Indian Journal of Agricultural Sciences **87** (11): 1487–98, November 2017/Article
https://doi.org/10.56093/ijas.v87i11.75726

Impact assessment of integrated walnut fruit sequencing in exposed subsoil on vegetative growth traits, soil quality indicators and biological diversity in rainfed ecological system

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Received: 26 March 2017; Accepted: 25 July 2017

ABSTRACT

This study monitored the impacts of integrated fruit-based cropping systems on vegetative growth traits, nutrient acquisition, microbial biomass and diversity in rhizosphere soil. Twelve cropping systems (CS) comprising Walnut-Peach-Sunhemp-Chrysanthemum (W_{PSC}), Walnut-Peach-Sunhemp-Tomato-Marigold (W_{PSTM}), Walnut-Nectarines-Sunhemp-Chrysanthemum (W_{NSC}), Walnut-Nectarines-Sunhemp-Tomato-Marigold (W_{NSTM}), Walnut-Plum- Sunhemp-Chrysanthemum ($W_{P\text{-}SC}$), Walnut-Plum-Sunhemp-Tomato-Marigold ($W_{P\text{-}STM}$), Walnut-Nectarines-Sunhemp-Chrysanthemum-Tomato-Marigold (W_{NSCTM}), Walnut-Apple-Sunhemp-Tomato-Marigold (W_{ASTM}), Walnut-Nectarines-Sunhemp-Soybean-Marigold (W_{NSSM}), Walnut-Nectarines-Sunhemp-Cucumber-Marigold (W_{NSCuM}), Walnut-Nectarines-Sunhemp-Cauliflower-Marigold (W_{NSCauM}) and Walnut-Nectarines (WN) have been demonstrated. The intercrop sequencing significantly improved vegetative growth and soil fertility indicators of exposed subsoils. Maximum moisture retention (25.2%), WHC (17.6%) and SOC (7.36 g/kg) was recorded in W_{NSCTM}. Available N, P, K, exchangeable Ca, Mg were improved by 30.1%, 34.3%, 20.5%, 92.1%, 78.3%, respectively, over walnut-nectarines. DTPA–extractable micronutrient cations (Fe, Cu, Zn, Mn) improved by 64.8%, 58%, 44.5%, 85.7%, respectively. Microbial biomass-C (MBC), microbial biomass-N (MBN), and the cultivable plate counts of *Bacillus* species, *Pseudomonas* species, soil fungi, *Azotobacter chroococcum* and actinobacteria exhibited significant variability. On an average, significantly higher cultivable microbial diversity was observed. Microbial communities of *Pseudomonas* (132.2%), *Bacillus* (141.4%), soil fungi (241.3%), *A. chroococcum* (222.1%) and actinobacteria (206.9%) improved significantly. Correlation analysis resulted in significant association ($P<0.05$). Principal component analysis (PCA) accounted for 96.1% of the total variance within CS. PCA also identified MBC, MBN, and the microbial communities as major drivers for the variability among CS. It can be inferred that integrated fruit based sequencing is a better option for improving agronomic performance in terms of nutrient supply, recycling and microbial biomass capacity to generate eco-friendly soil quality management for sustainability in rural farming ecosystem.

Key words: Biological diversity, Farm sequencing, Soil quality indicators

Sustainability for long-term cropping systems is determined by soil quality, directly related to the capacity of soil to function. Agricultural management practices such as tillage, crop rotation, stubble management and N fertilization have the potential to change soil biological indicators such as carbon mineralization and microbial biomass (Pankhurst *et al.* 2002). Despite the many benefits that no-till has on many soil properties it has inconsistent effects on soil biological properties (Bell *et al.* 2006). Traditionally, intercrop sequencing has been practised in many parts of world since long and has advantages over monoculture and sole cropping patterns (Karadag 2004). Crop sequencing with intercrops being a unique property of tropical and subtropical areas is popular now days among small farmers as it offers the possibility of yield advantage relative to sole cropping through yield stability and improved yield (Bhatti *et al.* 2006). Continuous monoculture cropping results in rapid decline in soil fertility and health, thus require a special attention. The experiment on cropping systems is the ultimate solution to overcome

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the drawbacks of mono-cropping system. Berry *et al.* (2002) investigated that cropping systems influenced plant growth and ecosystem productivity based on the natural availability of nutrients and green manure. Maximum, water holding capacity, moisture content, lower bulk density, and build-up of nutrient stocks can be obtained, with a favorable impact on vegetable production (Bulluck *et al.* 2002), pH stabilization and variation in soil microbial communities in organic cropping systems (Vogel *et al.* 2009). There is a need to identify walnut-based integrated fruit cropping systems that do not jeopardize the capacity of the soil to function over the long-term. Cropping systems with extended intercrop rotations with multiple crops offer the potential to meet this need, but information on how they impact soil fertility is scarce. The present study therefore, was conducted to assess the impact of various walnut based cropping systems on the soil health, in terms of SOC and other post harvest fertility indicators. The second objective of the investigation is to identify the change in nutrient dynamics and biological potential of the soil, eventually be considered as determinants of soil health and productivity to better understand the potentials on cropping behavior in rainfed ecosystem in the north-west Himalaya of India.

MATERIALS AND METHODS

The experiment was conducted on the Integrated Horticulture Model Farm' at the Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India (30°50'30" North latitude and 77°88'30" East longitude) for two consecutive years from 2012-2013. The site is located at an altitude of 1100 m above mean sea level, was under continuous fallow before the establishment of the experimental platform. The climate of the experimental area was typically a sub-temperate, wet and humid (north-west Himalaya). Heavy rainfall occurs in the monsoon and is scarce in other times. The southwest monsoon usually sets in June and withdraws in the end of September. July and August are the wettest months, contributed more than 75% to the annual rainfall in the region. From late October to mid of March, the minimum and maximum temperatures were in the lowest range, whereas, from mid of March onward up to mid of October, temperature was in the maximum range. However, the highest maximum temperature was recorded in May. The transition period from September to October forms the post-monsoon season. The periods from October to May are virtually dry.

