hectare than monocultures of the constitutive crops or other spatial arrangements at GKVK in 2016/17. Likewise, treatment T3+ resulted in higher yields for straw and grain as compared to the other treatments in 2016/17 at GKVK. For the intercropping treatments total biomass yield, straw yield and grain yield all declined from the T3+ to T6+. The results differed at the Kolli Hills site, where in 2016/17 PP (T2+) produced the highest yields for total biomass, straw and grain and where FM (T1+) and the different intercropping treatments produced lower yields with no significant differences among each other (Table 2). In summary this suggests that in 2016/17 we found a strong positive intercropping effect for total biomass yield, straw yield and grain yield at GKVK, where the intercropping effect were strongest in the 8:2 row-wise spacing. In contrast, no yield improvements by intercropping irrespective of the spatial arrangement were observed at the Kolli Hills site.

These observations are also reflected in LER values at GKVK, where values for total biomass were greater than one for T3+, T4+ and T5 and where T3+ had the highest LER value. Similarly for straw biomass, T3+ had higher LER values than T4+, T5+ and T6+. For gain biomass LER values were greater than one for the T3+ and T4+ treatment, equal to one for T5+ and less than one for T6+. At Kolli Hills LER values for all treatments were less than one (Fig. 4).

In 2017/18, intercropping and the spatial arrangement of the intercropping partners also had a strong and significant effect on the total biomass yield, straw yield and grain yield at GKVK (Fig. 5). As in 2016/17 the treatment T3- and T3+ produced significantly more biomass per hectare than monocultures of the constitutive crops or other spatial arrangements when compared to the respective treatments with and without biofertilizer. Importantly, the application of biofertilizers enhanced the total biomass yield, straw yield and grain yield in all treatments and this effect was consistent irrespective of experiment site, mono or intercropping (Table 3). At Kolli Hills, we also found significant treatment

effects (Fig. 3). However, intercropping treatments did not produce higher yields for total biomass and straw than any of the other treatments with or without biofertilizer. Yet, treatment T5+ was equal in total biomass yield than the most productive monoculture (T3+). For grain yield FM monoculture exceeded the productivity of PP (Fig. 5f) and in intercropping T3-, T3+ and T5+ grain yield was similar to monoculture of FM with or without biofertilizer. The effects of biofertilizers on total biomass yield, straw yield and grain yield that we detected at the GKVK site were also observed at the Kolli Hills site and this effect was again consistent across all treatments. We did not find a significant interaction between treatment and biofertilizers nor a significant three way interaction between treatment, biofertilizers, and site. However, as indicated above the effects of biofertilizers at Kolli Hills resulted in total biomass yield, straw yield and grain yield that were of the same magnitude in some intercropping treatments as the highest yield in the corresponding monocultures (e.g. T5+ for total biomass yield, and straw yield, and T3+ and T5+ for grain yield) (Fig. 5). In summary this suggests that in 2017/18 we found a strong positive intercropping effect for total biomass yield, straw yield and grain yield at GKVK. In Kolli Hills, no such intercropping effect was found. Interestingly, however, biofertilizers improved the yields of crops in both sites and independently of treatment. Despite the nonsignificant biofertilization – treatment interaction, intercropping treatments at Kolli Hills showed a trend to be more enhanced through biofertilizers than monocultures to an extent that they produced similar yields that the most productive monoculture, which we did not observe without biofertilizers.

These observations were also confirmed by LER values for 2017/18 at both sites (Fig. 6). LER was greater than one at GKVK for all treatments. Also, LER values at GKVK were largest for T3+ and declined in the other treatments. Interestingly, biofertilizers had a negative effect on LER values in all spatial arrangements at GKVK. At Kolli Hills, LER values in treatments without biofertilizers were either equal to or less than one. Biofertilizers increased, however, the LER values in all spatial arrangements to values of one or greater than one and the largest values were observed for T3+ and T5+.

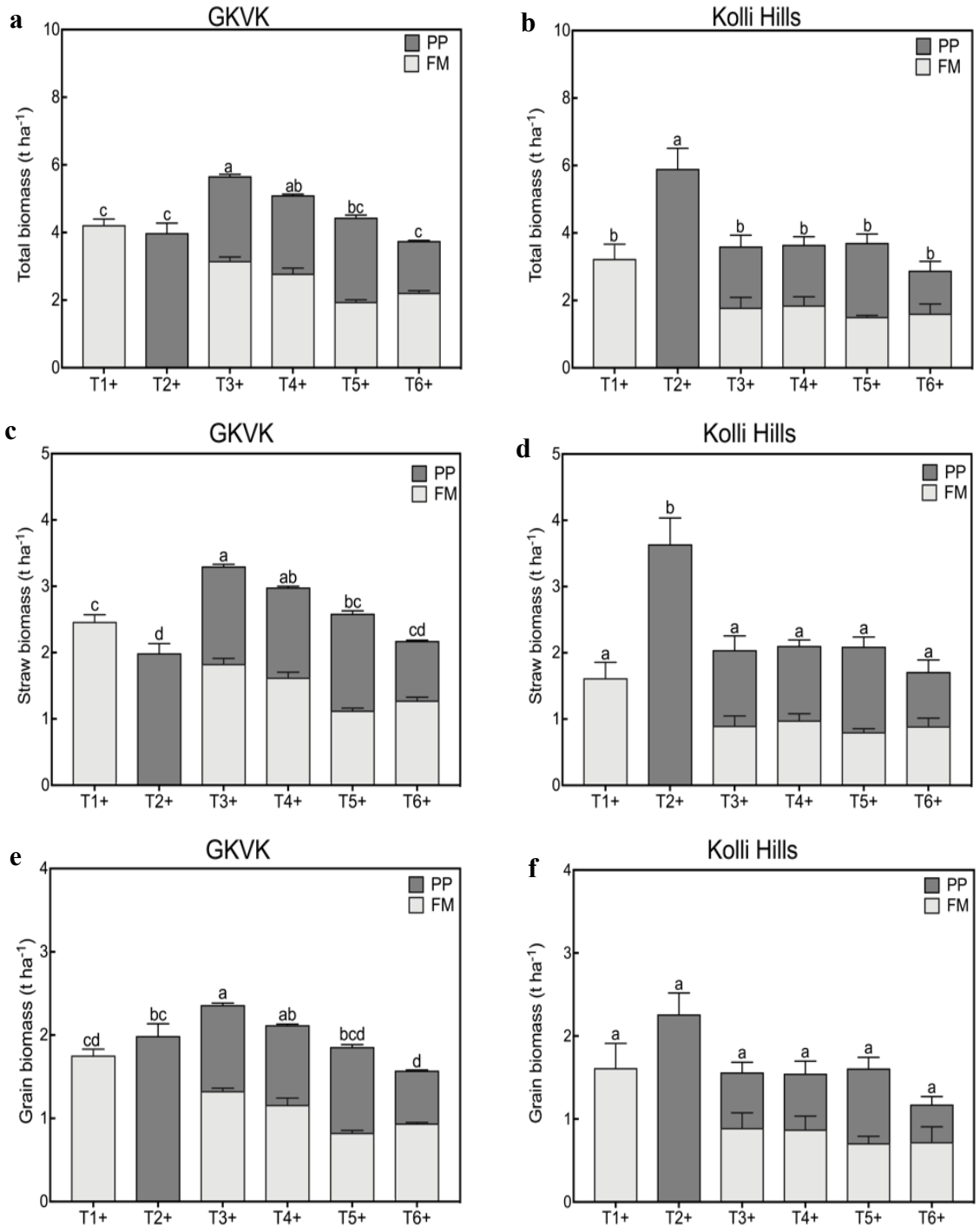


Fig. 3 Total biomass, straw and grain biomass at GKVK (Fig. 3a, 3c & 3e) and Kolli Hills (Fig. 3b, 3d & 3f) during year 2016/17. Bars represent average of four replicate with standard error of mean. One-Way ANOVA followed by Tukey`s test (posthoc test) was used for multiple comparison, separately for each site, and values with same letters are not significantly different from each other at $p>0.05$.

Table 2 ANOVA table to compare effect of experiment site and treatments, using total biomass, straw and grain biomass from GKVK and Kolli Hills field trial from 2016/17. Total biomass includes straw and grain biomass of PP and FM. While, straw biomass represent total straw biomass of PP and FM combined. Similarly, grain biomass represent total grain biomass of PP and FM combined.

Total biomass	**DF**	**SS**	**MS**	**F-value**	**P-value**
Site	1	5.8590	5.85902	11.38	0.0018
Treatments	5	14.3254	2.86508	5.57	0.0007
Site*Treatment	5	18.7519	3.75037	7.29	<0.0001
Error	36	18.5302	0.51473		
Total	47	57.4665			
Straw biomass	**DF**	**SS**	**MS**	**F-value**	**P-value**
Site	1	1.7442	1.74422	10.24	0.0029
Treatments	5	4.8492	0.96984	5.69	0.0006
Site*Treatment	5	10.7644	2.15288	12.64	<0.0001
Error	36	6.1321	0.17034		
Total	47	23.4899			
Grain biomass	**DF**	**SS**	**MS**	**F-value**	**P-value**
Site	1	1.1970	1.19701	8.51	0.0061
Treatments	5	2.6461	0.52923	3.76	0.0077
Site*Treatment	5	1.3798	0.27595	1.96	0.1083
Error	36	5.0664	0.14073		
Total	47	10.2893			

