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Cereal clover bi-cropping for sustainable forage production in the Himalayan region

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ABSTRACT

We studied the integration of white clover (*Trifolium repens*) with nitrogen management practices in wheat (*Triticum aestivum* L.) - maize (*Zea mays* L.) cropping sequence to find optimum level of N and to enhance forage production. This experiment was laid out in Randomized Block Design with three replications comprising of 12 treatments viz., (T_1) wheat-maize; (T_2) white clover + wheat -maize (0 N); (T_3) white clover (sole); and (T_4 to T_{12}) 50, 75 and 100 % of recommended N to wheat, maize or both. Application of 100 % N to white clover + wheat - maize (bi-cropping) recorded the highest crude protein in white clover. Wheat equivalent yield (WEY) was computed by multiplying the white clover and maize yields with their respective per unit price to compare the system productivity. The system productivity was found 2.67 (56.92 %) and 5.93 t/ha (315.42 %) higher in 100 % N application to bi-cropping over wheat-maize (0 N) and sole white clover, respectively. Highest net returns (US $ 188.5 and 224.0/ha at farmers' field and research farm, respectively) were also recorded in the same bi-cropping treatment. Bi-cropping along with N application resulted in higher Land equivalent ratio (LER) (18.01–57.65%) over sole cropping. The treatment and treatment x environment (TTE) bi-plot also explained 99.84, 98.70 and 99.63 % of treatment and treatment × environment variation for WEY, Benefit: Cost Ratio (BCR) and LER, respectively. Soil fertility improved significantly in sole white clover and bi-cropping treatments with N application. However, when upto 75 % N of recommended dose of fertilizers (RDF) was applied to bi-cropping system resulted in higher WEY, BCR and LER, which were at par with 100 % N. Thus, cereal-clover bi-cropping system along with 75 % N of RDF can be recommended for sustainable forage and food production; and better soil health in temperate, sub-temperate climatic regions of the world.

1. Introduction

The Indian Himalayan region is spread over 12 Indian states stretching across 2500 km (length) and 250–300 km (width) (Samal et al., 2003). It occupies 7.0 % area of India, wherein mixed farming and livestock rearing is an integral part of rural living (Tewari, 2016). Livestock not only provide milk, meat, and supplementary income to the rural households, but also reduce the vulnerability to climate change. Hence, livestock rearing is largely considered as the mainstay of the mountain economy (Genovese et al., 2017) and it plays an important role in supplementing family income and providing additional gainful employment especially for marginal (<1.0 ha) and small (1.0–2.0 ha) category farmers. Grasslands are the main forage resource base for the livestock in the region, however the productivity of grasslands in Himalayan states of India, particularly of Himachal Pradesh state is far below from their actual potential (Dev et al., 2006). Due to poor productivity of grasslands, there is shortage of fodder in the state (Dev, 2001; Dev et al., 2006; Singh et al., 2010). An annual shortage of 26 and

Abbreviations: FF_I, Farmers' field 1st year; FF_II, Farmers' field 2nd year; MGY, Maize grain yield; MSY, Maize stover yield; RF_I, Research Farm 1st year; RF_II, Research Farm 2nd Year; TTE, Treatment effect plus treatment × environment interaction effect; WEY, Wheat equivalent yield; WGY, Wheat grain yield; WSY, Wheat straw yield.

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54 % of green and dry fodder, respectively was reported by (Dev et al., 2006) in Himachal Pradesh of Himalayan region. Due to land constraints in the region, the sparing of the cultivable land for fodder production is not possible to bridge the existing fodder demand and supply gap.

Wheat - maize cropping system is one of the predominant systems practiced in the region and there is a possibility to integrate fodder cultivation (white clover) with this cropping system to overcome the problem of fodder shortage. White clover is a short-lived perennial that can re-seed itself under favorable conditions, grows rapidly and spreads via stolons. Seeds of white clover can be sown during September/ October or it can be transplanted. Pastoral systems in many temperate regions of the world use white clover due to its feed quality benefits for livestock and its inputs of nitrogen through biological N fixation (Gibson and Cope, 1985; Ledgard and Steele, 1992). White clover is generally regarded as a temperate species, however is also widely adapted to regions from the arctic to the subtropics, and has a wide altitudinal range, reportedly up to 6000 m in the Himalayan region (Sareen, 2003). The integration of leguminous fodder crops with the extant farming system (wheat-maize cropping sequence) not only increase the availability of quality fodder, but could also improve the productivity of associated crops though N fixation and other associated benefits (Jensen et al., 2020). Intercropping of cereal–legume offers many tangible benefits e.g. enhanced nitrogen supply through biological nitrogen fixation (BNF), increased use efficiency of nitrogen sources, irrigation and other inputs (Jensen et al., 2020; Liang et al., 2020). It also reduced external inputs such as nitrogenous fertilizers (Rodriguez et al., 2020), production cost (Mamine and Farès, 2020), improved weed control through a denser crop stand (Anil et al., 1998), higher productivity and profitability (Kumar et al., 2017b). In addition, it can improves soil quality (Rodriguez et al., 2020), conserve resources and overcome the problem of soil and water erosion. Legumes in the crop rotation can supply biologically fixed N and thus serve as an N source in agroecosystems (Anglade et al., 2015; Ashworth et al., 2015).

Bi-cropping is a planned crop diversity strategy in space and time, which involves the growing of two dissimilar crops on the same production unit (Vandermeer et al., 1998). This cropping practice may increase production per unit area over sole cropping because of better utilization of resources such as light, water and nutrients (Jalilian et al., 2017; Kumar et al., 2017a). Bi-cropping system, also, offers simultaneous cropping and soil fertility building (nitrogen buildup through BNF), effective nutrient cycling, protection against wind and soil erosion, increased water permeation as well as stable habitat for burrowing earthworms and mycorrhizae (Wahbi et al., 2016). This cropping system produces higher yields and forage biomass than sole cropping due to biological nitrogen fixation by the legume component (Bedoussac and Justes, 2010; Corre Hellou et al., 2011) and simultaneously reduces weed growth (Bahadur et al., 2015). The basic principle of the cereal clover system is that the legume supplies the cereals with nitrogen and simultaneously reduces nutrient leaching by providing a permanent soil cover (Thorsted et al., 2002). The clover under a cereal crop could make conditions unfavourable for pest and disease organisms (Jones and Clements, 1991). The mixtures of diverse plant species use resources more efficiently in nutrient poor environments (Hector, 1998). Introduction of legumes with grasses as an intercrop increases forage productivity and quality (Bork et al., 2017; Kumar and Machiwal, 2017; Papadopoulos et al., 2012). White clover with organic wheat improves ecological services viz., nitrogen provisioning and weed control (Vrignon-Brenas et al., 2018). Intercropping of wheat and white clover also reduces dose of nitrogen application by 20 % without any impact on grain and biomass production and its losses and increases sustainability of wheat cultivation (Kintl et al., 2018).

White clover and its integration with cereal cropping systems has been studied intensively in the temperate regions especially in European countries (Bergkvist, 2003; Enriquez-Hidalgo et al., 2018; Heshmati et al., 2020; Kintl et al., 2018; Miller et al., 2020), but no information on these cropping systems is available for the Himalayan region. Our objectives were to investigate the impact of white clover in wheat-maize systems under varying levels of N fertilization on wheat and maize yields, white clover fodder quantity and quality, soil fertility, and cropping system profitability.

Thus, keeping in view the above objectives of the study, our hypothesis was that this cropping sequence and management practice will enhance the soil fertility and productivity of forage as well as associated crops, and overall profitability of the cropping system.

2. Materials and methods

2.1. Experimental location, climate and soil characteristics

The experiment on cereal clover bi-cropping was carried out simultaneously at two fixed locations *viz.*, at the research farm of regional station, ICAR-Indian Grassland and Fodder Research Institute, Palampur (32° 6′ 24″ N latitude, 76°33′ 42″ E- longitude and 1340 m altitude) and at the farmers' field (32° 08′ 01″ N latitude, 76°32′ 31″ and 1397 m altitude), Palampur (Kangra district) in Himachal Pradesh, India (Fig. 1) consecutively for two years (2010/11 and 2011/12). Thus, four environments (two locations × two years) were considered for statistical analysis. The experimental sites lie in the Palam valley of Kangra district representing mid hill wet temperate conditions (As per Köppen climate classification, the region fall in Cwa; which stand for C (Temperate), w (Dry winter) and a (Hot summer); overall it is mentioned as a monsoon-influenced humid subtropical climate). The average annual rainfall of the area is around 2493 mm and average temperature ranges from 9.9 °C (January) to 27.1 °C (June), with minimum of 5.8 °C (January) to a maximum temperature of 31.9 °C (June). Soil of both the experimental sites was clay loam in texture and low in fertility. The initial (before study) soil pH, organic carbon (%), available N, P and K (kg/ha) at research farm was 5.44, 0.69, 213, 12.3 and 185, respectively. The corresponding values at farmer's fields were 5.46, 0.72, 225, 13.6 and 178, respectively. Taxonomically, the soils of the study areas falls under order *Alfisol* and sub-group Typic Hapludalf.