The predominant soil at the site is texturally sandy loam with an average particle-size distribution for the surface 15 cm soil depth of 36.2% sand, 27.8% silt and 29% clay. Water holding capacity (WHC) and moisture content at field capacity (M_cF_c) at 15 cm depth were 28.8% and 21.1%, respectively. The soil used for experiment was towards neutral (pH 6.86) in reaction with low electrical conductivity (EC, 0.12 d/Sm), 4.00 g/kg of soil organic carbon (SOC). The site had an initial available N (110.3 mg/kg), available P ($NaHCO_3$-extractable, 4.04 mg/kg) and available K (101.6 mg/kg). Diethylenetriaminepentaacetic acid (DTPA)–extractable micronutrient cations in the soil were in sufficiency range namely, zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu) were 1.38, 41.9, 54.1 and 1.74 mg/kg, respectively. The experimental soil also exhibited an initial viable microbial plate counts (colony forming units, CFU/g of the moist soil) of *Bacillus* species (5.2×10^4), *Pseudomonas* species (12.7×10^4), soil fungi (12.2×10^4), *Azotobacter chroococcum* (11.3×10^4) and actinobacteria (11.4×10^4) along with very-very less indigenous viable AM fungal spore population.

The study was applied in an integrated experimental and modeling approach, where measurements of the key indicators of productivity and nutrient cycling were carried out. Multiple fruit-based crop sequencing with walnut test crop included the trees of uniform age group (2 year) were spaced at distance of 8 m × 8 m apart. The trees were receiving the current applications for nutrition and other horticultural package of practices, irrigated at field capacity level through drip fertigation system with four emitters per plant with 8 liter discharges per emitter per h. At every winter season, the trees were prune to remove weak and dead limbs and trained with proper shape.

Twelve integrated fruit-based cropping system treatments in crop sequence (CS) and residue management were evaluated. The main CS components (companion crops) were *Chrysanthemum indicum* L. (chrysanthemum), *Solanum lycopersicum* L. (tomato), *Tagetes erecta* L. (marigold), *Cucumis sativus* L. (cucumber), cauliflower (*Brassica oleracea* var *Botrytis* cultivar group) and soybean [*Glycine max* (L). Merr.]. CS treatment combinations were comprised of Walnut-Peach-Sunhemp-Chrysanthemum (W_{PSC}), Walnut-Peach-Sunhemp-Tomato-Marigold (W_{PSTM}), Walnut-Nectarines-Sunhemp-Chrysanthemum (W_{NSC}), Walnut-Nectarines-Sunhemp-Tomato-Marigold (W_{NSTM}), Walnut-Plum-Sunhemp-Chrysanthemum ($W_{P\text{-}SC}$), Walnut-Plum-Sunhemp-Tomato-Marigold ($W_{P\text{-}STM}$), Walnut-Nectarines-Sunhemp-Chrysanthemum-Tomato-Marigold (W_{NSCTM}), Walnut-Apple-Sunhemp-Tomato-Marigold (W_{ASTM}), Walnut-Nectarines-Sunhemp-Soybean-Marigold (W_{NSSM}), Walnut-Nectarines-Sunhemp-Cucumber-Marigold (W_{NSCuM}), Walnut-Nectarines-Sunhemp-Cauliflower-Marigold (W_{NSCauM}) and Walnut-Nectarines (WN). The test crop plum was not performed well in the intercropping. Crop sequencing with intercrops was performed very well continuously. CS treatments for the whole plots were considered. Each CS was replicated thrice in the production scale plots, each 3 m × 4 m size with 1 m between plots and 2 m between blocks, in a randomized complete block design. All CS treatments were cultivated with standard management practice as recommended in mid hill sub-temperate ecosystem. The intercrop components were planted in the space between two rows. Sunhemp (*Crotalaria juncea* L.), a green manure crop was sown in July in the respective CS in the first year only. The subsequent intercrop, chrysanthemum (cv. Yellow Star) was transplanted as cuttings (5-7 inch length) in

February; marigold (cv. Pusa Narangi) was transplanted at 40 cm × 40 cm under intensive cultivation conditions in February; seeds of tomato (cv. Solan Gola) were sown at 90 cm × 30 cm in March (0.500 kg/ha), soybean (local variety) shown in April (70-75 kg/ha); seeds of cucumber (cv. KH-1) were sown at 50 cm × 75 cm in April (3-4 kg/ha) and cauliflower (cv. Pusa Snowball 1) was transplanted at 60 cm × 45 cm in July (0.600 kg/ha) in the represented CS. All the experimental plots were kept fixed during the entire period of study.

Walnut trees were supplemented with 20 kg of farm yard manure (FYM), 500 g of NPK mixed fertilizes (19:19:19) was applied in December at 30 cm away from the tree trunk as broadcast application. The intercrop components were supplemented with the fertilizers calcium ammonium nitrate (CAN), single super phosphate (SSP) and muriate of phosphate (MOP) with 30 g/m^2 each of N (half of N as basal dose and the remaining half dose after 45 days of planting was applied), P_2O_5, and K_2O in chrysanthemum; 400 kg ha−1 CAN, 475 kg ha−1 SSP, 90 kg ha−1 MOP in tomato; 600 kg/ha CAN, 1200 kg/ha SSP, 333 kg/ha MOP in marigold; 15-20 tonnes/ha FYM, 50-60 kg/ha MOP in soybean; 400 kg/ha CAN, 315 kg/ha SSP, 100 kg/ha MOP in cucumber, and 600 kg/ha CAN, 625 kg/ha SSP, 90 kg/ha MOP in cauliflower. Irrigation and other intercultural operations were carried out as and when necessary. At harvest of each intercrop, the stubbles were obtained from the residues, including the roots. All the loose and anchored (stubbles) residues of all intercrop after each harvest were retained in all the plots. Following the line sowing of chrysanthemum in W_{PSC}, W_{NSC} and $W_{P\text{-}SC}$, tomato and marigold in W_{PSTM} and W_{ASTM}, chrysanthemum, tomato, marigold in W_{NSCTM}, soybean and marigold in W_{NSSM}, cucumber and marigold in W_{NSCuM}, cauliflower and marigold in W_{NSCauM} into the left str over of these crops, all the residues were lying on the soil surface. The amount of crop residues either retained on the soil surface or in the soil was further recycled and incorporated naturally.

Plant growth traits were recorded annually in September during two years of the study. Tree spread was determined according to Westwood (1978). Plant canopy volume was calculated using the formula 'πr^2' in which radius mean was measured from all the directions of the crown. The representative sample size of fully expanded and matured leaves over the tree canopy was taken for the leaf area estimation on leaf area meter model LI-COR-3100, expressed in square centimeter (cm^2). Total chlorophylls (/g fresh weight) of walnut leaves were estimated according to Halfacre *et al*. (1968).