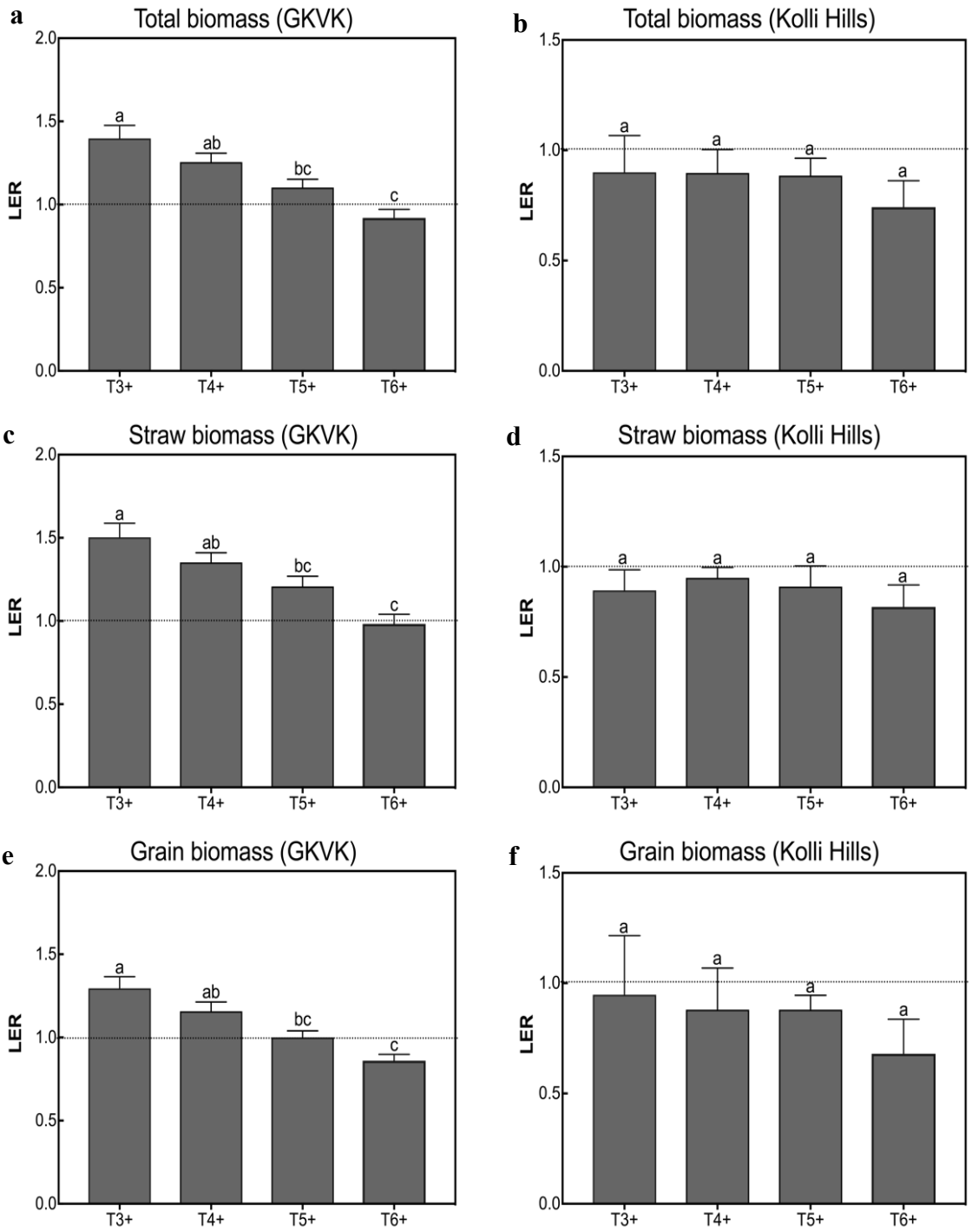


Fig. 4 Land equivalent ratio (LER) in different intercropping treatments during 2016/17 at GKVK (Fig. 4a, 4c & 4e) and Kolli Hills (Fig. 4b, 4d & 4f) site. Bars represent average of four replicate with standard error of mean. Tukey`s test (one-way ANOVA) was used for multiple comparison, separately for each site, and values with same letters are not significantly from each other different at $p>0.05$.

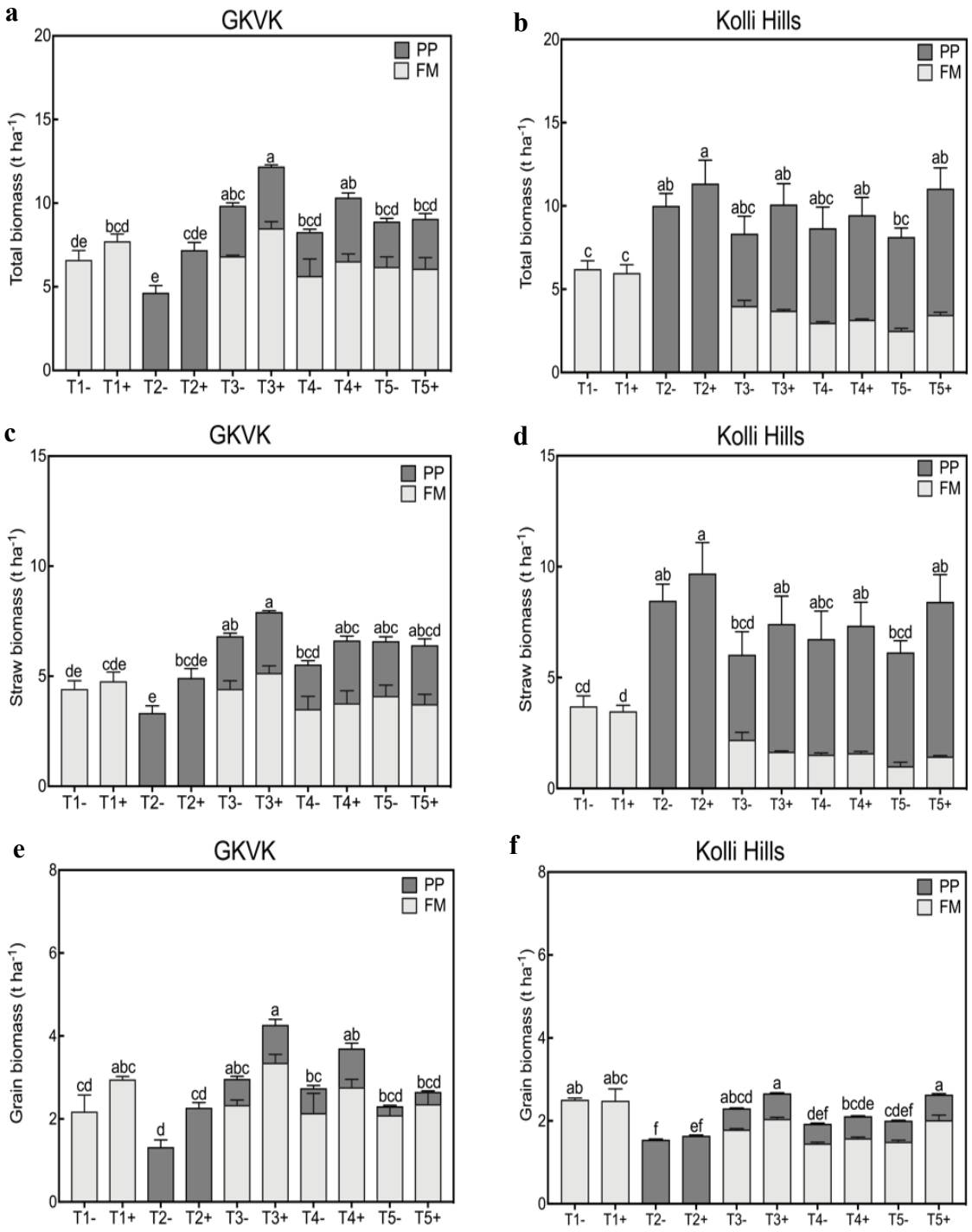


Fig. 5 Total biomass, straw and grain biomass at GKVK (Fig. 5a, 5c & 5e) and Kolli Hills (Fig. 5b, 5d & 5f) during year 2017/18. Bars represent average of four replicate with standard error of mean. One-way ANOVA followed by Tukey`s test (posthoc test) was used for multiple comparison, separately for each site, and values with same letters are not significantly different from each other at $p>0.05$.

Table 3 ANOVA table to compare effect of experiment site and treatments, using total biomass, straw and grain biomass from GKVK and Kolli Hills field trials data from 2017/18. Total biomass includes straw and grain biomass of PP and FM. While, straw biomass represent total straw biomass of PP and FM combined. Similarly, grain biomass represent total grain biomass of PP and FM combined.

Total biomass	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Biofertilization	1	43.471	43.4713	14.64	0.000
Treatments	4	112.438	28.1095	9.47	0.000
Site	1	4.002	4.0024	1.35	0.250
Biofertilization*Treatments	4	6.510	1.6274	0.55	0.701
Biofertilization*Site	1	0.572	0.5722	0.19	0.662
Treatments*Site	4	105.660	26.4150	8.90	0.000
Biofertilization*Treatments*Site	4	12.216	3.0541	1.03	0.400
Error	60	178.172	2.9695		
Total	79	463.042			
Straw biomass	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Biofertilization	1	16.736	16.7358	6.43	0.014
Treatment	4	94.892	23.7231	9.12	0.000
Site	1	19.999	19.9991	7.69	0.007
Biofertilization*Treatment	4	4.349	1.0872	0.42	0.795
Biofertilization*Site	1	0.383	0.3826	0.15	0.703
Treatment*Site	4	90.122	22.5304	8.66	0.000
Biofertilization*Treatment*Site	4	6.498	1.6246	0.62	0.647
Error	60	156.119	2.6020		
Total	79	389.098			
Grain biomass	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Biofertilization	1	6.2617	6.2617	33.13	0.000
Treatment	4	15.4984	3.8746	20.50	0.000
Site	1	6.1080	6.1080	32.31	0.000
Biofertilization*Treatment	4	0.4671	0.1168	0.62	0.652
Biofertilization*Site	1	1.8906	1.8906	10.00	0.002
Treatment*Site	4	5.0820	1.2705	6.72	0.000
Biofertilization*Treatment*Site	4	1.0130	0.2533	1.34	0.266
Error	60	11.3415	0.1890		
Total	79	47.6623			