2.2. Experimental design and treatments

The experiment at both the environments was laid out in Complete Randomized Block Design with three replications. The plot size was 5m × 4.0m. The experiment consisted of 12 treatments which are detailed in Table 1.

2.3. Crop management

Table 2 presents the practices adopted for raising of the experimental crops at both the environments. The legume component (white clover) was established by broadcasting in the plots as per treatment details during October 2010. Wheat during 1st year was sown in the pre-sown white clover plots and as sole crop. White clover was sown only once, however sward was maintained during both the cropping cycles for over two year in the experimental plots. Wheat and maize (two crops/year) were grown during both the years. Same treatments were allocated in the same plots during second years. The plots were maintained weed free and the experimental crops were raised following standard package of practices (Table 2). Nitrogen in each experimental crop was applied as per treatment details through urea (46 % N), whereas recommended dose of phosphorus (P) and potash (K) to white clover, wheat and maize were applied through single super phosphate (SSP) (16 %) and muriate of potash (MOP) (60 %), respectively.

2.4. Data recording

Ten plants of white clover from each plot were randomly selected and tagged for recording plant height and leaf area. Plant height was measured from base to the tip of longest leaf with meter scale. For

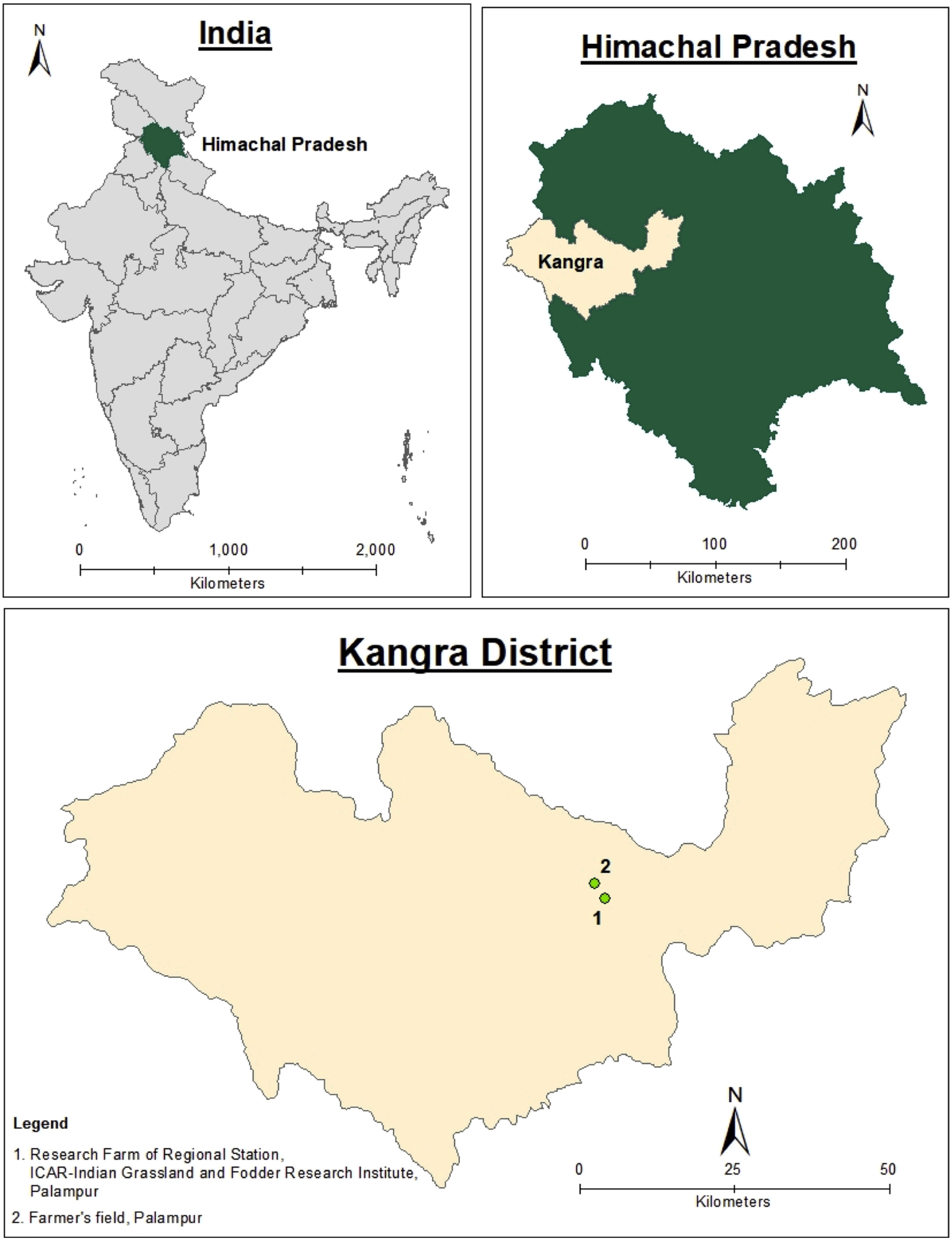

Fig. 1. Map of India with Himachal Pradesh state (Green) and study locations in Palampur, Kangra district in Himachal Pradesh (Light Yellow).

recording leaf area, tagged plants were harvested and taken to the lab and leaf area of all the separated leaves of ten plants was recorded using Li 3100 leaf area meter (Li-cor, Lincolin, NE, USA). Ten vigorous plants from each plot were randomly selected and uprooted for recording root length and number of nodules per plant. Roots of uprooted plants were gently washed followed by root length measurement and nodules per plant counted manually. White clover biomass accumulation was measured by placing a 0.5 by 0.5 m square at three randomly selected locations within each plot and cutting above ground biomass at the soil surface. The white clover production was obtained only from two cuts that is in March and April of each year and for recording dry biomass accumulation. The white clover biomass air dried for four days and then

Table 1
List of the treatments and doses of N applied to wheat and maize in bi-cropping system.

Treatment code	Treatment detail
T_1*	Wheat – Maize (Standard check) – recommended NPK
T_2**	White clover + Wheat – maize (without any N fertilizer)
T_3	White clover- sole
T_4	White clover + Wheat (50 % N) – Maize (50 % N)
T_5	White clover + Wheat (50 % N) – Maize (75 % N)
T_6	White clover + Wheat (50 % N) – Maize (100 % N)
T_7	White clover + Wheat (75 % N)– Maize (50 % N)
T_8	White clover + Wheat (75 % N) – Maize (75 % N)
T_9	White clover + Wheat (75 % N) – Maize (100 % N)
T_{10}	White clover + Wheat (100 % N) – Maize (50 % N)
T_{11}	White clover + Wheat (100 % N) – Maize (75 % N)
T_{12}	White clover + Wheat (100 % N) – Maize (100 % N)

Recommended dose of fertilizer (RDF) for wheat = 120:60:60 (NPK kg/ha); RDF for maize = 120:60:40 (NPK kg/ha).
50 % N=60 kg/ha; 75 % N=90 kg/ha; 100 % N = 120 kg/ha.
0:30:30 (NPK) was applied as basal dose in white clover plots at the time of sowing.
* White clover was not grown in T_1, hence it was omitted in statistical analysis of wheat and maize growth and yield parameters.
** Wheat and maize crops were not grown in T_3, hence it was omitted in statistical analysis of white clover proximate composition, growth and yield parameters.

oven dried at 60 ± 1 °C till constant weight. The white clover biomass from each plot were collected and used for analyzing fodders quality parameters viz., crude protein (CP), crude fibre (CF), total ash, neutral detergent fibre (NDF) and acid detergent fibre (ADF). The quality parameters were analyzed as per procedure described by (AOAC, 1980).