Composite soils samples (1 kg each) on transect of each plot at 0-30 cm soil depth taken with three replicate cores were collected. Essential soil physico-chemical and biological parameters that are relevant to soil fertility indicators were analyzed using standard laboratory analytical procedures. Soil moisture, and WHC were estimated according to Keen-Raczkowski Box method suggested by Piper (1966). The textural class was determined according to the United States Department of Agriculture (USDA) system. Samples were analyzed for pH (1:2.5 H_2O), EC, SOC (wet oxidation method, Walkley and Black 1934), available N (Subbiah and Asija 1956), Olsen P (0.5 M $NaHCO_3$ extractable pH 8.5, Olsen et al. 1954), available K (1N neutral NH_4OAc extractable K emission by spectrophotometer suggested by Merwin and Peach 1951), meso-nutrients (exchangeable Ca, Mg) according to ammonium acetate method (Black 1957). DTPA extractable (0.005 M) micronutrient cations (Fe, Cu, Zn, Mn), buffered at pH 7.3±0.05 were estimated according to Lindsay and Norvell (1978), and then analyzed using atomic absorption spectrophotometer model-4141. Soil microbial biomass carbon (MBC) was estimated using chloroform-fumigation incubation method (Jenkinson and Ladd 1981). MBC was determined as per the equation: $MB_C = (F_c\text{-}UF_c)/K_c$, where, F_c=CO_2 evolved from fumigated soil, UF_c=CO_2 evolved from unfumigated soil, K_c=0.45. Similarly, the amount of soil microbial biomass nitrogen (MBN) was calculated as per the equation: $MB_N = (F_{N\text{-}UFN})/K_N$, where, F_N=NH_4-N mineralized during 10 days from fumigated soil, UF_N=NH_4-N mineralized during 10 days from unfumigated soil, K_N=0.54 (Jenkinson 1988).

To estimate cultivable microorganisms, the plate count method, soil sample dilutions were prepared by adding field-moist 10 g of soil to 90 ml of sterile distilled water. Each soil sample was analyzed in two replicates. Suspensions were homogenized for 1 h on a horizontal shaker. After that serial dilutions were prepared, and 0.1 ml of dilutions 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilution were used. One ml of each sample dilution was spread on specified medium (Subba Rao 1986). Pure and viable microbial strains from the roots and rhizosphere soil of field-grown walnut trees of *Pseudomonas* and *Bacillus* species, soil fungi, actinobacteria and *A. chroococcum* were isolated on nutrient agar medium, Martin's Rose Bengal medium, Kenknight and Munaires medium and Jensen's N-free agar medium (Jensen 1954), respectively. The plate counts for bacterial and fungal population (CFUs) were assessed after 2-4 days of the incubation at 25±2°C, whereas, for actinobacteria, the incubation was done at 28oC for 10-14 days and were expressed g^{-1} soil. AM fungal spores present in the soil samples of field under different intercrop sequencing of were recovered through wet sieving and decanting (Gerdemann and Nicolson 1963), spore count via most probable number method (Porter 1979) and Gaur and Adholeya (1994). The colonization on roots was measured according to the gridline intersect method (Giovannetti and Mosse 1980).

Statistical analyses of the data using general linear model of the standard errors of the mean were carried out. Cropping systems were treated as fixed factors and replications were treated as random. The mean values for the respective parameter in different cropping system were tested at the 5% level of probability, wherever the results were significant. Duncan Multiple Range Test (DMRT) was also tested for each soil quality indicator to compare with its value under different systems of intercrop sequencing studied for multiple comparisons at $P < 0.05$ was carried

out according to DSAASTAT version 1.101 (Onofri 2007). The principal component analysis (PCA) through which the number of independent variables could be reduced and problems of multi-collinearity solved, were worked out according to XLSTAT version 2014.6.02. PCA of soil fertility indicators was also worked out to evaluate the differences induced in CS. The graphical interpretation is done by constructing biplots to map the original variables into principal component (PC) space because the angles between variables express their correlation, with the original variables drawn as Eigen vectors that summarize the correlation between the variable and both illustrated axes. Sample values are then projected into the new PC by computing PC scores for each sample CS.

RESULTS AND DISCUSSION

Morphometric traits and total leaf chlorophylls

Plant height, shoot length and stem girth of walnut trees was recorded in September annually. Among different intercrop sequencing systems, the plant height significantly increased in over Walnut-Nectarines CS (Table 1). The CS W_{NSCTM} produced plants with maximum height (1.68 m) followed by W_{NSTM}, W_{PSTM}, W_{PSC} compared to WN sequencing. W_{SCTM} resulted in 34.4% higher plant height over walnut-nectarine CS. Contrary to this, W_{NSCTM} displayed significant annual shoot growth and stem girth in an intermediate value in compared to other CS treatments studied. Maximum annual shoot growth and stem girth (39.4 cm, 24.9 cm) was exhibited in W_{NSCTM} followed by W_{PSTM} (38.7 cm, 23.8 cm) and W_{NSTM} (37.5 cm, 23.5 cm). The number of leaves were significantly increased in over walnut-nectarine CS. Maximum increase in canopy volume (144.7%) and leaf area (42.4%) was recorded in W_{NSCTM} CS compared to walnut-nectarine CS. Total leaf chlorophyll content was also significantly influenced with W_{NSCTM} (7.41 mg/g) followed by $W_{P\text{-}STM}$ (6.85 mg/g), W_{PSTM} (6.81 mg/g) and W_{PSC} (6.75 mg g^{-1}). Maximum nutrients availability especially N, stimulated growth and increased intermodal length through increased plant height due to better root development and uptake of water and nutrients and the production of growth hormones (Nazeri *et al.* 2010). In the earlier studies, the beneficial effects of intercrop sequencing have been well documented, cauliflower (Yildirim and Guvenc 2005), cabbage (Guvenc and Yildirim 2006), cucumber (Yildirim and Guvenc 2004), indicated that different CS with different vegetable crops was more productive and profitable due to their complementary effects than monoculture CS. Addition of fertilizers and irrigation to intercrops ameliorated nutrient and moisture content in CS which further enhanced tree growth (Shweta *et al.* 2015). Jose *et al.* (2004) reported that deeper roots of trees acted as a safety net by capturing nutrients that leached below the rooting zone of the crops and recycled them back into the system by tree component. Other studies revealed that yield are influenced by edaphic factors (Padmapriya and Chezhiyan 2009).