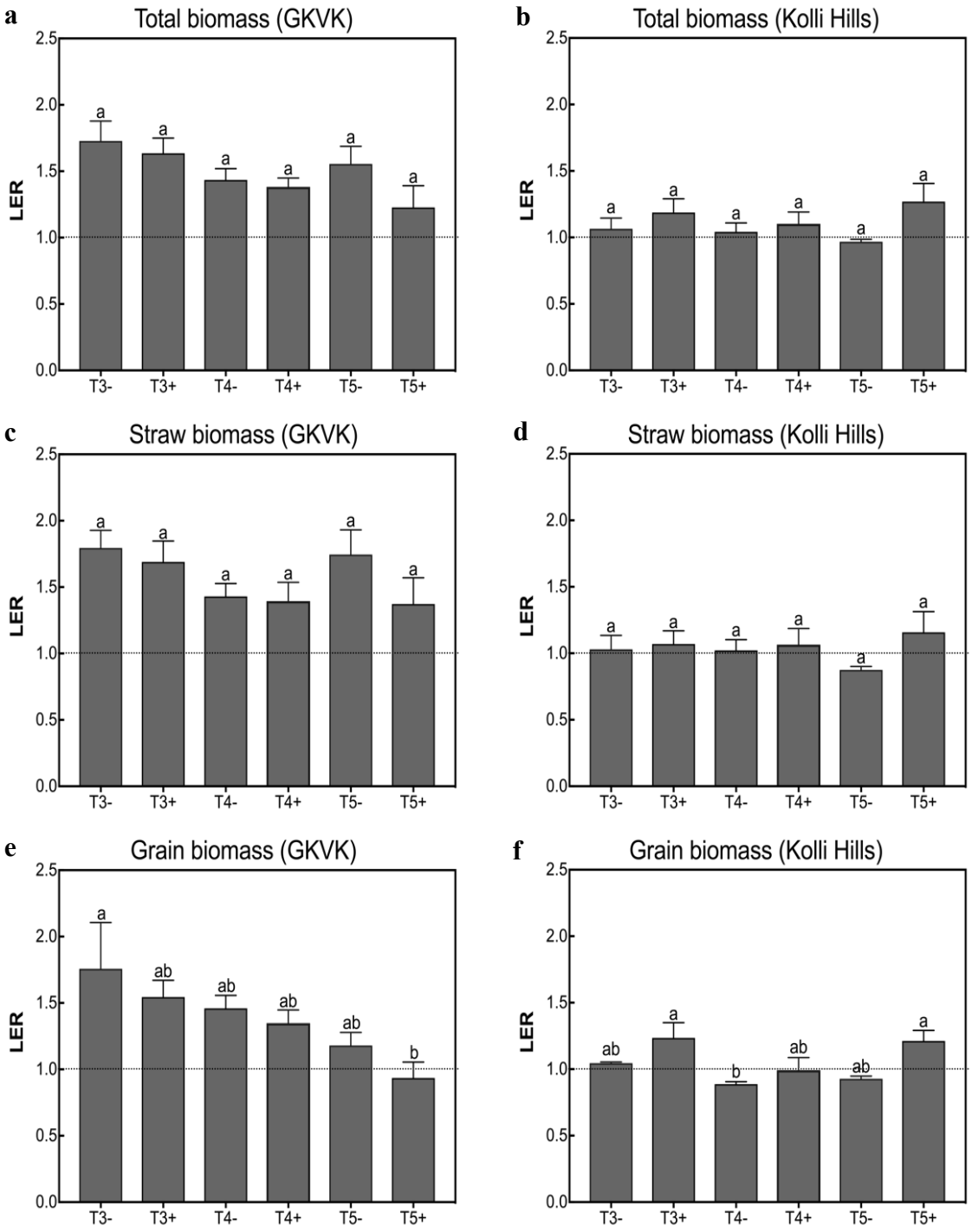


Fig. 6 Land equivalent ratio (LER) of total grain yield in different intercropping treatments during 2017-18 at GKVK (Fig. 6a, 6c & 6e) and Kolli Hills (Fig. 6b, 6d & 6f) site. Bars represent average of four replicate with standard error of mean. Tukey`s test (one-way ANOVA) was used for multiple comparison, separately for each site, and values with same letters are not significantly different from each other at $p>0.05$.

Per plant biomass yield of PP and FM

We found a significant effect of the intercropping treatments on total biomass per plant, total straw yield per plant and total grain yield per plant of PP and FM in GKVK but not in Kolli Hills in 2016/17 (Fig. 7, Table 4 & 5). At GKVK, total biomass per plant in FM was highest in the monoculture (T1+), the 2:8 treatment (T3+) and the 1:4 treatment (T4+). The biomass of the individual plants was significantly reduced in the mosaic treatments (T5+ and T6+) compared to monoculture (T1+) and row-wise intercropping (T3+ and T4+, Fig. 8a). PP showed highest total biomass in the mosaic treatment T6+, followed by other intercropping treatments and lowest biomass in the monoculture T2+ (Fig. 7c). At Kolli Hills, total biomass per plant in PP and FM did not differ significantly among treatments (Fig. 7b &7d). However, the trend was similar to GKVK site where FM showed reduction in biomass in mosaic treatments while PP showed increase in biomass in mosaic treatments.

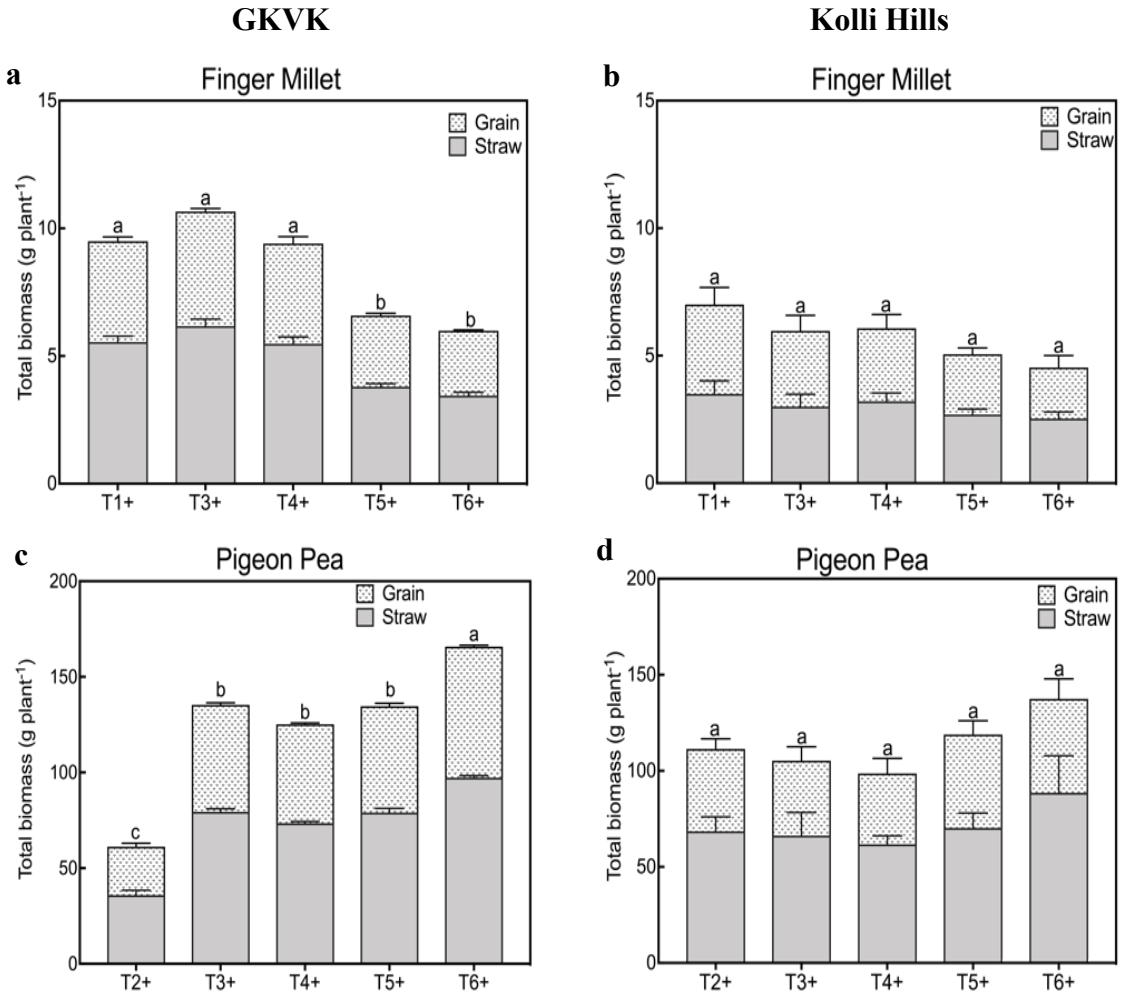


Fig 7 Total biomass per plant of FM and PP at GKVK site (Fig. 7a & 7c) and Kolli Hills site (Fig. 7b & 7d) during 2016/17 field trial. Bars represent average of four replicate with

standard error of mean. One-way ANOVA followed by Tukey`s test (posthoc test) was used for multiple comparison, separately for each site, and values with same letters are not significantly different from each other at $p>0.05$.

Table 4 ANOVA table for total, straw and grain biomass of FM per plant during 2016/17 field trial. Total biomass includes both straw and grain biomass of FM.

Total biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	72.819	72.8190	47.46	<0.0001
Treatment	4	65.741	16.4353	10.71	<0.0001
Site*Treatment	4	14.598	3.6495	2.38	0.0740
Error	30	46.031	1.5344		
Total	39	199.189			
Straw biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	36.1190	36.1190	93.12	<0.0001
Treatment	4	18.4602	4.6151	11.90	<0.0001
Site*Treatment	4	6.7410	1.6853	4.34	0.0069
Error	30	11.6364	0.3879		
Total	39	72.9566			
Grain biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	6.3680	6.36804	10.46	0.0030
Treatment	4	14.6037	3.65094	6.00	0.0011
Site*Treatment	4	1.8169	0.45421	0.75	0.5685
Error	30	18.2694	0.60898		
Total	39	41.0580			

Table 5 ANOVA table for total, straw and grain biomass of PP per plant during 2016/17 field trial. Total biomass includes both straw and grain biomass of PP.

Total biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	1019.1	1019.09	1.43	0.2409
Treatment	4	17964.4	4491.11	6.31	0.0008
Site*Treatment	4	9337.3	2334.32	3.28	0.0241
Error	30	21354.1	711.80		
Total	39	49674.9			
Straw biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	37.5	37.54	0.14	0.7123
Treatment	4	6844.3	1711.09	6.32	0.0008
Site*Treatment	4	3019.8	754.96	2.79	0.0443
Error	30	8128.2	270.94		
Total	39	18030.0			
Grain biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	665.45	665.448	5.38	0.0274
Treatment	4	2698.58	674.646	5.46	0.0020
Site*Treatment	4	1826.69	456.673	3.69	0.0147
Error	30	3710.10	123.670		
Total	39	8900.82			

In 2017/18 we also found a significant treatment effect on the total biomass, straw yield and grain yield of FM and PP at GKVK but only for PP at Kolli Hills (Fig. 8, Tables 6 & 7). At GKVK, total biomass of FM plants in T3+ was significantly larger than total biomass of plants in treatments T1-, T1+ and T4-. Total biomass of PP plants were largest in T3+ and T5+ compared to T2-, T2+, and T4-. At Kolli Hills total biomass per plant in FM did not show any significant difference among intercropping and monoculture. For PP, in contrast, total biomass per plant was largest in treatments T4+ and T5+ compared to T2- and T2+ (Fig. 8d).