For recording wheat and maize plant height, ten plants of each crop from individual plot were randomly selected and tagged. Plant height was measured at 60 DAS (at 49 and 59 BBCH scale code for wheat and maize, respectively) and at harvest (at 89 BBCH scale code for both the cereal crops) from base to the tip of longest leaf with the help of meter scale. Both the crops were manually harvested at maturity stage from net plot area. The harvested crops were sun dried in the field for five days and there after bundled and brought to the threshing floor. Bundle weight of each crop was recorded plot wise using portable weighing balance to obtain biological yield. Threshing of the crops was done manually, and grain was weighed manually to obtain grain yield (not adjusted for moisture). Straw/stover yield of both the crops were recorded by subtracting grain yield from biological yield. The harvest index was computed using the following equation described by (Donald and Hamblin, 1976).

$$\text{Harvest index } (\%) = \frac{\text{Economic yield (t/ha)}}{\text{Biological yield (t/ha)}} \text{x}100 \tag{1}$$

Since, the market price of each experimental crop was different, hence their combined (system) productivity was worked out by converting the white clover and maize yields into wheat equivalent yield (WEY) as per following formula:

$$\text{WEY (t/ha)of WC} = \textit{Biomass yield of WC x Price of WC/Price ofwheat} \tag{2}$$

Where, WC is white clover

$$\text{WEY (t/ha)of maize} = \textit{Maize grain yield x Price of maize grain/Price ofwheat} \tag{3}$$

$$\text{WEY (t/ha) of wheatstraw} = \textit{WheatstrawyieldxPriceofwheatstraw/Priceofwheat} \tag{4}$$

Note: For calculating WEY of maize straw, maize straw yield and maize straw price was considered

Further, WEY of maize and white clover was added to WEY of wheat to compute the system productivity. To quantify the benefits of bi-cropping, the land equivalent ratio (LER) was also calculated as per formula described by (Mead and Willey, 1980) and (Seserman et al., 2018)

$$LER = \frac{A1}{A2} + \frac{B1}{B2} \tag{5}$$

Where, A1- WEY of white clover in bi-cropping system; A2-WEY of white clover in sole system; B1- WEY of wheat-maize in bi-cropping system; B2- WEY of wheat-maize in sole system. If LER is greater than 1.0, intercropping is advantageous, lesser than 1.0, the intercropping is

Table 2
Crop management practices at both the sites.

Package of practices	Crops		
	White clover	Wheat	Maize
Variety	RRCP-L-10 (Collection)	UP 2338	HIM 123
Date of sowing	7th October, 2010 (first year only)	25th November (2010); 21st November (2011)	23rd June, 2011; 28th June, 2012
Spacing (Row x Row)	Broadcasted	22.5 cm	60.0 cm
Method of sowing	Broadcasting	Manual	Manual
$N:P_2O_5:K_2O$ application (kg/ha)	0:30:30 (7th October, 2010)	Basal application of P and K (60 and 60 kg) on 25th November (2010) and 21st November (2011); N as per treatments detail in three splits (basal dose, crown root initiating stage: 16 December, 2010 & 14 December, 2011) and milking stage: 17 February, 2011 & 15 February, 2012.	Basal application of P and K (60 and 40 kg) on 23rd June, 2011; 28th June, 2012; N as per treatments details in three splits (basal dose, just prior to tasseling; 21 July, 2011 & 24 July, 2012 and grain filling stag: 18 August, 2011 & 20 August, 2012.
Irrigation management	Pre sowing irrigation	Pre sowing irrigation followed by five irrigations at critical growth stages (crown root initiating stage; late tillering, late jointing, flowering and milk stage)	Maize was grown as rainfed crop in rainy season, hence no water deficit was observed during its growing period
Weed management	Pendimethalin @ 1.25 kg a.i. per ha as pre-emergence application	*One hand weeding at 30–35 days after sowing (at BBCH scale code 16)	*One hand weeding at 25–30 days after sowing (at BBCH scale code 15)
Harvesting	First cut in second fortnight of March (21 March, 2011 & 24 March, 2012) and Second cut in second fortnight of April (25 April, 2011 & 27th April, 2012) (both years)	Second fortnight of May (both years) (29 May, 2011 and 26 May, 2012)	First fortnight of October (both years) (13 October, 2011 and 10 October, 2012).
Soil sample collection	6 October, 2010 and 16 October, 2012		

* For sole wheat and maize treatment, beside hand weeding pendimethalin @ 1.25 kg a.i. per ha was also applied as pre emergence herbicide.

disadvantageous, while equal to 1.0 express intercropping neither advantageous nor disadvantageous.

Soil samples from 0−15 cm soil depth using soil augur were collected from each plot before and after completion of the study. The collected samples were further processed and analyzed for available N (Subbiah and Asija, 1956) P (Olsen et al., 1954) and K (Hanway and Heidel, 1952), organic carbon (Walkley and Black, 1934), pH and electrical conductivity (EC). The material and labour inputs, in terms of number of the man-days used for different operations, were recorded for financial analysis of the bi-cropping system. The input costs were calculated according to the current market prices of input items and services. The gross returns were calculated according to the minimum support prices (MSP announced by CACP, Government of India every year) (2011 and 2012) of the produce, while net returns was obtained by subtracting inputs cost from gross returns. The prevailing market rates were considered for those produces (white clover, straw of wheat and maize), which are not covered under MSP announced by Government of India. The benefit-cost ratio (BCR) was worked out by dividing net returns by input cost.

2.5. Data analysis and statistical methods

Data were subjected to analysis of variance using SASv9.4 (SAS, 2016) and R statistical software (R Core Team, 2016). The experiment was conducted on two environments (research farm and farmer's field) for two years, hence total four environments (RF_I-research farm 1st year; RF_II-research farm 2nd Year; FF_I- farmers' field 1st year and FF_II-Farmers' field 2nd year) were considered in statistical analysis. Environments and treatments were analyzed as fixed effects model. Multivariate stability statistics (GGE biplot) was computed using the 'GGEBiplotGUI' package with the support of R studio (RStudio, 2014) in R statistical software. In this study instead of GGE biplot, TTE (Treatment, Treatment × Environment) bi-plot term was used to assess the interaction between environments and treatments and rank the treatment/management practice based on stability and mean (Yan and Kang, 2003). The bullet graphs were also generated for graphical summary of stability statistics and mean of different treatments within and across the environment using SAS PROC GPLOT in conjugation with SAS v9.4.

3. Results

3.1. Growth parameters

White clover plant height was in the range of 8.94–23.82 cm across the treatments (Table 3). Bi-cropping treatment (T_2) without N application had significantly higher white clover plant height as compared to the sole treatments (T_3). Application of N to wheat/maize in bi-cropping system recorded significant increase in white clover plant height as compared to its sole treatment. Leaf area of white clover was significantly influenced by the treatments (Table 3). Sole white clover treatment (T_3) performed better over other treatments, and recorded significantly higher (45.14, 39.56, 36.55 and 33.68 %) leaf area to T_2, T_4, T_5 and T_6, respectively but was statistically at par with remaining treatments. Root length and number of nodules per plant indicated that sole white clover (T_3) proved significantly superior to bi-cropping treatments, recorded higher root length (19.92–25.58 %) and nodule number per plant (17.97–47.32 %) over the bi-cropping treatments. Plant height of wheat recorded in the range of 13.06–16.20 cm (at BBCH Scale code 49) and 59.05–73.29 cm (at BBCH Scale code 89) and corresponding values for maize crop were 69.91–114.43 cm (at BBCH Scale code 59) and 93.08–152.36 cm (at BBCH Scale code 89). The maximum plant height of wheat and maize at both the growth stages were recorded in T_1- wheat–maize (standard check)-recommended NPK, which was found to be significantly greater than T_2, T_4, T_5 and T_6 (in wheat crop) and to T_2, T_4, T_7 and T_{10} (in maize crop), however remained statistically at par with rest of the treatments (Table 3).

Table 3
Growth parameters of white clover, wheat and maize in cereal-clover bi-cropping (mean of two years).

Treatments	White clover					Wheat		Maize	
	Plant height (cm)		Leaf area (cm^2)	Root length (cm)	Nodules/ plant (no.)	Plant height (cm)		Plant height (cm)	
	1st cut	2nd cut				60 DAS (BBCH-Scale Code 49)	at harvest (BBCH-Scale Code 89)	60 DAS (BBCH-Scale Code 59)	at harvest (BBCH-Scale Code 89)
T_1- Wheat – Maize (Standard check) – recommended NPK	–	–	–	–	–	16.20	73.29	114.43	152.36
T_2- White clover + Wheat – maize (without any N fertilizer)	11.97	20.87	1.75	16.01	15.90	13.18	59.05	69.91	93.08
T_3- White clover- sole	8.94	17.09	2.54	19.98	23.10	–	–	–	–
T_4- White clover + Wheat (50% N) – Maize (50% N)	11.04	21.89	1.82	15.91	15.68	13.09	60.48	77.77	103.40
T_5- White clover + Wheat (50% N) – Maize (75% N)	11.81	21.01	1.86	16.66	15.82	13.06	61.50	102.46	136.42
T_6- White clover + Wheat (50% N) – Maize (100% N)	11.68	22.28	1.90	16.34	15.70	13.40	63.95	105.15	140.45
T_7- White clover + Wheat (75% N)– Maize (50% N)	11.11	23.82	2.41	15.83	18.87	15.58	71.70	79.75	106.49
T_8- White clover + Wheat (75% N) – Maize (75% N)	12.21	21.96	2.20	16.52	18.79	15.22	71.11	103.30	138.23
T_9- White clover + Wheat (75% N) – Maize (100% N)	11.55	23.57	2.28	16.44	19.43	15.42	70.83	104.09	138.92
T_{10}- White clover + Wheat (100% N) – Maize (50% N)	12.79	20.94	2.51	16.60	19.58	15.85	72.22	81.42	107.36
T_{11}- White clover + Wheat (100% N) – Maize (75% N)	11.21	23.66	2.26	16.33	19.00	16.19	72.80	101.74	135.47
T_{12}- White clover + Wheat (100% N) – Maize (100% N)	11.32	23.12	2.34	16.32	19.39	16.17	73.29	109.16	144.79
$SE_{(m)+}$	0.87	1.56	0.17	0.37	0.51	0.65	2.99	5.59	7.41
LSD (p = 0.05)	2.09	3.68	0.43	1.08	1.50	1.67	7.86	13.89	18.50