Soil texture, moisture content and water holding capacity

Sand, silt, clay, moisture content and water holding capacity were significantly influenced by different CS (Fig 1). Variability in water holding capacity ascribed generally to the fluctuation in variable retention of moisture content and plant-soil relationship in different CS. Improved

Table 1 Growth traits and tot al chlorophylls in walnut based multiple crop sequencing

Cropping treatment	Plant height (m)	Tree spread (m)		Shoot growth (cm)	Stem girth (cm)	Canopy volume (cm^3)	Number of leavers/shoot	leaf area (cm^3)	Total chloro-phylls (mg/g)
		E-W	N-S						
W_{PSC}	1.52	1.73	1.54	34.9	21.4	34.0	24.2	11.9	6.75
W_{PSTM}	1.54	1.87	1.68	38.7	23.8	37.9	25.3	12.5	6.81
W_{NSC}	1.50	1.79	1.60	36.8	20.7	32.5	23.5	13.4	6.73
W_{NSTM}	1.58	1.84	1.65	37.5	23.5	39.4	24.4	12.2	6.55
$W_{P\text{-}SC}$	1.43	1.61	1.42	30.6	15.9	23.8	19.2	13.1	6.51
$W_{P\text{-}STM}$	1.41	1.59	1.40	31.7	15.4	22.7	19.7	13.9	6.85
W_{NSCTM}	1.68	1.92	1.73	39.4	24.9	43.8	26.6	14.1	7.41
W_{ASTM}	1.46	1.63	1.44	32.3	17.6	26.9	21.5	13.4	6.33
W_{NSSM}	1.48	1.70	1.51	35.6	19.7	30.5	22.2	12.2	6.25
W_{NSCuM}	1.47	1.66	1.47	33.8	18.2	28.0	21.3	12.9	6.31
W_{NSCauM}	1.44	1.64	1.45	33.3	17.4	26.2	20.6	13.9	6.55
Walnut-Nectarines	1.25	1.28	1.09	26.1	13.7	17.9	17.5	9.9	4.91
LSD (p=0.05)	0.08	0.13	0.13	2.91	2.77	5.83	2.09	0.89	0.45

W_{PSC}, Walnut-Peach-Sunhemp-Chrysanthemum; W_{PSTM}, Walnut-Peach-Sunhemp-Tomato-Marigold; W_{NSC}, Walnut-Nectarines-Sunhemp-Chrysanthemum; W_{NSTM}, Walnut-Nectarines-Sunhemp-Tomato-Marigold; $W_{P\text{-}SC}$, Walnut-Plum- Sunhemp-Chrysanthemum; $W_{P\text{-}STM}$, Walnut-Plum-Sunhemp-Tomato-Marigold; W_{NSCTM}, Walnut-Nectarines-Sunhemp-Chrysanthemum-Tomato-Marigold; W_{ASTM}, Walnut-Apple-Sunhemp-Tomato-Marigold; W_{NSSM}, Walnut-Nectarines-Sunhemp-Soybean-Marigold; W_{NSCuM}, Walnut-Nectarines-Sunhemp-Cucumber-Marigold; W_{NSCauM}, Walnut- Nectarines-Sunhemp-Cauliflower-Marigold; East; W, West

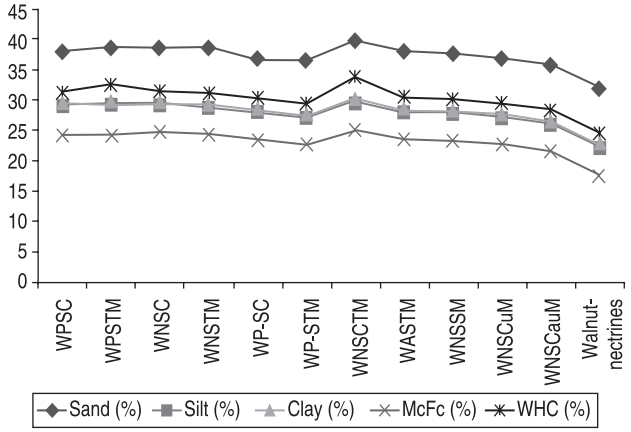

Fig 1 Post-harvest soil physical indicators under integrated fruit based crop sequencing systems of exposed subsoil in the 0-30 cm soil profile

moisture retention and WHC in different CS by ornamentals and their residues influenced growth traits positively through increased N-uptake by the trees. Highest and lowest moisture content and water holding capacity was observed in W_{NSCTM} and walnut-nectarine. Contrary to this, none of the tillage methods have beneficial effects on soil moisture content (Gicheru *et al.* 2004, Landers 2008). The crop residues incorporation in CS improved soil texture and moisture retention capacity of the surface soil (Sarkar *et al.* 2003). High clay content of soils also retained with maximum organic matter. Intercrop sequencing system is generally more productive than monoculture cropping system (Zhang *et al.* 2007, Ijoyah *et al.* 2013) which could be a way to save water and retain more moisture level and increased water holding capacity of subsoil (Tsubo *et al.* 2005). Grant *et al*. (2002) noticed cropping systems that significantly reduce tillage could conserve moisture level to support continuous cropping, particularly when rotations include deep rooted crops that diversify both SOM contributions and resource uptake.

Soil pH, EC and OC

The mean values of soil chemical indicators were recorded the highest in W_{SCTM} which further reduced through W_{PSTM}, W_{PSC}, while, the WN CS has the least mean value (Table 2). The significant effect of CS on soil pH and EC but the differences were very small. CS treatments changed pH of the soil towards neutral. The intercrop sequencing in W_{NSCTM} had a pH of 7.1, which was closest towards neutrality in compared to walnut-nectarine CS with pH of 6.7. The increase of pH could be explained by the large amount of plant debris which improved soil pH by the decomposition of the protein to ammonium in the CS (Petcu *et al.* 2014). Contrary to this, Hulugalle and Weaver (2005) recorded a decrease of pH resulted from the decomposition of crop residues on account of the production of organic acids and microbial respiration. CS treatments were also effective in decreasing soil EC. The data presented on SOC content indicated that it is significantly increased due varied intercrops after all the crop sequences tested. Maximum soil OC build up was recorded in W_{SCTM} and increased when the crop residues are incorporated through total decay into the soil. In general, the extent of SOC was higher when sunhemp, chrysanthemum, tomato and marigold were intercropped along with the test crop. Maximum SOC increased by 84% in W_{NSCTM} followed by W_{PSC} (73.5%), W_{PSTM} (71%), W_{PSTM} (70.5%) and W_{NSCuM} (70.3%) CS over initial, ascribed to the accumulation of root residues and shedding of leaves by the intercrop used.