A two-way ANOVA analysis was performed to find out the effect on per plant yield due to spatial arrangement and biofertilization (Table 8 & 9). At both sites, in 2017/18 field trial,

FM yield did not show any significant effect of biofertilizer application. However, PP showed a strong significant effect of biofertilization at GKVK, and at Kolli Hills site effect was slightly significant. At both site, effect of biofertilization did not differ among treatments due to spatial arrangement of component plants in intercropping system.

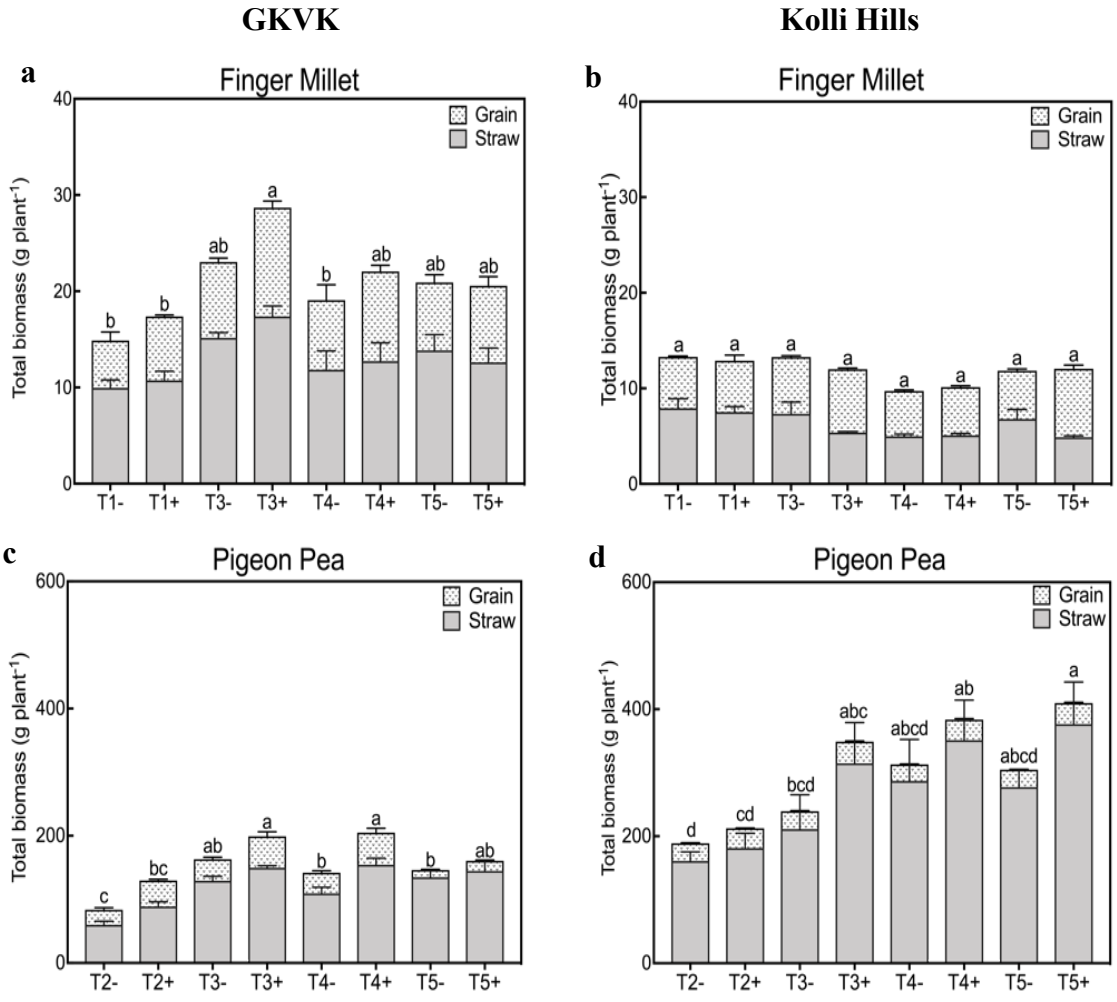


Fig. 8 Total biomass per plant of FM and PP at GKVK site (Fig. 8a & 8c) and Kolli Hills site (Fig. 8b & 8d) during 2017/18 field trial. Bars represent average of four replicate with standard error of mean. One-way ANOVA followed by Tukey`s test (posthoc test) was used for multiple comparison, separately for each site, and values with same letters are not significantly different from each other at $p>0.05$.

Table 6 ANOVA table for total, straw and grain biomass of FM per plant during 2017/18 field trial. Total biomass includes both straw and grain biomass of FM.

Total biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	1285.67	1285.67	160.25	0.000
Treatment	3	202.91	67.64	8.43	0.000
Biofertilization	1	21.91	21.91	2.73	0.105
Site*Treatment	3	224.50	74.83	9.33	0.000
Site*Biofertilization	1	37.26	37.26	4.64	0.036
Treatment*Biofertilization	3	13.34	4.45	0.55	0.648
Site*Treatment*Biofertilization	3	25.66	8.55	1.07	0.372
Error	48	385.10	8.02		
Total	63	2196.35			
Straw biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	740.38	740.384	151.85	0.000
Treatment	3	66.13	22.044	4.52	0.007
Biofertilization	1	0.63	0.628	0.13	0.721
Site*Treatment	3	110.34	36.779	7.54	0.000
Site*Biofertilization	1	11.68	11.679	2.40	0.128
Treatment*Biofertilization	3	10.62	3.540	0.73	0.541
Site*Treatment*Biofertilization	3	8.35	2.782	0.57	0.637
Error	48	234.03	4.876		
Total	63	1182.16			
Grain biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	74.758	74.758	45.08	0.000
Treatment	3	45.212	15.071	9.09	0.000
Biofertilization	1	29.962	29.962	18.07	0.000
Site*Treatment	3	25.016	8.339	5.03	0.004
Site*Biofertilization	1	7.216	7.216	4.35	0.042
Treatment*Biofertilization	3	3.063	1.021	0.62	0.608
Site*Treatment*Biofertilization	3	7.102	2.367	1.43	0.246
Error	48	79.608	1.658		
Total	63	271.936			

Table 7 ANOVA table for total, straw and grain biomass of PP per plant during 2017/18 field trial. Total biomass includes both straw and grain biomass of PP.

Total biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	343257	343257	61.85	0.000
Treatment	3	119086	39695	7.15	0.000
Biofertilization	1	54798	54798	9.87	0.003
Site*Treatment	3	31971	10657	1.92	0.139
Site*Biofertilization	1	5584	5584	1.01	0.321
Treatment*Biofertilization	3	3371	1124	0.20	0.894
Site*Treatment*Biofertilization	3	8617	2872	0.52	0.672
Error	48	266379	5550		
Total	63	833063			
Straw biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	353809	353809	64.35	0.000
Treatment	3	122601	40867	7.43	0.000
Biofertilization	1	38317	38317	6.97	0.011
Site*Treatment	3	25252	8417	1.53	0.219
Site*Biofertilization	1	8419	8419	1.53	0.222
Treatment*Biofertilization	3	3302	1101	0.20	0.896
Site*Treatment*Biofertilization	3	6893	2298	0.42	0.741
Error	48	263920	5498		
Total	63	822513			
Grain biomass per plant	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Site	1	79.88	79.88	2.23	0.142
Treatment	3	2059.46	686.49	19.18	0.000
Biofertilization	1	1470.15	1470.15	41.07	0.000
Site*Treatment	3	2105.29	701.76	19.61	0.000
Site*Biofertilization	1	289.85	289.85	8.10	0.006
Treatment*Biofertilization	3	116.45	38.82	1.08	0.365
Site*Treatment*Biofertilization	3	142.42	47.47	1.33	0.277
Error	48	1718.03	35.79		
Total	63	7981.52			

Table 8 ANOVA table of total biomass per plant of FM and PP at GKVK Bengaluru, India, in 2017/18 field trial.

Total biomass per plant of FM	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Biofertilization	1	45.678	45.6780	2.81	0.1109
Spatial Arrangement	2	145.371	72.6854	4.47	0.0265
Biofertilization*Spatial Arrangement	2	36.050	18.0249	1.11	0.3513
Error	18	292.494	16.2497		
Total	23	519.593			
Total biomass per plant of PP	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Biofertilization	1	8596.1	8596.11	16.27	0.0008
Spatial Arrangement	2	3292.8	1646.42	3.12	0.0688
Biofertilization*Spatial Arrangement	2	2391.0	1195.49	2.26	0.1328
Error	18	9508.2	528.23		
Total	23	23788.1			

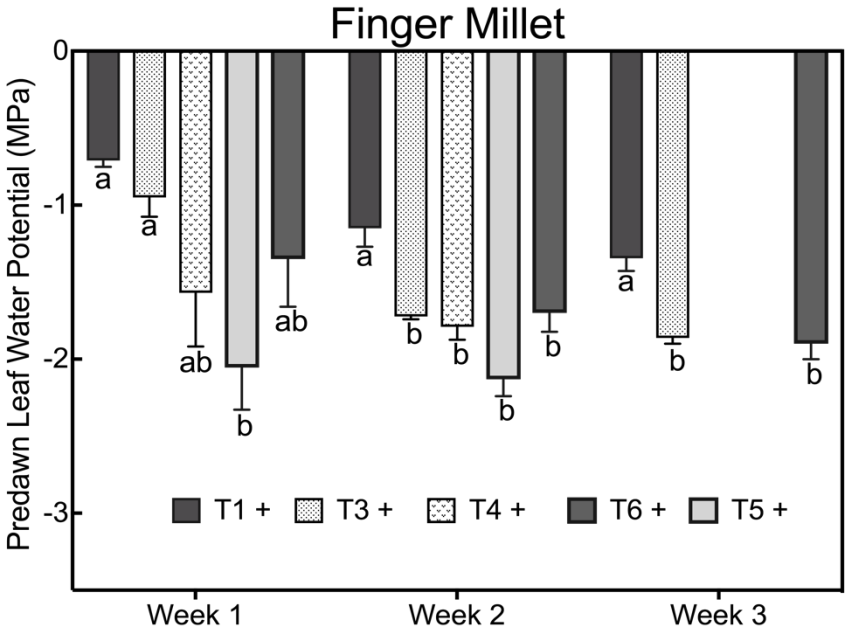
Table 9 ANOVA table of total biomass per plant of FM and PP at Kolli Hills, India, in 2017/18 field trial.