3.2. Proximate composition of white clover

Crude protein (CP), crude fibre (CF), total ash, Acid detergent fibre (ADF) and Neural detergent fibre (NDF) are very important fodder quality parameters. Nitrogen treatments significantly influenced CP, CF, total ash and ADF of white clover fodder (Fig. 2). Among treatments, the highest (20.35 %) and lowest (18.03 %) CP content was recorded in T_{12} and T_4, respectively. T_{12} recorded significantly higher (0.99–2.32 %) CP content to T_2, T_3, T_4, T_5, T_6 and T_8, but was found statistically at par to T_7, T_9, T_{10} and T_{11}. The higher CP signifies the better quality of fodder. Across the treatments, the maximum (21.16 %) and minimum (18.10 %) CF content was recorded with T_3 and T_8, respectively. Sole white clover recorded significantly higher CF thanT_4, T_7, T_8, T_9, T_{10}, T_{11} and T_{12}, but was found statistically at par with rest of the treatments. T_6 recorded significantly lowest (5.55 %) total ash content to T_7, T_8, T_9, T_{10}, T_{11} and T_{12}, but it remained statistically at par with T_2, T_3, T_4 and T_5. No significant differences were observed w.r.t. NDF, however concerning to ADF, T_4 performed better over rest of the treatments and recorded significantly lower ADF than T_{10}, but proved statistically at par to rest of the treatments (Fig. 2).

3.3. White clover biomass, wheat grain yields and maize grain yields

Mean white clover biomass, wheat (grain and straw), maize (grain and straw) and system productivity (WEY) have been explained in bullet graphs and box plots (Figs. 3 & 4). Biomass production of white clover was significantly affected by treatments (Table 4). Slightly higher biomass yield was recorded at research farm as compared to the farmers' field (Fig. 3a). Among treatments, sole white clover (T_3) recorded significantly higher (11.16–44.86 % at research farm and 26.45–75.00 % at farmers field) biomass yield than other treatments. Across N applications to wheat-maize system, white clover yield in six treatments (T_7, T_8, T_9, T_{10}, T_{11} and T_{12}) were found superior over control (T_2) at both the locations. However, only five treatments (T_2, T_3, T_7, T_8 and T_9) showed the fair stability and rest of the treatments showed poor stability (Fig. 3a).

Grain and straw yield of wheat was significantly influenced by different treatments in bi-cropping sequence (Fig. 3b and c). Wheat grain yield (WGY) varied in the range of 1.13 (T_2-white clover + wheat-maize (without any N fertilizer) to 2.69 t/ha (T_1-wheat-maize (standard check)-recommended NPK) across the treatment at research farm and farmers' field. T_1 recorded significantly higher grain yield to T_2, T_4, T_5, T_6, T_8 and T_{11}, however it was found statistically at par with T_7, T_9, T_{10} and T_{12}. Only two treatments i.e. T_6 and T_{11} showed good stability concerning to grain yield and rest of the treatments reported fair stability. Average across the treatments, WGY at research farm was recorded 0.25 t/ha higher over the WGY at farmers' field (1.95 t/ha). Likewise, results were observed w.r.t straw yields in all environments. Harvest Index (HI) of wheat was not significantly influenced due to N treatments with or without bi-cropping sequence across the environments (Fig. 4).

With respect to maize grain yield (MGY), T_9 recorded significantly higher grain yield (13.77–76.55 %) to T_2, T_4, T_7, T_{10} and T_{11}, but was found statistically at par with rest of the treatments across the environments (Figs. 3d and 4). In bullet graph (Fig. 3d), two treatments (T_{11} and T_{12}) showed good stability as compared to other treatments. Among all the treatments, the highest (3.27 t/ha at research farm) and lowest (1.73 t/ha at farmers' field) stover yield was recorded with T_{12} and T_2, respectively (Figs. 3e and 4). T_{12} -white clover + wheat (100 % N)–maize (100 % N) was found significantly superior to T_2, T_4, T_5, T_7, T_{10} and T_{11} treatments, but remained statistically at par with rest of the treatments. Maize stover yield (MSY) in T_{12} increased by 76.22 % over the stover yield recorded under T_2.

Wheat equivalent yield (WEY) was significantly influenced by treatments at both the experimental locations (Fig. 5). T_{12} had the highest WEY and T_3 the lowest. Comparatively research farm had higher WEY than farmers' field (Fig. 3f). T_{12} proved significantly superior to T_1, T_2, T_3, T_4, T_5, T_6, T_7 and T_{10} at research farm and T_1, T_2, T_3, T_4, T_5 and T_6 at farmers' field, but it was statistically at par with rest of the treatments. WEY increased by 3.2 (69.41 %) and 5.93 t/ha (315.42 %) under T_{12} as compared to T_2 and T_3 treatments, respectively at research farm, whereas at farmer's field, it increased by 2.67 (56.92 %) and 5.49 t/ha (293.58 %).

3.4. Financial analysis and Land equivalent ratio

Across the environments, significantly higher economic returns (gross and net) were obtained in T_{12} as compared to T_1, T_2, T_3, T_4, T_5 and

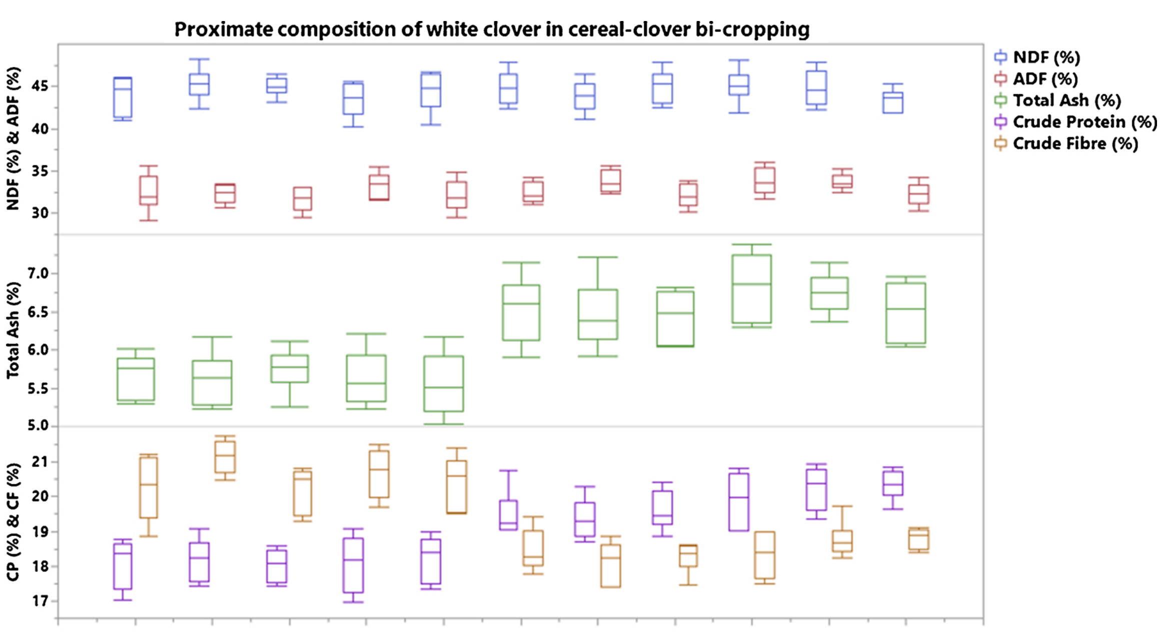

Fig. 2. Box plot of proximate composition of white clover in cereal-clover bi-cropping (mean of two years). *(T_1- wheat-maize (no clover), hence proximate composition not shown in figure).*