Available macro-, meso- and micronutrients cations

W_{NSCTM} recorded the maximum available macro- (N, P, K), meso- (exchangeable Ca, Mg) and DTPA-extractable micronutrient cations (Fe, Cu, Zn, Mn) followed by W_{PSTM}, W_{NSTM} and W_{PSC} compared to Walnut-Nectarine CS which recorded the least (Table 2). The initial available N content of the soil was 110.3 mg/kg. Progressive increase in available N status after harvest was observed with the inclusion of CS crops in the rotation. Among the CS tested, the availability of N significantly improved being the highest in W_{NSCTM} (39.6%) followed by W_{PSTM} (35.5%), W_{NSTM} (34.9%) and W_{PSC} (34.3%) over initial. The comparison of available P (13.9 mg/kg) and available K (135.4 mg/kg) under different CS treatments showed that W_{NSCTM} exhibited maximum values for these chemical indicators compared to walnut-nectarine CS. The result also showed that in W_{NSCTM} CS, the availability of exchangeable Ca and Mg content increased with corresponding values of 92.1% and 78.3% over walnut-nectarine CS. Concerning to the availability of DTPA extracted Fe, Cu, Zn, Mn; W_{NSCTM} increased 64.8%, 58%, 44.5%, 85.7%, respectively, over walnut-nectarine CS. Furthermore, the improved N_2 fixation is also incorporated in crop rotations using mixed cropping systems. It is well documented that the preceding crop species can have a beneficial or detrimental effect on the performance of the succeeding crop. The well known beneficial effects of preceding crop are also found in multiple cropping systems varies with management practices (Porpavai *et al.* 2011). Crop residue is more important than residue management practices affected N mineralization in soils (Kumar and Goh 2000). The cultivation of sunhemp as green manure legume is viewed more as a soil fertility improver than as independent crops grown for growth measurements output due to self-sufficiency in N supply. The results also demonstrated that among different CS, the mineralization processes were stronger, the fact that required a stricter control of the soil supply with nutritive elements; they changed rapidly under the influence of soil compaction and other technological processes that tended to increase with higher temperature and moisture level in the soil (Bolinder *et al.* 2007). Moreover, CS with soybean obtained 80% of its total nitrogen requirement from biological nitrogen fixation (Salvagiotti *et al.* 2008).

Table 2 Post-harvest indicators of exposed subsoil in the 0-30 cm soil profile under integrated fruit based farm sequencing

Cropping treatment	pH	EC (dS/m)	OC (g/kg)	MB_C (mg/kg)	MB_N (mg/kg)	MB_C/MB_N	Available macronutrient (mg/kg)			Exchangeable cations (mg/kg)		DTPA micronutrient cations (mg/kg)			
							N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
W_{PSC}	7.02	0.154	6.94	280.6	19.4	14.5	148.1	12.4	130.4	25.3	24.5	64.2	2.21	1.72	52.1
W_{PSTM}	7.05	0.163	6.84	285.9	18.7	15.3	149.5	12.0	132.3	28.3	27.6	69.3	2.40	1.90	56.7
W_{NSC}	6.96	0.144	6.63	279.1	18.6	15.0	145.2	12.8	129.5	24.8	23.3	60.5	2.19	1.78	50.8
W_{NSTM}	6.94	0.165	6.36	268.3	16.3	16.5	148.9	11.9	131.2	26.9	25.2	65.3	2.38	1.84	54.3
$W_{P\text{-}SC}$	6.92	0.154	6.74	286.9	17.6	16.3	137.9	12.2	118.4	18.9	17.2	55.3	1.85	1.48	40.0
$W_{P\text{-}STM}$	7.04	0.153	6.78	277.8	17.7	15.7	133.9	11.1	116.2	18.0	17.2	55.0	1.80	1.44	39.4
W_{NSCTM}	7.10	0.152	7.36	329.7	19.9	16.6	154.0	12.9	135.4	29.4	28.7	70.7	2.56	1.98	59.6
W_{ASTM}	6.97	0.143	6.82	268.3	16.8	16.0	143.9	10.5	126.3	23.1	22.4	58.0	2.06	1.62	44.9
W_{NSSM}	6.92	0.166	6.72	282.7	17.4	16.2	144.0	11.8	127.6	24.6	23.1	60.5	2.11	1.69	49.8
W_{NSCuM}	6.90	0.157	6.81	279.3	18.8	14.9	139.5	11.2	124.2	21.8	20.3	59.4	2.01	1.60	42.9
W_{NSCauM}	6.89	0.155	6.69	257.2	15.8	16.3	138.8	10.8	120.2	19.5	18.4	57.7	1.97	1.58	40.4
Walnut-Nectarines	6.70	0.163	5.98	136.3	9.9	13.8	118.3	9.6	112.3	15.3	14.1	42.9	1.62	1.37	32.1
LSD (*P=0.05*)	0.08	0.01	0.25	34.9	2.02	0.69	7.21	1.07	5.51	3.37	3.44	5.68	0.21	0.14	6.35

W_{PSC}, Walnut-Peach-Sunhemp-Chrysanthemum; W_{PSTM}, Walnut-Peach-Sunhemp-Tomato-Marigold; W_{NSC}, Walnut-Nectarines-Sunhemp-Chrysanthemum; W_{NSTM}, Walnut-Nectarines-Sunhemp-Tomato-Marigold; $W_{P\text{-}SC}$, Walnut-Plum- Sunhemp-Chrysanthemum; $W_{P\text{-}STM}$, Walnut-Plum- Sunhemp-Tomato-Marigold; W_{NSCTM}, Walnut-Nectarines-Sunhemp-Chrysanthemum-Tomato-Marigold; W_{ASTM}, Walnut-Apple-Sunhemp-Tomato-Marigold; W_{NSSM}, Walnut-Nectarines-Sunhemp-Soybean-Marigold; W_{NSCuM}, Walnut-Nectarines-Sunhemp-Cucumber-Marigold; W_{NSCauM}, Walnut-Nectarines-Sunhemp-Cauliflower-Marigold; EC, electrical conductivity; OC, organic carbon; MB_C, microbial biomass carbon; MB_N, microbial biomass nitrogen.