Total biomass per plant of FM	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Biofertilization	1	0.2993	0.2993	0.15	0.7017
Spatial Arrangement	2	31.6316	15.8158	8.00	0.0033
Biofertilization*Spatial Arrangement	2	3.3286	1.6643	0.84	0.4471
Error	18	35.5713	1.9762		
Total	23	70.8307			
Total biomass per plant of PP	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Biofertilization	1	54247	54246.9	3.97	0.0618
Spatial Arrangement	2	18668	9333.8	0.68	0.5179
Biofertilization*Spatial Arrangement	2	1829	914.4	0.07	0.9355
Error	18	246126	13673.7		
Total	23	320869			

Water relations of PP and FM in intercropping treatments

Measurement of predawn leaf water potential (LWP) at GKVK, showed the effect of spatial arrangement on the water relations of FM in different intercropping treatments (Fig. 9, Table 10). In 2016/17 at week 1 (1st week of November 2016) of measurement, FM in the mosaic treatment T5+ had the lowest predawn LWP of -2.5 MPa which is significantly lower than the predawn LWP of -0.70 MPa in monoculture (T1) and the row-wise intercropping treatment (T3+, -0.95 MPa). At week 2 (2nd week of November 2016), FM in monoculture (T1+) maintained significantly higher predawn LWP of -1.15 MPa than other intercropping treatments (Fig. 9a). At week 3, (3rd week of November 2016) FM in treatments T4+ and T5+ were dead (desiccated & drooped), while FM in T3+ and T6+ showed a significantly lower LWP of -1.89 and -1.90 MPa than FM in monoculture with -1.34 MPa. Predawn LWP data showed that FM in monoculture maintained higher LWP during the measurement phase than FM in all intercropping treatments.

a



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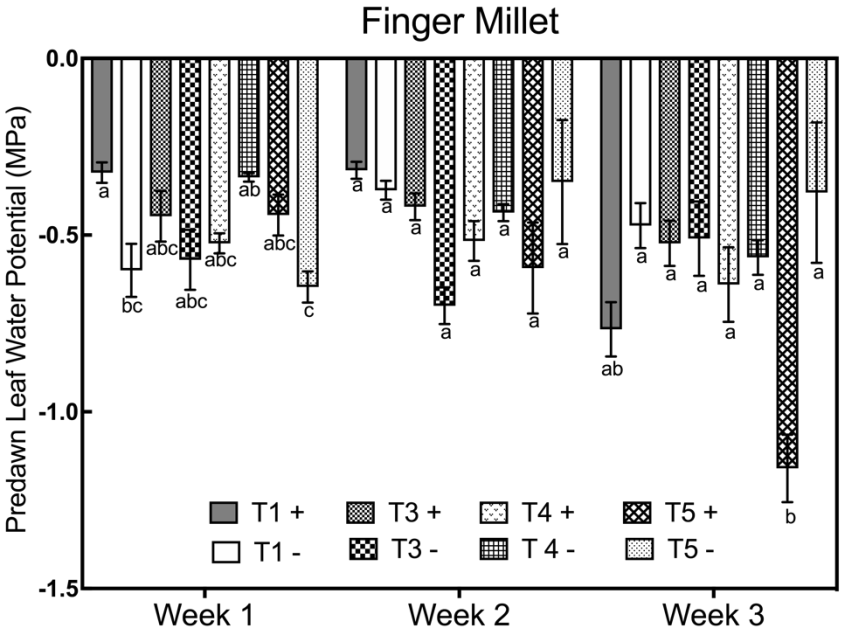

Fig. 9 Predawn leaf water potential of FM in different intercropping treatments during 2016/17 (Fig. 9a) and 2017-18 (Fig. 9b) field trial. Weeks represent first, second and third week of November in 2016 and 2017, during which measurement was done. Bars represent average of four replicate with standard error of mean. Tukey`s test (One-way ANOVA) was used for multiple comparison and values with same letters are not significantly different from each other at $p>0.05$.

In 2017/18, predawn LWP of FM in monoculture with biofertilizer (T1+) had LWP of -0.32 MPa which is significantly higher than monoculture without biofertilizer (T1-) with -0.60 MPa LWP (Fig. 9b) at week 1 (1st week of November 2017). At later weeks, FM did not show any significant difference among intercropping treatments, but treatments without biofertilizer showed a deceasing trend in LWP as compared to its respective treatment with biofertilizer. The biofertilizer application did not have a significant effect on LWP of FM, but intercropping treatments showed a strong significant effect (Table 10). Effect of biofertilizer showed significant interaction with intercropping treatments, as we observed in Fig. 9, treatments T1-, T1+, T5- and T5+ consistently showed a large difference in LWP of FM with or without biofertilizer.

Table 10 ANOVA table for predawn leaf water potential of FM in 2016/17 and 2017/18 at GKVK site. Multifactor ANOVA analysis was performed to find out the effect of different factors and its interaction on water-relations of FM.

Predawn leaf water potential (2016/17)	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Treatment	4	3.7650	0.941252	7.52	0.0002
Time	2	1.5043	0.752163	6.01	0.0057
Treatment*Time	8	1.4607	0.182585	1.46	0.2075
Error	35	4.3793	0.125123		
Total	49	13.0806			
Predawn leaf water potential (2017/18)	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Treatment	3	0.31029	0.10343	7.77	0.000
Biofertilizer	1	0.01628	0.01628	1.22	0.274
Time	2	0.41462	0.20731	15.58	0.000
Treatment*Biofertilizer	3	0.21259	0.07086	5.33	0.003
Treatment*Time	6	0.27380	0.04563	3.43	0.007
Biofertilizer*Time	2	0.39849	0.19924	14.97	0.000
Treatment*Biofertilizer*Time	6	0.37823	0.06304	4.74	0.001
Error	46	0.61205	0.01331		
Total	69	2.73278			

Discussion

The results obtained from the field trials during 2016/17 and 2017/18 showed that intercropping can improve the straw and grain yield in PP–FM intercropping compared to the respective monocultures but that intercropping effects vary depending on the site characteristic such as climate and soil type. Spatial arrangement of component plants affected the total, straw and grain biomass in intercropping treatments but this effect also varied across site. The results from 2017/18 clearly demonstrated a positive effect of biofertilizer on biomass gain and this effect was irrespective of site, spatial arrangement, mixed or monoculture. Yet, biofertilizers at Kolli Hills enhanced the 2017/18 yields in the intercropping treatments to the same levels as the constitutive monocultures. Despite the positive effect of intercropping and biofertilization on FM and PP yield, water-relations of FM were not enhanced in the intercropping treatments or by biofertilizers. Most likely this is due to interspecific competition for soil moisture in topsoil layer between PP and FM. On the basis of these results, we could remark that spatial arrangement of component crops is a key factor that affect the productivity in intercropping, and application of biofertilizer enhances the yield of intercropping system. However, facilitative effect of bioirrigation and its facilitation through CMN may not be significant due to interspecific competition for soil resource (such as water) between intercropping partners.

Is PP – FM intercropping beneficial over monocropping?

At one of our research sites, the intercropping treatments (T3+ and T4+) produced higher yields (Fig. 3 & 5) than monoculture, but the response depends on the cultivation season (climatic conditions) and soil type at experiment site. This is illustrated in Kolli Hills, where in 2016/17 there was no significant effect of intercropping, but 2017/18 resulted into a strongly significant intercropping effect. The total rainfall in year 2017 was 1690 mm, while 2016 faced a severe drought with total rainfall of 281.7 mm. These results indicate that low amount of rainfall could be the factor due to which intercropping effect was not significant in 2016/17. In addition, PP plant variety used at Kolli Hills in 2016/17 and 2017/18 was different. At Kolli Hills site in 2016/17 field trial there was no significant effect of intercropping on total yield when PP variety Vamban-3 was used (Fig. 3b). While, in 2017/18, PP variety SA-1 produced a significant effect of intercropping on total yield (Fig. 5b). At GKVK site, intercropping treatments T3+ and T4+ produced 1.42 and 1.28

times higher, respectively, than monoculture of PP, and in 2017/18 field trial the yield in T3+ and T4+ were 1.69 and 1.43 times higher than monoculture of P, respectively. Furthermore, LER values clearly demonstrate that PP – FM intercropping was beneficial over monocropping. As at GKVK site, row-wise intercropping was more advantageous than mosaic. While a Kolli Hills, in 2017/18, row-wise (T3+) and mosaic treatment (T5+) showed similar LER and yield per hectare.

The yield advantage in intercropping system is typically assigned to resource sharing and facilitation (Huston, 1997; Loreau and Hector, 2001). The facilitation occurs through increased availability of soil resources such as water, nutrients (Zhang et al., 2010). Intercropping systems with legume (such as PP in this study) can increase agricultural productivity through providing increased nitrogen availability through N_2 fixation. Legumes are key factor in intercropping system, and it is one of the most frequently used intercrop species (Altieri et al., 2012; Hauggaard-Nielsen and Jensen, 2005). Nonlegumes (such as cereals) in an intercropping system with legume plants obtains additional N released by legume into soil, and legumes can contribute up to 15% of the N in intercropped cereal (Long Li et al., 2007; Zuo et al., 2004).

Effect of spatial arrangement on yield in PP - FM intercropping

The effect of spatial arrangement is well reflected on total straw and grain yield as well (Fig. 3 & 5). At GKVK site, straw and grain yield (per hectare) showed similar trend where row-wise intercropping treatments produced higher yield than mosaic during 2016/17 and 2017/18 field trial. The row-wise intercropping has been consistently advantageous at GKVK site, but the results from Kolli Hills site differed in its response upon changing the PP variety in 2017/18 field trial. At Kolli Hills site, spatial arrangement did not show any significant effect on total straw and grain yield in 2016/17, but in 2017/18 field trial, both row-wise treatments (T3+ and T4+) and mosaic treatment (T5+) produced higher straw yield and total yield than FM monoculture (T1- and T1+) (Fig. 5d). This study clearly demonstrated that PP–FM intercropping gives advantage of yield increase over monoculture of FM and PP. Spatial arrangement of component plants in an intercropping system is a crucial factor that affects the overall yield and competitiveness (both intra and inter specific) of an intercropping system.