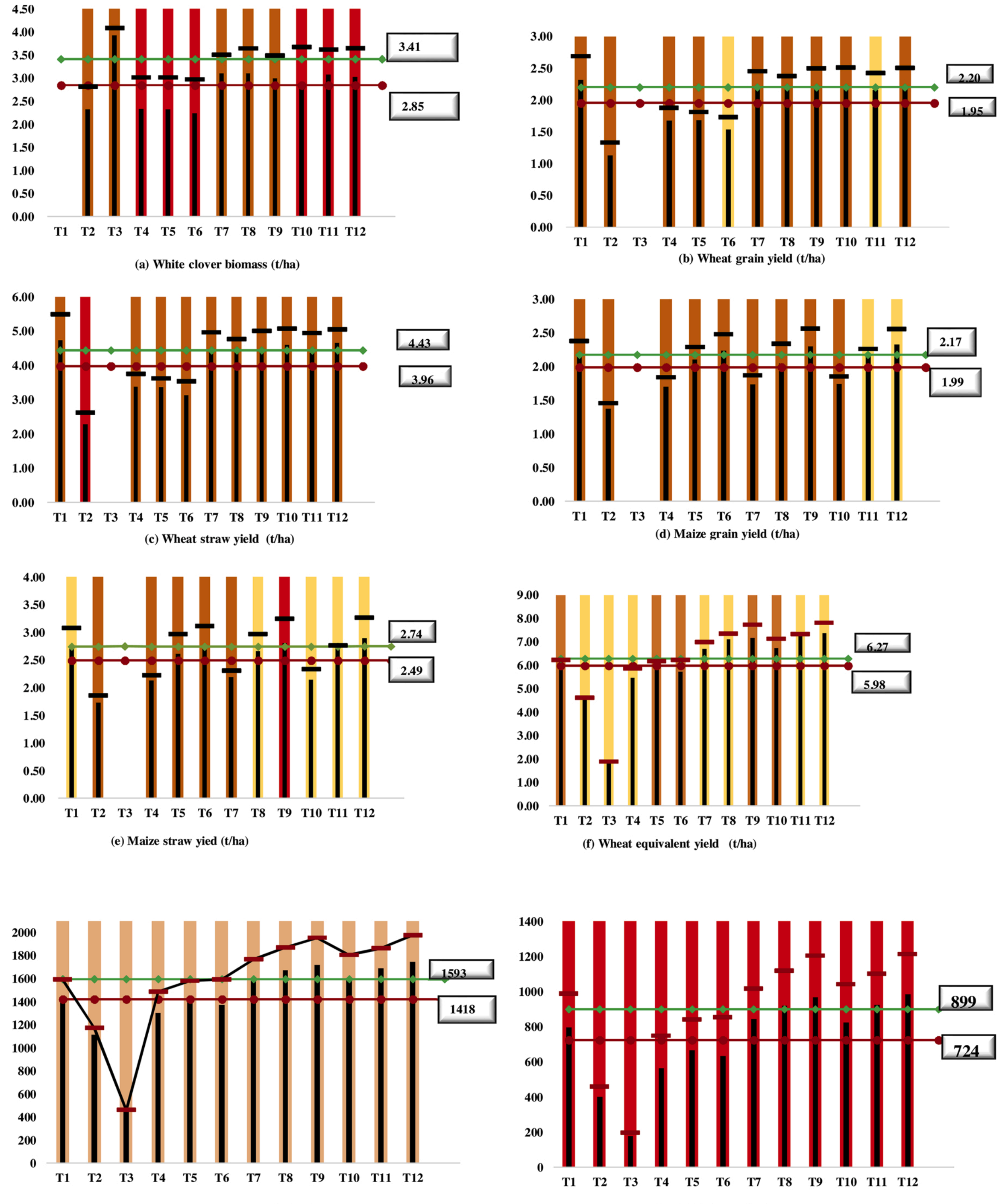

Fig. 3. Bullet graph summarizes the stability statistics, mean yields of white clover biomass, wheat (grain and straw), maize (grain and straw) gross and net returns from systems across the treatments (different nitrogen management doses) at Farmer's field and Research farm tested in 2 years. The vertical bars represent different treatments (T_1–T_{12}). Back ground fill colour of yellow, orange and red within each vertical bar represent good, fair and poor stability. The vertical and horizontal black line within each vertical bar measure mean parameter value at farmer's field and research farm, respectively, on quantitative scale (Y-axis, t/ha). The red and green horizontal lines across vertical bar represent mean parameter value at farmer's field and research farm across the treatments, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the webversion of this article.).

T_6 (Figs. 3g, h and 5). ANOVA for wheat equivalent yield (WEY), gross and net returns and BCR has been presented in Table 5. In general N application increased the yields. Higher economic returns (US$ 175 per ha) were obtained at research farm as compared to farmers' field. The presence of white clover sward in T_{12} increased the net returns (US $ 188.5 and 224.0 per ha at farmers' field and research farm, respectively) over the T_1- wheat–maize (standard check)–recommended NPK. All the treatments showed fair stability for gross returns, while poor stability for net returns. The highest gross and net returns were obtained in T_{12}. However, the highest BCR was recorded in T_9 - white clover + wheat (75 % N) - maize (100 % N) (Fig. 5), which remained statistically at par withT_1, T_8, T_{11} and T_{12}. Treatment T_{12} had the highest LER and T_2 the lowest (Fig. 5). Treatments which received N had greater LER 18.69–52.84 % (research farm) and 18.01–57.65 % (farmers field),

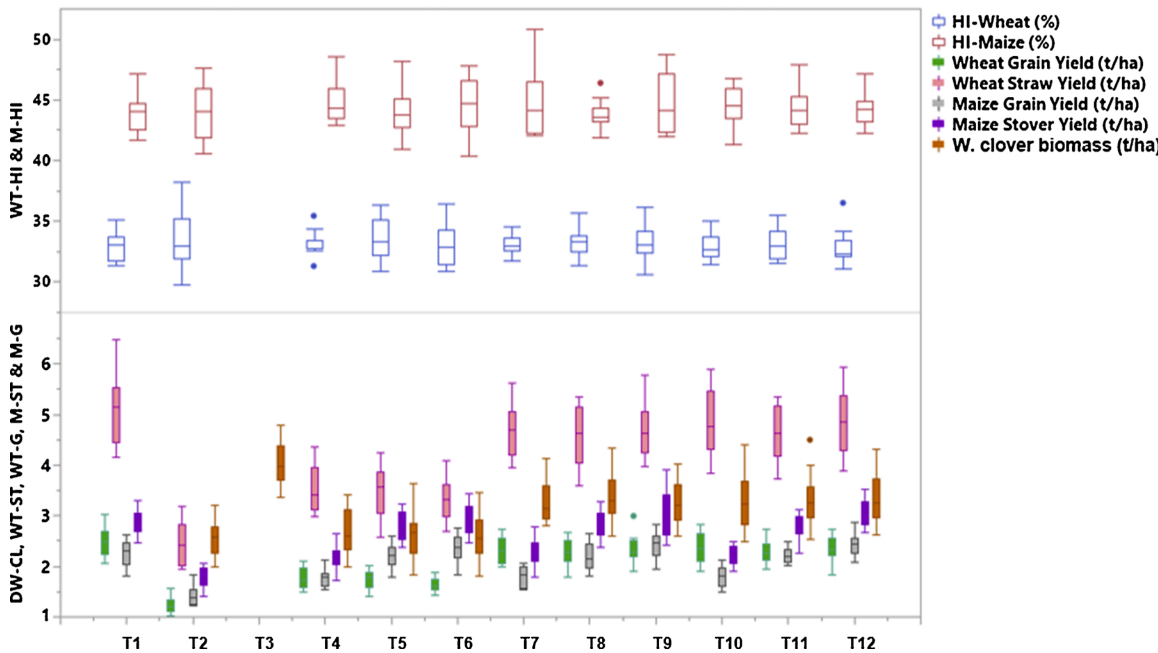

Fig. 4. Box plot of White clover biomass production (DW clover) and crop yields. *(T_3- Sole clover, hence not shown in figure).*

Table 4
Analysis of variance (ANOVA) for white clover biomass, wheat grain (WGY) and straw (WSY) yields, maize grain (MGY) and straw (MSY) yields.

Source of Variation	DF	W. clover biomass	WGY	WSY	MGY	MSY
Replication	2	0.3225	0.212	1.5635	0.3555	0.254
Treatments (T)	10	2.132**	1.09**	4.572**	0.321**	0.458**
Environment (E)	3	0.057	0.004	0.036	0.009	0.049
T × E	30	0.768**	0.409**	1.768**	0.401**	0.701**
Error	86	0.067	0.037	0.247	0.037	0.059
Total	131					

Note: In ANOVA for dry biomass of white clover T_1 (Wheat – Maize – recommended NPK) was omitted and for wheat and maize yields T_3 (White clover sole) was omitted.

respectively. LER values showed that bi-cropping system is better than sole cropping system in terms of productivity.