Soil microbial biomass

The experimental results showed that MB_C and MB_N were significantly influenced under different CS systems (Table 2). Among different CS, MB_C was highest (329.7 mg/kg) in W_{NSCTM} followed by 286.9 mg/kg in $W_{P\text{-}SC}$, 285.9 mg kg^{-1} in W_{PSTM} and 282.7 in W_{NSSM}. MB_C content are mostly higher under W_{NSCTM} CS ascribed to more crop residues under this cropping system coupled with more microbial incorporation and/or decomposition. Past reports have also described that microbial communities provided useful data to study both applied and basic environmental events (Pankhurst *et al.* 2002). Moreover, the stubble retention from each intercrop grown in different CS increased soil microbial activity and biomass (Gupta and David 2003). Changes in residue management altered MB_C. The studies also demonstrated that larger root systems and greater MB_C caused greater soil CO_2-C emissions from sub-soil surfaces with chrysanthemum, marigold and soybean cropping systems than sub-soil with monoculture rotation. This difference in emissions attributed to a larger MB_C pool, which led to greater OC mineralization in sub-soils within cropping systems (Kaisi and Grote 2006). The amount of MB_C within the pool of SOC increased with time and drive the balance between the release of SOC and its sequestration in soil organic matter (Lange *et al.* 2015) and therefore, altered the amount of soil microbial biomass that changed carbon dynamics in soil (Bardgett *et al.* 2008). Similarly, MB_N content of the soil was also recorded significantly higher in different intercrop sequenced plots. The average MB_N content under W_{NSCTM}, W_{PSC}, W_{PSTM} and W_{NSC} were 19.9, 19.4, 18.7, 18.6 mg/kg respectively. Comparatively, the W_{NSCTM} CS had 82.6% higher soil MB_N content as compared to those observed under walnut-nectarine CS. MB_N content in soil improved N mineralization and nutrient cycling due to microbial activities in rhizosphere soil. Furthermore, these results are also in line with those of Daniel *et al.* (2013) who reported that when tomato crop grown after bean had a highest microbial biomass carbon and nitrogen in comparison with other cropping systems ascribed to the high fresh biomass incorporated in the bean plot before the plantation of main crop. The MB_C/MB_N ratio is an indication of the relative proportion of fungi to bacteria. Consequently, the wider MB_C/MB_N of W_{NSCTM} (16.6) would suggest that W_{NSCTM} have a greater proportion of fungal compared to bacterial biomass than walnut-nectarines ascribed to high available nutrient content in the soil under different CS.

Microbial communities' structures in rhizosphere soils

The effect of different integrated fruit-based CS on the total cultivable microbial population is presented in Fig 2. The plate count of *Pseudomonas* spp., *Bacillus* spp., *A. chroococcum*, soil fungi and actinobacteria was significantly improved which was varied between Walnut-Nectarine and W_{NSCTM} with respective values of 12.7×10^4 -29.5×10^4 CFU/g soil, 8.2×10 -19.8×10^4 CFU/g soil, 11.3×10 -36.4×10^4 CFU/g soil, 9.2×10^4-31.4×10^4 CFU/g soil and 12.5×10^4-39.2×10^4 CFU/g soil. Similarly, AM fungal spore population ranged between 197.2 and 421.2 per 100 g soil. Walnut-Nectarine CS had only 9.2% colonization (Fig 3) which ascribed to the variation in effectiveness due to viable AM fungal spore count differences (Sharma *et al.* 2015). In this study, the cultivable fungi ($\leq 10^4$ CFU/g) in CS even if they are a versatile group able to adapt and grow under extreme environmental conditions (Anand *et al.* 2006). It is well documented that soil microorganisms governed the numerous nutrient cycling reactions in soils; the higher mineral availability due to higher microorganism activities. P and K fluxes through the microbial biomass are faster in organic soils, and more minerals are normally bound in the microbial biomass (Chirinda *et al.* 2008). Velmourougane (2016) confirmed the long-term impacts of organic and conventional methods on soil physical, chemical, biological, and microbial diversity in coffee farming. Indigenous microbial count in soil is of fundamental importance for ecosystem functioning in managed agricultural soils due to soil structure formation, organic matter decomposition and nutrient cycling.

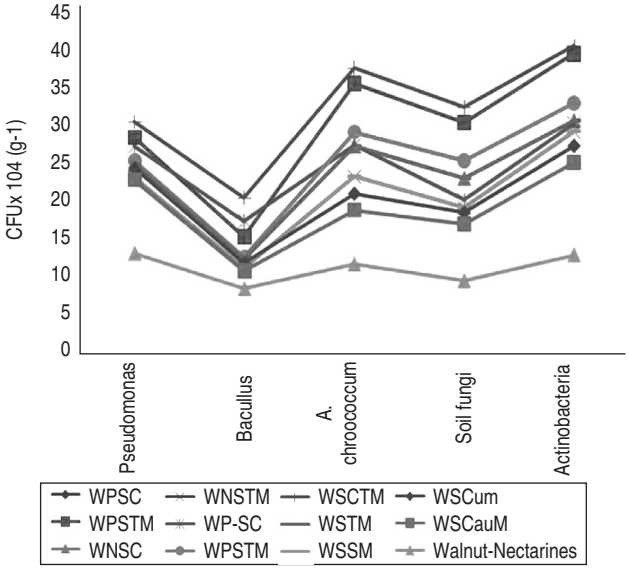

Fig 2 Soil microbial communities (between 1 to 5% of the total microbial count cultured on artificial media) in integrated fruit based multiple crop sequencing.

Correlation matrix among soil quality attributes

Correlation matrix of the different soil chemical and biological attributes resulted in a significant correlation (P□0.05) (Table 3). In general, the Pearson's correlation coefficients relations between moisture content, WHC with soil pH, SOC, MB_C and MB_N, available N, P, K, exchangeable Ca, Mg and micronutrient content has shown positive and significant correlation among all physical-chemical and biological soil indicators correlated. Further, available N, P, K, Ca, Mg, Fe, Cu, Zn and Mn content in the soil had positive and significant impact on the total number of actinobacteria, soil fungi, AMF and root colonization, whereas, it was positive and

Table 3 Correlation matrix (Pearson, n) of soil quality parameters in integrated fruit based farm sequencing