Effects of spatial arrangement can be explained by intra- and interspecific competition as illustrated in per plant biomass (Fig. 7 & 8), however, the results we obtained showed high variability at two different experimental sites (GKVK and Kolli Hills). Results from GKVK site clearly indicate that PP profits a lot, in terms of per plant biomass, in intercropping treatments and it is mainly due to reduction in intra-specific competition that PP faces in monoculture. In contrast, FM faces higher inter-specific competition in mosaic treatments that leads to reduction in per plant biomass in mosaic treatments (T5+ and T6+). The field trial results from Kolli Hills, however, did not show any significant effect of spatial arrangement of plants on per plant biomass in PP and FM during 2016/17 trial (Fig. 7b & 7d). While, during 2017/18, only PP showed a significant increase in per plant biomass in intercropping treatments T4+ and T5+ than monoculture treatments T2- and T2+. The change in PP response at Kolli Hills during 2017-18 trial could mainly be attributed to change in variety. The effect of spatial arrangement on FM per plant biomass was not significant and it was consistent during both years at Kolli Hills.

The results of this study has shown consistently that PP growth is favoured in intercropping systems due to reduction in intra-specific competition, while FM faces higher inter-specific competition in mosaic intercropping than row-wise intercropping pattern which is again subjected to variety of intercropped PP, soil quality and local weather. There are several factors, such as light, soil moisture and nutrient, that affects the yield of each component crop in intercropping (Härdter and Horst, 1991; Jahansooz et al., 2007). The difference in penetration of light into canopy is considered to be a key factor affecting the photosynthesis and ultimately the growth that results into yield (Gwathmey and Clement, 2010; Kaggwa-Asiimwe et al., 2013). In our study, the reduction in light availability to short FM plants standing next to taller PP (see supplementary data) plant in mosaic intercropping treatments (T5 & T6) could be a factor impacting the growth, since in all row-wise intercropping designs PP and FM rows are well spaced to avoid lighting effect, which is not the case in mosaic design. Similar results were reported by Martin and Snaydon (1982) and Dubey et al. (1995), who reported highest yield for barley/beans and sorghum/soybean in row-wise intercropping than mosaic (mixed within rows), respectively.

Furthermore, for intercropping designs tested in this study row-wise intercropping treatment T3+ (2:8 with biofertilizer) showed a consistent positive performance over others, due to release of intra-specific competition. In addition, 2:8 row-wise intercropping

offered enough space between the rows of PP to reduce the interspecific competition as well. The spatial arrangement of component plants in intercropping has shown to be species specific, e.g. Chen et al. (2004), Lauk and Lauk (2008) and Aynehband et al. (2010) showed that mixing of component plant within rows (mosaic pattern) to be the best arrangement for barley/peas, maize/soybean and maize/amaranth, respectively. In contrast, Martin and Snaydon (1982) and Dubey et al. 1(995) reported higher yields for barley/beans and sorghum/soybean sown in alternate rows than in mixed within rows, respectively. Interspecific competition could occur when two species are planted together, and such competition could lead to decrease in plant growth and yield (Jensen, 1996). In a cereal-legume intercropping system there is a significant number of days for overlapping growth period, and interspecific competition between component crops could lead to decrease in yield (Clément et al., 1992; Karasawa and Takebe, 2012; Oljaca et al., 2000), therefore, spatial arrangement between the plants needs to be carefully optimized.

Effects of biofertilizers

In 2017/18 field trial, at both experimental sites, effect of biofertilizer application was positive and showed increase in total yield (Fig. 8). The positive effect of biofertilization did not differ among intercropping treatments with different spatial arrangement (Table 8 & 9). The effect of biofertilization was specific to each component plants in PP–FM intercropping system. Total biomass and straw yield per plant in FM did not have any significant effect of biofertilizer (Table 6), while grain yield per plant showed a significant effect. In case of PP, effect of biofertilization was significant on total biomass, straw and grain yield per plant. In this study, a combination of AMF strains, PGPR, and rhizobia were applied as biofertilizer. Research studies suggest that PGPR have a strong influence on growth of AMF, and AMF prefers to colonize roots already colonized with N_2 fixing bacteria. AMF hyphae improve plants nutrient uptake by increasing the plant-soil contact area that leads to yield advantage (Vande Broek and Vanderleyden, 1995). In our study, significant effect of biofertilizer on PP could be assigned to better colonization by AMF and symbiosis with PGPR and rhizobia. AMF hyphae could grow into small soil pores not accessible by root hairs to enhance nutrient uptake, therefore, crop species (such as wheat, oat and millet etc.) with well-developed fine roots and abundant root hairs do not get significantly affected by AMF application (Graham and Ryan, 2002), as we observed in case of FM in this study, only grain yield showed a significant effect. The increase in grain

yield in both component plants (FM and PP) in intercropping was the result of an increased number of panicle and grain weight per panicle in FM and number of pod and pod weight per plant in PP (see supplementary data). Since, the process of pod and panicle formation is influenced by light availability, nutrients and soil moisture (Härdter and Horst, 1991), the mechanism for yield improvement in row-wise intercropping could be attributed to efficient utilization of nutrient through applied biofertilizer.

Effect of intercropping and biofertilizers on water relations of FM

In this study, the water relations (predawn LWP) of FM decreased significantly in mosaic treatments as compared to row-wise and monoculture treatments (Fig. 9a & 9b). At GKVK site during 2016/17 field trial, predawn LWP data shows that FM in monoculture maintains highest LWP for continuous three weeks, while FM in mosaic treatment T5 showed lowest LWP of -1.25 MPa at week 1, at week 2 all intercropping treatments showed significantly lower LWP, and at week 3 only FM in monoculture and T3+ and T5+ could survive (Fig. 9a). These results suggests that PP provided a strong competition to FM for soil moisture in mosaic treatments and 1:4 row-wise treatment T4+. The trend in predawn LWP can also be compared with the trend in per plant biomass production, therefore, competition for water could be the limiting factor here which influenced the yield and effectiveness of intercropping treatments at GKVK site. Our results suggest that there exists an important degree of below-ground competition for water between PP and FM, and the facilitative effect of bioirrigation is suppressed. Similar results have been reported by Ludwig et al. (2004) that HL performing trees extracted significant amount of water from topsoil layer that resulted in lower LWP in understorey grasses, however, grasses were able to absorb deep soil moisture released by tree.

A general hypothesis of intercropping is that the root systems of component crops occupy to some extent different soil layers that leads to complementary use of soil resources such as water (Schroth, 1998). However, few studies (Rao et al., 1997; Singh et al., 1989) have reported the limitation of different intercropping systems in semiarid tropics, where below ground competition for water overwhelmed the positive effect of intercropping. Ong et al. (1991) and Jose et al. (2004) have reported similar observations where competition for water decreased crop yields in semiarid tropics. As we observed in this study, the lower

LWP of FM in intercropping is mainly the result of a strong interspecific competition between PP and FM, where PP extracted significant amount of water from topsoil layer.

In this study, one of our objective was to find out if CMN can facilitate transfer of bioirrigated water from PP to FM and improve the water-relations of FM in intercropping treatments. The results from 2017/18 field trial showed that CMN did not affect the water relations (predawn LWP) of FM in intercropping treatments. However, at Week 1 and 2 (first and second week of November 2017) FM in T3+ had higher, but not significant, LWP than T3-. Similarly, FM in monoculture treatment showed a higher (less negative LWP) with CMN than without CMN (Fig. 9b). Since, we observe similar effect of CMN in both monoculture and 2:8 row-wise intercropping, we cannot assign bioirrigation factor here. The effect of CMN changed over time and at week 3 (third week of November 2017) treatments T1+, T3+, and T5+ with CMN had lower LWP than without CMN. The biofertilization effect change over time and showed significant interaction with different treatments (Table 10).

In this study, we could not find out if the positive intercropping effect by CMN was due to bioirrigation. The average hyphal spread rate of *Glomus* species is 0.7 – 0.8 mm per day (Jakobsen et al., 1992), Since, we did not check for spread of CMN between PP and FM, it is possible the AMF introduced through biofertilizer could not cover the distance of 45 cm between PP and FM in intercropping treatments and thus facilitative effect of bioirrigation through CMN was not observed.

Conclusions

In this study, we showed that intercropping has positive effect on total yield of PP and FM but this effect varies across site based on site characteristic such as soil type and weather. The extent of intra- and interspecific competition between PP and FM varies in row-wise and mosaic intercropping treatments. The effect of biofertilizer was positive on total yield of intercropping and monocropping, but increase in yield was not supported by CMN facilitated bioirrigation as we observed FM having lower LWP in intercropping treatments than in monoculture. In conclusion, the answers to our three research questions are as follows: (i) spatial arrangements of intercropping partners does affect the straw and grain yield in a FM – PP intercropping system, and optimal spatial arrangement for PP – FM

intercropping system depends on location (local weather conditions) and plant variety. In general, row-wise treatment (T3+) was more productive than mosaic treatments at GKVK site, while, at Kolli Hills site in 2017/18, both row-wise treatment (T3+) and mosaic treatment (T5+) performed equally. (ii) application of biofertilizer promotes yield in intercropping system, but spatial arrangement of component plants do not affect the effect of biofertilization. The effect of biofertilization mainly derives from PP, as only PP (per plant biomass) was significantly affected by the biofertilization. (iii) spatial arrangement of plants is a key factor that affect the competition for topsoil moisture between PP and FM, it this study, effect of CMN (biofertilizer) was positive on intercropping yield and it significantly influenced the PP yield. But, LWP of FM was significantly reduced in intercropping treatments. Therefore, we do not see a positive effect on water-relations of FM in an intercropping system.

Further research with different verities of PP, and different spatial arrangement including the planting distance between PP and FM would provide crucial information to design bioirrigation based intercropping model for rainfed areas in semiarid tropics. In order to establish a CMN facilitated bioirrigation based intercropping system, a deep investigation into the rhizosphere ecology of intercropping partner, and establishment of mycorrhizal network between component crop is required.

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Supplementary data of chapter 3

Table S1, Seed weight, plant height, number of panicle per plant, and grain weight per panicle has been shown for finger millet from field trial at GKVK during 2016-17.