3.5. Polygon view (Which-Won-Where) of treatments and treatment × environment (TTE) biplots

The "which-won-where" polygon view of the treatment × environment (TTE/GGE) biplots (Yan et al., 2000) is one of the most effective tool in mega-environment analysis. Irregular polygons are drawn by joining extreme genotypes or the treatments of the biplot (Fig. 6). A set of lines drawn from the biplot origin and intersecting each of the sides at right angles, thus creates different sectors in biplot (Yan et al., 2007). If management practices or the treatments fall into different sectors, then the particular treatment/s won in that sector, and a treatment × environment interaction exists. Polygon view of treatment effects plus treatment ×environment interactions of biomass yield of white clover, WGY and MGY, WEY, BCR and LER has been presented in Fig. 6. Across the environment, the polygon view of TTE biplot explained 98.99, 99.78, 99.00, 99.84, 98.70 and 99.63 % of the treatment and treatment × environment variation for biomass yield of white clover, WGY, MGY, WEY, BCR and LER, respectively. In TTE biplot of white clover biomass yield, treatments are distributed in three sectors and T_3, T_7, T_8, T_9, T_{11} and T_{12} has come under single mega environment (Fig. 6). In TTE biplot of WGY, all the environments fallen under two sectors and T_1 was winner followed by T_9, T_{10}, T_{12} and T_7. Similarly, two mega environments have been created for MGY and in first sector of RF_I and FF_I, T_6 was winner, whereas in second sector of RF_II and FF_II, T_{12} was observed winner followed by T_9 and T_{11}. However, in case of WEY, all the environments came under single mega environment with T_{12} as winner. All the four environments have fallen in single sector in TTE biplot of BCR and LER, where T_1 won the sector followed by T_9, T_{12}, T_8, T_{11}, T_7 and T_{10} in BCR TTE biplot and in TTE biplot of LER, T_{12} was winner followed by T_9, T_8, T_{11}, T_{10} and T_7 treatments.

3.6. Mean performance vs stability of the treatments

The 'average environment coordinate' (AEC) abscissa has one direction, with the arrow pointing to greater treatment main effect focused on singular value partitioning (SVP = 1) (Yan et al., 2007). Mean vs Stability TTE biplot facilitates treatment comparisons based on mean performance and stability of treatment across the environments. The TTE biplot of mean vs stability explained 98.99, 99.78, 99.00, 99.84, 98.70 and 99.63 % of treatment and treatment × environment variation across the environment under different treatments for biomass yield of white clover, WGY, MGY, WEY, BCR and LER, respectively (Fig. 7). The arrow shown in circle of each biplot on the AEC abscissa point in the direction of higher performance of the treatment in terms of respective parameter (yield, BCR and LER) and rank the treatment w.r.t performance. Thus, T_3 (sole clover) ranked first with less stability in white clover biomass yield followed by T_8 with high stability. In TTE biplot of wheat grain yield (WGY), T_1 observed with mean highest yield followed by T_{10} and T_{12}. However, T_{12} performed better in TTE biplot of both maize grain yield (MGY) and wheat equivalent yield (WEY) (Fig. 7). T_1 ranked 1st in TTE biplot of BCR with high stability followed by T_9 and T_{12} and lowest stability was observed in T6. However, in TTE biplot of LER, T_{12} ranked first with high stability. The stability of each treatment was shown by its projection onto the AEC vertical axis. The treatment closest to AEC abscissa (near zero projection) is most stable and

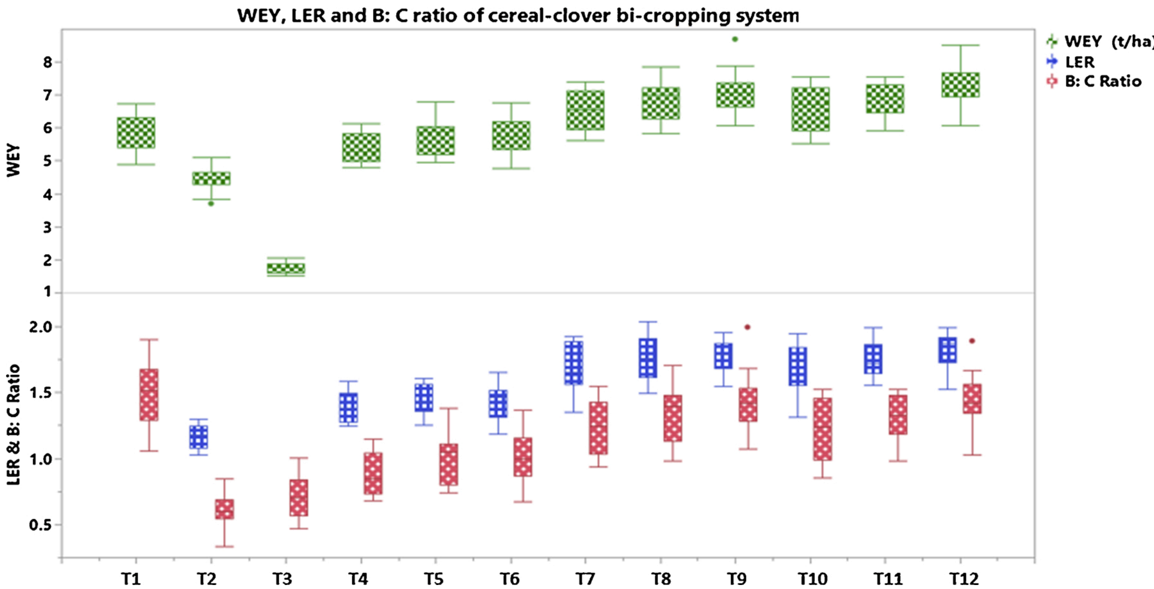

Fig. 5. Box plot of wheat equivalent yield (WEY), land equivalent ratio and B: C ratio of cereal-clover bi-cropping system.

Table 5
Analysis of variance (ANOVA) for wheat equivalent yield (WEY), gross and net returns and Benefit: Cost ratio.

Source of Variation	DF	WEY	Gross Return	Net Return	B: C Ratio
Replication	2	1.82	267.60	267.54	0.21
Treatments (T)	11	28.06**	4059.45**	2009.71**	1.05**
Environment (E)	3	5.88**	2483.01**	1528.16**	0.81**
T × E	33	0.11	23.44	20.35	0.01
Error	94	0.19	27.08	27.08	0.02
Total	143				

vice-versa.

3.7. Soil fertility

Available N, P and organic carbon (OC) in soil were significantly influenced by the treatments after two years of experimentation at both experimental locations (Table 6). Organic matter increased over initial value across the treatments irrespective of N doses. The highest increase was recorded with treatments which produced higher biomass/yields at both experimental locations. Compared to initial value, available N at research farm and farmers field was found to be increased in the range of