Variables	McF_C	WHC	pH	EC	OC	Soil MB_C	Soil MB_N	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	P_{smas}	*Bacillus*	A_{Chro}	Soil fungi	Actino	AMF	RC
McF_C	**1**																						
WHC	**0.966**	**1**																					
pH	**0.858**	**0.899**	**1**																				
EC	-0.321	-0.249	-0.323	**1**																			
OC	**0.725**	**0.783**	**0.850**	-0.396	**1**																		
Soil MB_C	**0.914**	**0.901**	**0.874**	-0.291	**0.876**	1																	
Soil MB_N	**0.901**	**0.883**	**0.870**	-0.334	**0.867**	**0.952**	**1**																
N	**0.940**	**0.948**	**0.808**	-0.157	**0.717**	**0.855**	0.826	1															
P	**0.745**	**0.813**	**0.627**	0.055	0.497	**0.605**	**0.619**	**0.859**	**1**														
K	**0.836**	**0.873**	**0.680**	-0.058	0.583	0.681	0.692	0.944	0.942	1													
Ca	**0.822**	**0.880**	**0.698**	0.018	0.571	**0.676**	**0.672**	**0.935**	**0.947**	**0.992**	**1**												
Mg	**0.813**	**0.881**	**0.728**	-0.009	**0.602**	**0.673**	**0.673**	**0.930**	**0.936**	**0.986**	**0.996**	**1**											
Fe	**0.874**	**0.931**	**0.823**	-0.012	0.718	0.824	0.809	0.967	0.898	0.932	0.944	0.946	1										
Cu	**0.805**	**0.875**	**0.705**	0.008	**0.584**	**0.682**	**0.655**	**0.934**	0.960	0.974	0.984	0.981	0.957	1									
Zn	**0.768**	**0.847**	**0.658**	0.020	0.536	**0.633**	**0.616**	**0.902**	**0.975**	**0.969**	**0.980**	**0.976**	0.931	0.991	1								
Mn	**0.840**	**0.899**	**0.740**	0.025	**0.588**	**0.700**	**0.693**	**0.935**	**0.963**	**0.977**	**0.991**	**0.987**	**0.952**	**0.982**	0.978	1							
P_{smas}	**0.878**	**0.917**	**0.910**	-0.343	**0.889**	**0.953**	**0.919**	**0.812**	0.571	**0.641**	**0.651**	**0.662**	**0.808**	**0.665**	**0.621**	**0.678**	1						
Bacillus	**0.613**	**0.738**	**0.692**	-0.255	**0.773**	**0.710**	**0.634**	0.563	0.466	0.455	0.488	0.501	**0.591**	0.530	0.500	0.526	**0.831**	1					
A_{Chro}	**0.824**	**0.912**	**0.947**	-0.308	**0.820**	**0.813**	**0.809**	**0.765**	**0.633**	**0.675**	**0.706**	**0.735**	**0.788**	**0.702**	**0.674**	**0.745**	**0.914**	0.827	1				
Soil fungi	**0.802**	**0.895**	**0.967**	-0.319	**0.843**	**0.802**	**0.815**	**0.772**	**0.660**	**0.697**	**0.723**	**0.760**	**0.811**	**0.725**	**0.698**	**0.759**	0.891	0.766	0.985	1			
Actino	**0.885**	**0.949**	**0.973**	-0.240	**0.850**	**0.900**	**0.884**	**0.850**	**0.680**	**0.735**	**0.762**	**0.783**	**0.873**	**0.760**	0.725	0.795	0.953	0.770	0.973	0.970	1		
AMF	**0.657**	**0.817**	**0.858**	-0.169	**0.763**	**0.661**	**0.654**	**0.688**	**0.683**	**0.674**	**0.722**	0.763	0.770	0.735	0.728	0.755	0.787	0.796	0.937	0.952	0.894	1	
RC	**0.710**	**0.845**	**0.853**	-0.209	**0.852**	**0.770**	**0.717**	**0.723**	**0.676**	**0.670**	**0.705**	**0.732**	**0.778**	**0.739**	**0.711**	**0.739**	**0.850**	**0.909**	**0.917**	**0.913**	**0.889**	**0.942**	1

Values in bold are different from 0 with a significance level alpha=0.05; M_cF_c, moisture content at field capacity; WHC, water holding capacity; MB_C, microbial biomass carbon; MB_N, microbial biomass nitrogen; P_{smas}, *Pseudomonas*; A_{Chro}, *A. chroococcum*; Actino, actinobacteria; AMF, arbuscular mycorrhizal fungi; RC, root colonization.

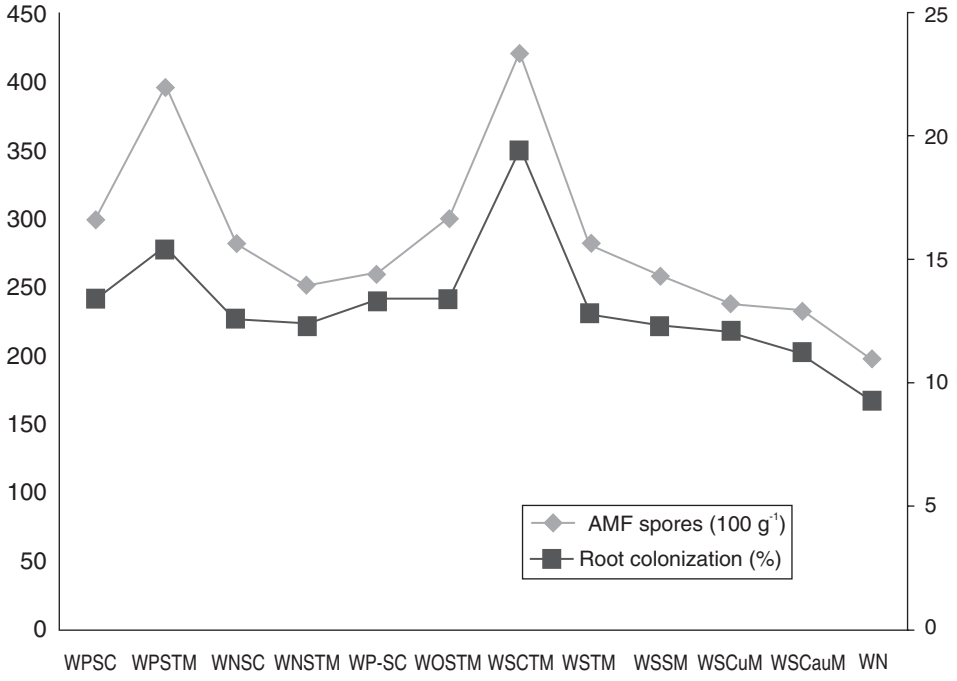

Fig 3 Variability in AM fungal spore population and root colonization in integrated fruit based multiple crop sequencing.