Treatments (finger millet)	1000 seed wt. (g)	Plant height (m)	Panicle/plant	Grain wt./Panicle (g/panicle)
T1+	3.33 ± 0.19^{a}	0.71 ± 0.08^{a}	4.00 ± 0.00^{a}	13.00 ± 2.31^{a}
T3+	3.38 ± 0.13^{a}	0.72 ± 0.03^{a}	3.75 ± 0.50^{a}	13.00 ± 1.83^{a}
T4+	3.35 ± 0.22^{a}	0.67 ± 0.05^{ab}	3.25 ± 0.50^{ab}	8.25 ± 2.22^{b}
T5+	3.25 ± 0.20^{a}	0.62 ± 0.05^{b}	2.75 ± 0.96^{b}	7.75 ± 4.99^{b}
T6+	3.26 ± 0.38^{a}	0.62 ± 0.05^{b}	3.25 ± 0.50^{ab}	7.00 ± 1.41^{b}

Table S2, Seed weight, plant height, number of pods per plant, and pod weight per plant has been shown for pigeon pea from field trial at GKVK during 2016-17

Treatments (pigeon pea)	100 seed wt. (g)	Plant height (m)	Pod/plant	Pod wt./plant (g/plant)
T2+	9.65 ± 0.10^{b}	1.55 ± 0.01^{a}	100 ± 7.93^{b}	108.5 ± 5.32^{a}
T3+	9.65 ± 0.20^{b}	1.55 ± 0.12^{a}	117.75 ± 15.65^{a}	116.75 ± 6.55^{a}
T4+	9.70 ± 0.77^{b}	1.52 ± 0.11^{a}	112.5 ± 3.70^{ab}	120.25 ± 32.22^{a}
T5+	10.27 ± 0.64^{ab}	1.52 ± 0.05^{a}	119 ± 1.41^{a}	119.75 ± 13.15^{a}
T6+	10.52 ± 0.31^{a}	1.50 ± 0.05^{a}	117.5 ± 13.38^{a}	103 ± 2.16^{a}

Table S3, Seed weight, plant height, number of panicle per plant, and grain weight per panicle has been shown for finger millet from field trial at GKVK during 2017-18

Treatments (finger millet)	1000 seed wt. (g)	Plant height (m)	Panicle/plant	Grain wt./Panicle (g/panicle)
T1+	2.93 ± 0.15^{a}	0.98 ± 0.08^{a}	1	3.45 ± 0.42^{b}
T1-	2.90 ± 0.27^{a}	1.05 ± 0.05^{a}	1	2.97 ± 0.25^{b}
T3+	3.04 ± 0.11^{a}	1.04 ± 0.07^{a}	1	5.11 ± 1.29^{a}
T3-	2.83 ± 0.17^{a}	1.02 ± 0.05^{a}	1	3.44 ± 0.53^{b}
T4+	3.08 ± 0.10^{a}	1.02 ± 0.08^{a}	1	3.55 ± 0.35^{b}
T4-	2.93 ± 0.17^{a}	1.03 ± 0.01^{a}	1	3.28 ± 0.36^{b}

Table S4, Seed weight, plant height, number of pods per plant, and pod weight per plant has been shown for pigeon pea from field trial at GKVK during 2017-18

Treatments (pigeon pea)	100 seed wt. (g)	Plant height (m)	Pod/plant	Pod wt./Plant (g/plant)
T1+	9.88 ± 0.59^{a}	N/A	N/A	N/A
T1-	9.48 ± 0.67^{a}	N/A	N/A	N/A
T3+	9.43 ± 0.48^{a}	N/A	N/A	N/A
T3-	9.34 ± 1.14^{a}	N/A	N/A	N/A
T4+	9.88 ± 0.68^{a}	N/A	N/A	N/A
T4-	9.40 ± 0.61^{a}	N/A	N/A	N/A

Table S5, Seed weight, plant height, number of panicle per plant, and grain weight per panicle has been shown for finger millet from field trial at Kolli Hills during 2016-17

Treatments (finger millet)	1000 seed wt. (g)	Plant height (m)	Panicle/plant	Grain wt./Panicle (g/panicle)
T1+	N/A	0.74 ± 0.03^{a}	1.15 ± 0.19^{a}	8.89 ± 2.63^{a}

T3+	N/A	0.71 ± 0.11^{a}	1.58 ± 0.33^{a}	6.38 ± 0.19^{b}
T4+	N/A	0.75 ± 0.10^{a}	1.23 ± 0.26^{a}	6.02 ± 1.17^{b}
T5+	N/A	0.70 ± 0.07^{a}	1.30 ± 0.38^{a}	6.96 ± 0.46^{ab}
T6+	N/A	0.69 ± 0.06^{a}	1.25 ± 0.25^{a}	7.07 ± 1.75^{ab}

Table S6, Seed weight, plant height, number of pods per plant, and pod weight per plant has been shown for pigeon pea from field trial at Kolli Hills during 2016-17

Treatments (pigeon pea)	**100 seed wt. (g)**	**Plant height (m)**	**pod/plant**	**Pod wt./plant (g/plant)**
T2+	11.80 ± 0.97^{a}	1.44 ± 0.18^{ab}	85.50 ± 6.86^{b}	93.50 ± 20.34^{b}
T3+	11.10 ± 0.75^{a}	1.39 ± 0.09^{ab}	118.75 ± 32.36^{ab}	112.75 ± 35.75^{ab}
T4+	12.10 ± 1.44^{a}	1.46 ± 0.10^{a}	120.25 ± 24.60^{ab}	126.00 ± 27.24^{ab}
T5+	11.40 ± 0.62^{a}	1.29 ± 0.06^{b}	158.25 ± 28.93^{a}	140.00 ± 43.89^{a}
T6+	10.90 ± 0.97^{a}	1.30 ± 0.07^{b}	133.50 ± 51.80^{ab}	135.75 ± 14.48^{ab}

Table S7, Seed weight, plant height, number of panicle per plant, and grain weight per panicle has been shown for finger millet from field trial at Kolli Hills during 2017-18

Treatments (finger millet)	**1000 seed wt. (g)**	**Plant height (m)**	**Panicle/plant**	**Grain wt./ear (g/panicle)**
T1+	3.38 ± 0.13^{a}	1.19 ± 0.08^{b}	1.35 ± 0.19^{ab}	3.25 ± 0.67^{bc}
T1-	3.30 ± 0.14^{a}	0.92 ± 0.03^{d}	1.05 ± 0.10^{d}	2.65 ± 0.19^{c}
T3+	3.40 ± 0.12^{a}	1.17 ± 0.02^{b}	1.45 ± 0.10^{a}	4.32 ± 1.58^{a}
T3-	3.35 ± 0.17^{a}	1.01 ± 0.04^{c}	1.25 ± 0.19^{abcd}	3.09 ± 0.47^{bc}
T4+	2.45 ± 0.29^{b}	1.17 ± 0.04^{b}	1.10 ± 0.12^{cd}	3.19 ± 0.32^{bc}
T4-	2.68 ± 0.34^{b}	0.97 ± 0.03^{cd}	1.30 ± 0.12^{abc}	2.93 ± 0.31^{c}
T5+	3.38 ± 0.10^{a}	1.39 ± 0.02^{a}	1.20 ± 0.16^{bcd}	3.04 ± 0.45^{bc}
T5-	3.53 ± 0.21^{a}	1.15 ± 0.02^{b}	1.10 ± 0.20^{cd}	3.96 ± 0.48^{ab}

Table S8, Seed weight, plant height, number of pods per plant, and pod weight per plant has been shown for pigeon pea from field trial at Kolli Hills during 2017-18

Treatments (pigeon pea)	100 seed wt. (g)	Plant height (m)	Pod/plant	pod wt./plant (g/panicle)
T2+	N/A	2.75 ± 0.10^{abc}	147.92 ± 12.76^{ab}	96.10 ± 10.05^{a}
T2-	N/A	2.70 ± 0.08^{abc}	134.00 ± 13.45b	87.88 ± 8.88^{a}
T3+	N/A	2.81 ± 0.10^{a}	162.33 ± 19.71^{ab}	100.93 ± 8.24^{a}
T3-	N/A	2.66 ± 0.05^{bc}	147.83 ± 43.99^{ab}	90.75 ± 6.62^{a}
T4+	N/A	2.76 ± 0.06^{ab}	151.92 ± 11.74^{ab}	93.18 ± 7.21^{a}
T4-	N/A	2.75 ± 0.04^{abc}	132.58 ± 22.93^{b}	90.82 ± 8.66^{a}
T5+	N/A	2.64 ± 0.09^{c}	170.50 ± 19.56^{a}	100.42 ± 16.23^{a}
T5-	N/A	2.64 ± 0.11^{c}	138.50 ± 24.89^{ab}	88.65 ± 12.54^{a}

Note: Values shown are average of four replicate ± standard deviation. Tukey`s test (One Way ANOVA) was used for multiple comparison, and values sharing same letters are not significantly different at $p>0.05$.

Concluding discussion

This thesis aimed to evaluate role of CMN as facilitator of bioirrigation in legume – millet intercropping system. To address the research questions of this thesis, experiments were carried to validate the hypothesis of CMN facilitated bioirrigation under controlled conditions inside the greenhouse at University of Basel, and to further evaluate the application of bioirrigation into real-world agriculture, field trials were performed at GKVK, University of Agriculture Sciences Bengaluru, and Kolli Hills, Tamil Nadu, India. The experiments inside the greenhouse were conducted three times, first two experiments were performed into pot size of 21 x 12.8 cm with different strain of AMF to develop CMN between PP and FM (chapter 1), and third experiment was conducted into a scaled up pot size of 70 x 30 cm to provide more realistic growth condition for PP and FM and mimic the onset of drought condition as it occurs under field condition (chapter 2). The results from pot experiments and field trials (chapter 3) conducted from June 2014 to July 2018 yielded important results to provide a proof-of-concept for CMN facilitated bioirrigation in PP–FM intercropping system, and importance of spatial arrangement of component crops in intercropping system.

Facilitation of bioirrigation through CMN

The results obtained from first two pot experiments (chapter 1) clearly showed that PP does perform HL and presence of CMN is an important factor in facilitation of bioirrigation between PP and FM. The shallow-rooted FM was able to absorb bioirrigated water in the presence of CMN but only in split-root set up where roots of PP and FM were intermingled. Furthermore, the effect of bioirrigation was reflected through water relations (stomatal conductance) of shallow-rooted FM as well, since FM in intercropping treatments with CMN were able to maintain higher water relations in intercropping treatments with CMN than without CMN or in monocrop during drought period. The presence of CMN in split-root treatment connects the rhizosphere of PP and FM thus provides a potential pathway for transfer of bioirrigated water. Some research studies have reported that a shallow-rooted plant growing in close vicinity of rhizosphere of HL conducting deep-rooted plant benefit from the process and maintain its water-relations (Ludwig et al. 2003; Prieto et al. 2011). Furthermore, higher mortality and foliar damage percentage of FM were observed in monoculture and intercropping treatments without CMN (Chapter 2), and it provides a second-line of evidence that FM was benefitted by the presence of CMN that results in

lowest mortality percentage in split-root treatment. However in scaled up pot experiment, the effect of CMN was not significant on water relations of finger millet, and all intercropping treatments showed similar trend for water relations.