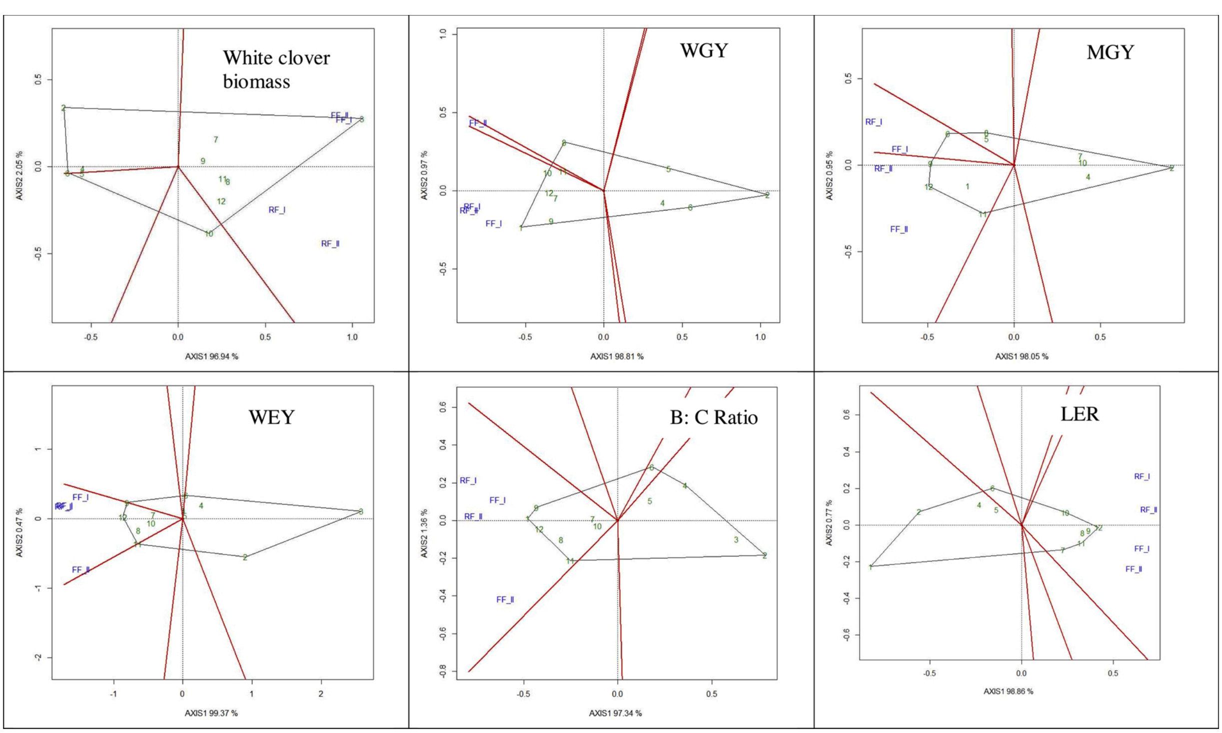

Fig. 6. The polygon (which-won-where) view of treatment effects plus treatment × environment interaction effect (TTE) biplot of yields (biomass and grain) of white clover, wheat and maize, wheat equivalent yield (WEY), B: C ratio and land equivalent ratio (LER). Key to the labels of management practices (treatments) and environment is presented in abbreviation section.

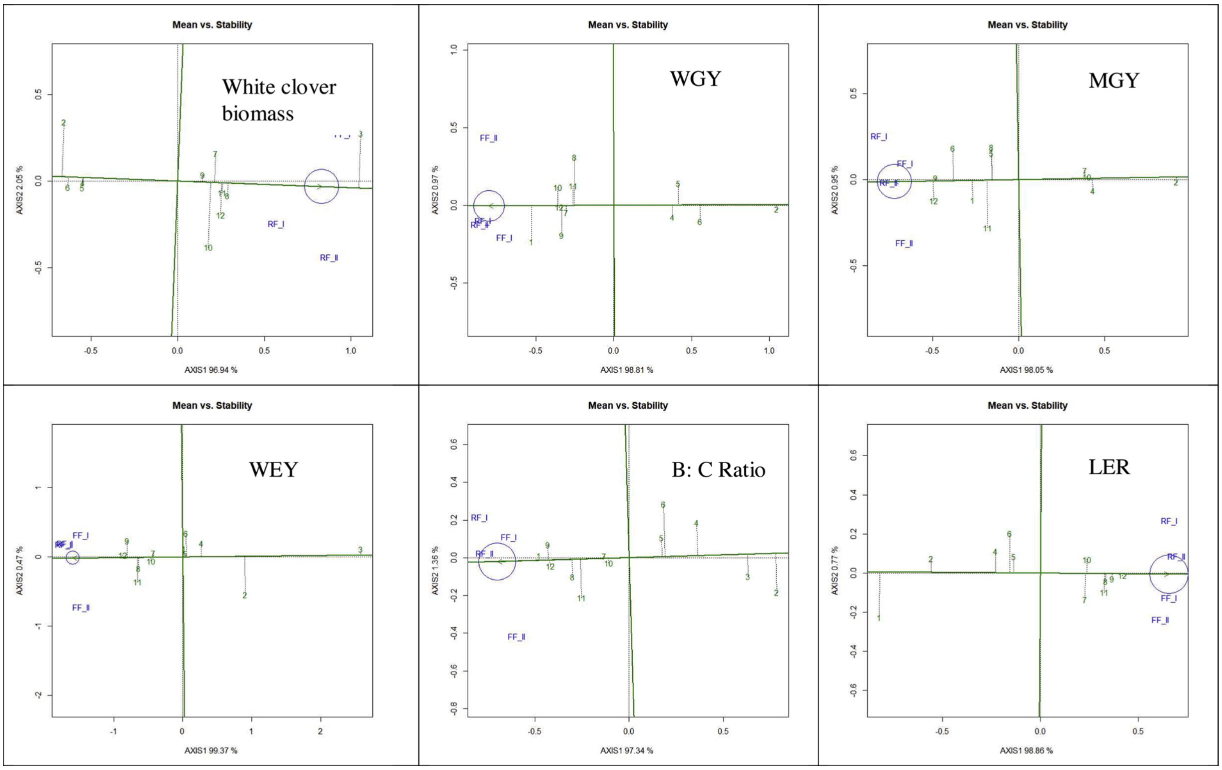

Fig. 7. The mean vs. stability view of treatment effects plus treatment × environment interaction effect (TTE) biplot of yields (biomass and grain) of white clover, wheat and maize, WEY, B: C ratio and LER. Key to the labels of management practices (treatments) and environment is presented in abbreviation section.

Table 6
Effect of cereal-clover bi-cropping system on soil properties.

Treatments	At research Farm					At farmers' field				
	pH	OC (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)	pH	OC (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
T_1- Wheat – Maize (Standard check) – recommended NPK	5.41	0.72	235	15.3	182	5.43	0.74	230	15.8	174
T_2- White clover + Wheat – maize (without any N fertilizer)	5.42	0.77	360	16.4	180	5.44	0.76	355	16.9	175
T_3- White clover- sole	5.39	0.75	390	17.2	178	5.42	0.77	398	17.4	182
T_4- White clover + Wheat (50% N) – Maize (50% N)	5.40	0.79	340	16.8	176	5.41	0.80	332	17.1	180
T_5- White clover + Wheat (50% N) – Maize (75% N)	5.41	0.81	342	17.6	172	5.43	0.83	328	18.7	177
T_6- White clover + Wheat (50% N) – Maize (100% N)	5.39	0.85	348	18.4	172	5.41	0.87	337	19.3	172
T_7- White clover + Wheat (75% N)– Maize (50% N)	5.41	0.82	362	16.9	176	5.42	0.85	366	18.4	180
T_8- White clover + Wheat (75% N) – Maize (75% N)	5.41	0.84	360	18.2	182	5.41	0.88	378	19.8	175
T_9-White clover + Wheat (75% N) – Maize (100% N)	5.38	0.86	363	18.5	175	5.42	0.91	367	20.4	179
T_{10}- White clover + Wheat (100% N) – Maize (50% N)	5.37	0.84	368	18.7	172	5.41	0.88	371	20.9	172
T_{11}- White clover + Wheat (100% N) – Maize (75% N)	5.38	0.86	372	19.1	177	5.40	0.90	382	21.4	173
T_{12}- White clover + Wheat (100% N) – Maize (100% N)	5.37	0.89	371	19.8	176	5.40	0.92	377	21.8	176
SE $_{(m)+}$	0.10	0.02	10.6	0.50	4.7	0.09	0.03	7.9	0.55	3.9
LSD (P = 0.05)	NS	0.07	31.1	1.47	NS	NS	0.08	23.2	1.62	NS
Initial value	*5.44*	*0.69*	*213*	*12.3*	*185*	*5.46*	*0.72*	*225*	*13.6*	*178*

10.32–83.09 and 2.22–76.88 %, respectively across the treatments. However, available P increase was observed in the range of 24.39–60.97 % and 16.17–60.29 % at Research farm and farmers field, respectively. The highest and lowest increase in available N and P over initial value was recorded in T_3 and T_1 and T_{12} and T_1, respectively at both experimental locations. Among the treatments, T_3 and T_{12} proved superior and recorded highest available N and P at both experimental locations.

4. Discussion

In this study, we integrated white clover and optimized N application with existing wheat-maize cropping sequence in Himalayan conditions. The study was conducted in two different locations viz., at research farm and at farmer's field for two consecutive years. Thus, study existed in total four environments (RF-I, RF-II, FF-I and FF-II). Hence, multi-environment data were partitioned among experimental factors and their interactions and *F* ratio of the fixed effect (treatment (T), environment (E) and T × E) was observed statistically significant.

Every year two cuttings of white clover were taken. The plant height of white clover at the time of every cutting was observed higher in bi-cropping than sole cropping. This might be due to competition between associated crops for light and availability of better nutrition (Wilman and Asiegbu, 1982; Xiao et al., 2006). In contrast to plant height, the higher leaf area, root length and number of nodules/plant of white clover were recorded in sole cropping as compared to bi-cropping treatments. This may be due to less competition for various growth resources viz., light, moisture, space and nutrients in sole crop (Lima Filho, 2000). Both cereals were taller in T_1 than in the other treatments. Nitrogen plays an important role in photosynthetic activities, plant growth and yield of cereal crops (Ali et al., 2018; Kumar et al., 2019).