significant between N and K with *Pseudomonas* count. In other investigations, positive impact of phosphorus was observed on soil saprophytic microfungi but negative on species richness of AMF in soil macrocosms (Huang *et al.* 2005). Such contradiction can be explained by the fact that the number of phosphate solubilizing microorganisms (*Pseudomonas* spp.) vary from soil to soil (Gyaneshwar *et al.* 2002). The present investigation has evidenced that this beneficial effect is variable AMF and percent root colonization suggesting fungal diversity in decreasing the adverse conditions for plant growth in acidic to neural soil. Borie *et al.* (2006) have reported the positive effect of CS on soil characteristics such as pH, EC, available N, P contents, levels of some P forms associated with organic matter, and its relationship with AM propagules, such as root colonization, spore number and active hyphal length in an Ultisol. In the present study, MB_C and MB_N showed a positive and significant correlation with *Pseudomonas* count (r=0.953, 0.919), *Bacillus* (r=0.710, 0.634), *A. chroococcum* (r=0.813, 0.809, soil fungi (r=0.802, 0.815), actinobacteria (r=0.900, 0.884), AM spore population (r=0.661, 0.654) and root colonization (r=0.770, 0.717). The data also showed the strong positive and significant relationship among all microbial plate count with available N, P, K, exchangeable Ca, Mg and Fe, Cu, Zn, Mn. The perusal of the correlation data indicated that AM spore population and root colonization had positive and significant correlation with available N, P, K, Ca, Mg, Fe, Cu, Zn and Mn content. Similarly, both AM spore population and per cent root colonization was also positively and significantly correlated with *Pseudomonas*, *Bacillus*, *A. chroococcum,* soil fungi and actinobacterial count. In our study, there is a significant positive relationship between soil organic C and total N. The microbial biomass N showed a significant positive correlation with microbial biomass C which coincides with the findings of Sharma *et al.* (2004) and Wright *et al*. (2005).

Principal component analysis (PCA)

PCA of soil fertility indicators evaluated the differences induced by CS studied. The correlation biplots and Scree plots of physical-chemical and biological soil indicators showing PC are presented in Table 4. PCA (PC1, PC2, PC3, PC4) for soil physico-chemical determinants identified for four factors (F1, F2, F3, F4) based on the Eigen value (>1) and explained 77.58, 89, 93.37 and 96.18% of the cumulative variance, respectively. The cumulative variance explained according to PC (100%), the first four principal components (PCs) was considered. From these PCs, highly weighted variables, i.e. factor loadings >0.40 were further selected according to Wander and Bolero (1999). The minimum data set suggested by PCA is M_cF_c, WHC, pH, EC, SOC, MB_C, MB_N, available N, P, K, exchangeable Ca, Mg, DTPA extractable Fe, Zn, Mn. PC4 accounted for maximum 96.18% of the total variance in all CS studied; therefore, no correlation was observed between the soil physico-chemical properties and the first, second and third PC (PC2, PC3, and PC4, respectively). PCA-F1 had the highest positive loadings from Fe, M_cF_c, actinobacteria, Cu, exchangeable Ca, followed for SOC, MB_C, MB_N, available N and P compared to PCA-F2 and PCA-F3. When the calculated factor scores of PCA-F1, PCA-F2 and PCA-F3 for soil physico-chemical attributes were analyzed, the significant differences were obtained. Scores of W_{NSCTM} were highest followed by W_{PSTM}, W_{PSC} and W_{NSC} for PCA-F1. PCA for the microbial count of *Pseudomonas*, *Bacillus*, *A. chroococcum,* soil fungi, actinobacteria, AMF spore population and root colonization was also identified for four factor loadings. Furthermore, similar patterns were also expressed in all PCAs, the differences between walnut based intercrop sequencing samples were not constant. It varied depending on crop rotation, season, and cultivar of the vegetables and ornamentals in the crop sequencing studied. This variation needs to be taken into a deeper consideration before any general conclusions can be made out of the results presented here.

This research demonstrated the adoption of cultivation and intercrop sequencing in walnut-nectarines-sunhemp-

Table 4 Factor loadings and principal component analysis of soil quality parameters and microbial communities (CFU/g) in rhizosphere subsoil of integrated fruit based farm sequencing

Principal component	PC1	PC2	PC3	PC4
Eigen value	17.84	2.63	1.01	0.65
Variability (%)	77.58	11.42	4.37	2.81
Cumulative variance (%)	77.58	89.00	93.37	96.18
Variables	*Factor loadings*			
	F1	*F2*	*F3*	*F4*
M_cF_c	0.92	-0.04	-0.32	0.07
WHC	0.99	-0.03	-0.09	0.02
pH	0.91	-0.28	0.00	0.03
EC	-0.21	0.60	0.49	0.60
OC	0.82	-0.42	-0.02	0.07
MB_C	0.88	-0.27	-0.22	0.28
MB_N	0.87	-0.26	-0.28	0.23
Available N	0.95	0.19	-0.22	0.06
Available P	0.85	0.46	0.02	-0.09
Available K	0.89	0.41	-0.13	-0.10
Exchangeable Ca	0.90	0.41	-0.03	-0.06
Exchangeable Mg	0.91	0.38	0.01	-0.09
Fe	0.96	0.22	-0.03	0.12
Cu	0.91	0.40	0.01	-0.07
Zn	0.88	0.44	0.03	-0.12
Mn	0.92	0.38	0.01	-0.04
Pseudomonas spp.	0.90	-0.37	-0.05	0.16
Bacillus spp.	0.74	-0.41	0.35	-0.01
A. chroococcum	0.92	-0.30	0.17	-0.06
Soil fungi	0.92	-0.26	0.16	-0.10
Actinobacteria	0.95	-0.22	0.06	0.09
AMF spore count	0.87	-0.15	0.41	-0.17
Root colonization	0.89	-0.23	0.34	-0.08

F1, Factor-1; F2, Factor-2; F3, Factor-3; F4, Factor-4; PC1, Principal Component-1; PC2, Principal Component-2; PC3, Principal Component-3; PC4, Principal Component-4.

chrysanthemum-tomato-marigold CS influenced cropping behavior, residue management to build and improve the soil fertility in terms of physico-chemical and microbiological diversity at both surface and subsurface soil. The culture and management system have produced some rapid changes in soil properties such as microbial biomass carbon and nitrogen content that are sensitive indicators for crop management regimens and indeed to be monitored on longer term to quantify cumulative effects of various cropping interventions. Microbial biomass and the plate counts of observed microbial communities were highest in integrated fruit based farm sequencing enforced soils at vegetative growth stage of walnut crop. Moreover, this study also revealed the impact of integrated fruit farm sequencing on soil fertility indicators during walnut-based CS, thus providing more reliable and predictable indicators to monitor the fertility sustainability in sub-temperate ecological conditions.

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