The higher water relations (stomatal conductance), low mortality and less foliar damage in FM during drought conditions reflect possible benefits that a shallow-rooted plant could obtain in CMN facilitated bioirrigation. However, facilitation through bioirrigation did not translate into plant growth due to interspecific competition between PP and FM as we observed reduced per plants biomass in intercropping treatments. The competition between PP and FM for soil moisture in topsoil layer was clearly demonstrated in scaled up (big) pot experiment (chapter 2). At onset of drought FM and PP both competed for soil moisture in topsoil layer and soil moisture was rapidly decreased to create a severe drought condition in all intercropping treatments, while soil moisture in FM monoculture decreased slowly that allowed FM to grow further in height and gain more biomass. But, few weeks later, when FM in monoculture treatments faced similar drought condition plant could not survive, while FM in intercropping treatments were able to maintain its water relations and low foliar damage percentage was observed.

As results of the pot experiments have clearly showed that CMN facilitates bioirrigation and have positive impact on plants survival during drought condition, but the interspecific competition between PP and FM should be addressed to promote growth and yield of crop. A number of studies have reported that facilitation and competition could be concurrent when roots of two plants are allowed to interact (Ludwig et al. 2003; Schoonmaker et al. 2007; Hawkins et al. 2009). The facilitation and competition could vary depending upon total water availability and number of plants competing for it in a confined area, therefore plant density needs to be optimized in order to reduce the competition. However, the results from the pot experiment (chapter 1 & 2) are encouraging from an agronomic perspective because it offers a potential solution where crop loss due to intermittent drought in rainfed areas could be reduced.

Effect of spatial arrangement and biofertilization on yield and bioirrigation efficacy in PP – FM intercropping system

The hypothesis of bioirrigation based intercropping system to reduce impact of drought on shallow-rooted FM was further tested through field trials conducted at GKVK and Kolli

Hills during 2016/17 and 2017/18 (chapter 3). The results from field trial clearly demonstrated that intercropping of PP–FM, in general, provides higher yield than its respective monoculture, and spatial arrangement of PP and FM is a crucial factor that affects productivity of intercropping. In PP–FM intercropping system, PP showed increase in per plant biomass in intercropping treatments due to reduced intraspecific competition, while FM faced significantly strong interspecific competition (in mosaic treatments) and its biomass (per plant yield) was reduced. The spatial arrangement of PP and FM had a significant effect on water relations (predawn leaf water potential) of FM as well. FM in intercropping treatments showed lower leaf water potential than monoculture, and reduction in per plant yield can be explained through interspecific competition for water between PP and FM. As was observed in pot experiment (chapter 2), FM in intercropping treatments had early drought stress since both PP and FM were competing for topsoil moisture, while FM in monoculture were still able to maintain its water relations and growth. The pattern of water relations recorded during field trial showed similarity with results of pot experiment and it can be inferred that presence of PP caused early onset of drought and due to interspecific competition FM in intercropping had lower leaf water potential than FM in monocropping.

A number of study, mainly in agroforestry, have reported similar competitive effects when grasses or other shallow-rooted species growing under the canopy of trees were able to utilize HLW but their leaf water potential were lower than plants growing away from tree (Belsky et al. 1989; Ludwig et al. 2001; Riginos 2009). The overlap between roots of tree and crops, mainly in the top 50 cm of soil, causes competition between tree roots and grasses for soil water and nutrients (Jonsson et al. 1988; Rao et al. 1993). Thus it is important to know, whether a complementarity for water use can be established when there is such overlapping of root system. However, the extent to which such competitive effect affected the facilitative effect of intercropping that resulted into increased yield in intercropping is unclear. The row-wise intercropping treatment at GKVK site was more effective in terms of yield and water-relations of FM in comparison to other intercropping treatments. In 2:8 row-wise treatment, two rows of PP were separated through 8 rows of FM that offered enough space between PP and FM thus reducing the interspecific competition. The interspecific competition for topsoil moisture by PP is a major limitation to our experimental design that needs to resolved through optimizing the distance between PP and FM and plants density in intercropping system.

In 2017/18 field trial, effect of biofertilizer application on yield and water relations of FM was compared. The biofertilizer (AMF, PGPR and rhizobia) had a positive effect on yield, in general, and it had similar impact on all treatments irrespective of spatial arrangement, mono or mixed cropping. And, the biofertilizer effect on yield was similar across both experimental site. However, per plant yield of FM and PP showed that only PP is significantly affected by biofertilizer application and FM did not show any significant effect. Therefore, yield increase that we observed among intercropping treatments was mainly derived from PP. Application of biofertilizer did not improve the water relations of FM in intercropping treatments, and treatments with and without biofertilizer showed similar trends. As observed from results of our pot experiments (chapter 1) that CMN introduced through biofertilizer played key role in facilitation of bioirrigated water that enabled FM in split-root treatment to maintain its water relations and survive the drought period. While such effect of CMN (applied through biofertilizer) was not reflected in water-relations of FM in field trials. The absence of CMN effect on bioirrigation facilitation indicate that applied AMF strains could not establish a mycorrhizal network between PP and FM. In field experiments, the distance between PP and FM was 45 cm, and AMF strains had to cover up this distance to facilitate bioirrigation. Since, we did not check for spread of CMN in topsoil layer between FM and PP, we can only put possibility for absence of CMN between PP and FM.

The AMF colonization results from pot experiments (chapter 1 & chapter 2) of this study showed that AMF colonization percentage was significantly lower in FM than PP, and AMF did not show any effect on plant biomass of FM. The major benefit that AMF provides to its host plant include enhanced uptake of nutrients, plant establishment and tolerance to abiotic stresses such as drought (Sanders 2004). Specifically, narrow hyphae that grows into small soil pores which are not accessible to root and root hairs, enhances plant nutrient uptake (O`Keefe and Sylvia 1991). Therefore, crop species such as cereals (wheat, barley, oats and millets) with well-developed fine roots and abundant root hairs remain little affected by the AMF colonization (Ryan and Graham, 2002). In order to achieve CMN facilitated bioirrigation in PP – FM intercropping system, a well-developed CMN should be established.

In conclusion, the results of this study provided a strong proof-of-concept for CMN facilitated bioirrigation in PP – FM intercropping system under controlled conditions where

PP supported water-relations and survival of FM during drought condition. However, interspecific competition between PP and FM for soil moisture in topsoil layer overwhelmed the facilitative effect of bioirrigation due which FM could not grow (plant height) during drought period and per plant yield in FM was significantly reduced. The results obtained from field experiments validated HL potential of PP and its potential to be used as bioirrigator, but when applied into intercropping system, interspecific competition between PP and FM reduced it facilitative effect as bioirrigator. In general, 2:8 row-wise intercropping treatment showed positive effect on yield at both experimental site, and application of biofertilizer had positive effect on yield irrespective of spatial arrangement, experiment site, mixed or monocropping. The outcome of this thesis suggests that if plant density and space between PP and FM are optimized, and a well-developed CMN is established between PP and FM then the competitive effect could be reduced to promote facilitative effect of bioirrigation by PP.

Outlook

Bioirrigation based intercropping model has potential to reduce drought induced yield loss of shallow-rooted crops in rainfed areas. For development of such model, suitable bioirrigators (plants with high HL efflux) must be identified. Further studies to assess the efficiency of CMN facilitated bioirrigation in intercropping system must focus of two key aspects: (i) optimal space between two plants, and (ii) establishment of CMN under field condition. Both aspects have close relation with each other, because space between two plant will affect the establishment of CMN between the plant. An average hyphal spread rate for *Glomus* sp. is about 0.7-0.8 mm per day (Jakobsen et al. 1992), therefore a large distance between two plant would not be optimal for CMN establishment and it will affect the efficacy of bioirrigation as well. Furthermore, field experiment with more attention on soil moisture profile of each row and water relations of plants are required, and these parameters should be monitored for following phase: before flowering, during flowering and grain filling or pod formation phase. The soil moisture profile during these phases would provide a clear picture of competition and facilitation for soil moisture. In addition, check points between plants should be established to check establishment of CMN.

The results obtained in this study offer a proof-of-concept for bioirrigation based intercropping that needs to be further validated in field with other legumes species. There are much scope for fine-tuning of the intercropping partner and ideal facilitator of bioirrigation. Depending on site-characteristic results might vary, therefore such intercropping model would be best if it is tested and developed considering local variety of crop plants for a particular region.

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Appendix

Table A1

Reference composition for Hoagland solution (Gamborg and Wetter, 1975) were taken and it was modified to reduce the phosphate content (to 25% P). Modified Hoagland solution was used for pot experiments carried at University of Basel, Switzerland.

Solution	**Mol. Weight**	**mg/100 ml**	**mg/1000 ml**	**mMol**
Solution A				
H_3BO_3	61.83	280.0	2800	45.285
$MnSO_4.H_2O$	169.00	238.4	2384	14.107
$CuSO_4.5H_2O$	249.68	10.0	100	0.401
$ZnSO_4.7H_2O$	287.54	22.0	220	0.765
Solution B		**Ml/100 ml**	**Ml/1000 ml**	
H_2SO_4		0.5	5	
Solution C		**g/500 ml**	**mg/1000 ml**	**mMol**
$Na_2EDTA.4H_2O$	372.24	3.725	7450	20.014
$FeSO_4.7H_2O$	278.02	2.785	5570	20.035
Solution D		**g/100 ml**	**mg/1000 ml**	**mMol**
$Ca(NO_3)_2.4H_2O$	236.15	9.4	9400	39.805
$MgSO_4.7H_2O$	246.48	5.0	5000	20.286
KNO_3	101.11	6.6	6600	65.275
$NH_4H_2PO_4$	115.03	0.3	300	2.600
Solution A	10.0		10.0 (ml)	
Solution B	1		1.0 (ml)	
Final Solution	**ml/1000 ml**			
Solution C	5			
Solution D	100			