Nitrogen application in wheat-maize sequence had positive impact on fodder quality parameters viz., CP and total ash content of associated white clover. N is essentially required for synthesis of amino acids, nucleic acid and enzymes, hence CP and total ash content increased with increasing N doses (Delevatti et al., 2019; Kering et al., 2011). White clover can supply 100 kg/ha of atmospheric nitrogen (equivalent to 200 kg of N in mineral fertilizers) to the soil (Andrews et al., 2007). In our study, NDF and ADF of white clover were not affected with different levels of N application in wheat-maize sequence (Fig. 2), but decrease of NDF and ADF with increasing N doses has been reported by (Kering et al., 2011) in Bermuda grass.

White clover had significantly higher biomass yield under sole cropping treatment in comparison to bi-cropping treatments possibly due to differences in sowing densities and competition with associated crops (Cannon et al., 2020). White clover recorded higher biomass yield (19.64 %) at research farm as compared to the farmer's field might be due to better management factors. Amongst bi-cropping treatments, six treatments (T_7, T_8, T_9, T_{10}, T_{11} and T_{12}) recorded higher white clover biomass yield than other treatments, which might be due to higher N application in the associated crops (Kintl et al., 2018). In TTE biplot (which-won-where), white clover biomass yield of different treatments is distributed in three sectors and T_3, T_7, T_8, T_9, T_{11} and T_{12} came under single mega environment (Fig. 6). (Thorsted et al., 2006) conducted a bi-cropping study at Research Centre Foulum in Denmark and reported the reduction of white clover yield in bi-cropping system with wheat due to competition for light and nutrients. However, in another study (Nyfeler et al., 2011) recorded overall higher biomass in bi-cropping system as compared to mono-cropping due to mutual stimulation of N uptake. In an experiment in Sweden, clover was undersown in spring barley and remained established in two consecutive crops of wheat in two field experiments. Clover decreased grain yield in the first crop of wheat and increased it in the second (Bergkvist, 2003).

Sole cropping (wheat-maize) treatment (T_1) with recommended dose of fertilizers recorded higher wheat grain and straw yields than bi-cropping treatments at both experimental locations, which might be due to optimum nutrition and minimal competition for above and below ground resources. In TTE biplot, grain yield of wheat in all the treatments fallen under two sectors and T_1 was winner, but it was statistically at par with four bi-cropping treatments (T_9, T_{10}, T_{12} and T_7). Almost similar research findings have also been reported by (Cannon et al., 2020). Intercropping of winter wheat and clover resulted in decreased wheat grain yield (10–25 %) as compared to sole wheat (Thorsted et al., 2006). However, in our study bi-cropping treatments except T_8 with 75 % N application recorded statistically at par wheat grain and straw yields in comparison to sole cropping treatment and 100 % N application. This could be ascribed to the fact that BNF by white clover might have supplemented N requirement of the companion crops at both experimental locations. (Kintl et al., 2018) also found wheat + white clover bi-cropping system more sustainable over wheat mono cropping because white clover in mix cropping can supplement the 20 % of total N requirement of wheat besides having weed suppression effects.

In maize crop, the treatment which received 100 % N resulted in higher yield as compared to treatment with lower doses of N in bi-cropping system. Among bi-cropping treatments, T_{12}-white clover + wheat (100 % N)–maize (100 % N) recorded highest maize grain and stover yields, which indicates that white clover had positive impact on yields at the same level (T_1) of N application.

The system yield here has been presented as wheat equivalent yield (WEY). Bi-cropping treatment (T_{12}) loaded with 100 % N application to both the cereal crops along with white clover recorded higher WEY in all the four environments. (Mall et al., 2014) and (Prasad et al., 2016) also advocated intensification of cropping sequences with inclusion of legumes for higher crop yield and profitability. N fixers benefit the associated plants by providing them atmospheric N, which contributes to better development of plant growth and biomass production (Singh et al., 2015). Inclusion of legumes component in wheat-maize cropping system not only improved soil aggregation, but also helped in increasing OC content in the soil (Hazra et al., 2019) and sustainability of the system (Sharma and Behera, 2009). Being a cereal system, wheat-maize cropping system is highly responsive to the N application (Liu et al., 2010) and even with use of optical sensor for N application, yield of maize and wheat can be increased by 14–20 % over recommended N application (Oyeogbe et al., 2018).

All the bi-cropping treatments recorded LER > 1 and it was increased with increasing N doses to both the cereal crops. LER in 75 % N treatments remained at par with 100 % N treatments which showed the N compensatory effect of white clover in cereal system. This might have improved overall yields besides suppressing weeds (Cannon et al., 2020). Bi-cropping treatment (T_{12}) with 100 % N application recorded higher LER (1.88 and 1.75) at both experimental locations which might be due to efficient utilization of environmental resources (Chi et al., 2019).

Among treatments, T_{12} recorded highest gross and net returns because of higher WEY. Treatment T_9 (75 % N in wheat and 100 % N in maize) recorded highest BCR due to beneficial effect of white clover in wheat-maize system in terms of biomass yield and N fixation. The mean vs. stability view of treatment effects plus treatment × environment interaction effect (TTE) biplot, T_{12} showed highest stability followed by T_9 treatment. (Kermah et al., 2017) opined that intercropping of legumes in cereals reduces the N requirement and also increases LER and economic profitability.

OC, available N and P increased in soil after two years study at both experimental locations due to inclusion of white clover in the wheat-maize cropping system. Increase in OC could be ascribed to higher root biomass additions and better soil aggregation (Hazra et al., 2019). The increased N status in soil might be due to more N fixation by white clover coupled with application of N through urea (Jones, 1992). The soils of both experimental sites were acidic in nature, which might be responsible for increased P content through P fixation.

Interactions of grass–legume stimulated acquisition of N symbiotic, N non-symbiotic and efficient transformation of N into biomass compared to monocultures (Nyfeler et al., 2011). Greater use of BNF in agricultural systems could reduce the reliance on synthetic N (Temperton et al., 2007). The symbiotic N fixation in agricultural grasslands would be an important contribution to sustainable and resource-efficient agricultural systems (Gruber and Galloway, 2008). Indeed, symbiotically fixed N in legumes ranged from 100 to 380 kg of N/ha/year, however exceptionally large amounts of more than 500 kg of N/ha/year has also been reported (Boller and Nösberger, 1987; Carlsson and Huss-Danell, 2003; Ledgard and Steele, 1992; Zanetti et al., 1997). In mixed grass–legume systems, N amounts of 10–75 kg of N/ha/year may additionally be transferred from legumes to grasses (Nyfeler et al., 2011)

5. Conclusions

Cereal–legume combinations (bi-cropping) considerably reduce the demand of N fertilizer to the associated crops and improve the soil properties. In our study, integration of white clover along nitrogen application in extant wheat-maize cropping sequence in sub temperate conditions not only increased the performance of associated cereal crops, but also improved fodder quality of white clover, system productivity, profitability and soil health. Key findings of the study are presented here under:

- The bi-cropping (white clover + wheat-maize) resulted in increased WEY by 3.2 (69.41 %) and 5.93 t/ha (315.42 %) under 100 % N application as compared to wheat-maize (0 N) and sole white clover treatments, respectively at research farm, whereas at farmer's field, the respective increment was 2.67 (56.92 %) and 5.49 t/ha (293.58 %). Application of N upto 75 % of RDF also resulted statistically at par WEY, BCR and LER with 100 % N application to bi-cropping system.
- Bi-cropping with 100 % N to wheat and maize crops increased the net returns by US $ 188.5 (farmer's field) and 224.0 (research farm) per ha, over wheat–maize (standard check). However, when N application to the system decreased upto 75 % N of RDF, the returns obtained were statistically at par with 100 % N application.
- Soil organic carbon, available N and P increased significantly in bi-cropping system over sole wheat-maize cropping sequence.

Therefore, the cereal-clover bi-cropping system along with minimum application of 75 % N of RDF can be recommended for getting higher and quality forage production in all temperate regions of the world. Further, in future the studies may be conducted in different environmental conditions to see the effect of white clover swards and bi-cropping systems on runoff & soil loss, micro climate changes and ecosystem services generated.

CRediT authorship contribution statement

Inder Dev: Conceptualization, Investigation, Supervision. **Asha Ram:** Writing-Original draft, Formal analysis. **Sudesh Radotra:** Resources. **B.K. Misri:** Resources, methodology. **Sindhu Sareen:** Conceptualization. **Pardeep Kumar:** Investigation, Formal analysis. **Deepak Singh:** Software, Formal analysis. **Sushil Kumar:** Data curation. **Naresh Kumar:** Data curation. **Ramesh Singh:** Writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eja.2021.126354.

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