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Effects of organic supplementation to reduced rates of chemical fertilization on soil fertility of *Zanthoxylum armatum*

Abstract: Soil acidification, fertility depletion, food insecurity, and environmental pollution all are consequences of the extensive use of chemical fertilizers (CF) in intensively managed plantations. Recent studies have shown that incorporating organic manure (OM) to partially replace CF can help to maintain productivity and soil health. There are no reports on integrated fertilizer management of *Zanthoxylum armatum*, for optimal ecological services and economic profitability. A pot experiment with *Z. armatum* was performed to investigate the possibility of incorporating OM in reducing CF use, enhancing soil fertility, and increasing plant growth. For this, seven treatments were designed: NPK, PK, NP, NK, OM, MNPK (50%NPK+50%OM), and control (CK, no fertilizer application). The results showed that NPK application significantly ($P < 0.05$) increased plant growth, soil nitrate-N, ammonium-N and available K compared to CK. However conventional CF application induced soil acidification. OM application raised the soil pH from 5.50 (CK) to 6.58 and significantly enhanced soil fertility by increasing alkali-hydrolysable N (2.3 times), ammonium-N (1.4 times), available P (4.3 times), the activity of invertase (1.5 times), catalase (2.8 times), acid phosphatase (1.5 times), and count of soil culturable microbes (2.9 times) compared to CK. Conversely, sole OM application did not result in optimum plant growth. Integrating OM with NPK (MNPK), on the other hand, not only provided the highest growth comprehensive value (0.84), but also substantially higher soil fertility comprehensive value (0.71) than traditional CF treatments. Correlation analysis also indicated a positive and significant correlation between soil microbes, enzyme activities and available nutrients. Therefore, OM+NPK could be an optimum measure to get maximum benefits regarding soil fertility, growth of *Z. armatum*, fertilizer savings and sustainable agroecology.

Keywords: fertilization, soil fertility, nutrient availability, organic manure, culturable microbes

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Introduction

Zanthoxylum armatum, also known as 'Green Sichuan pepper, or Green Huajiao', a semi-deciduous tree in the Rutaceae family is widely observed throughout southwestern China (Xu et al., 2019). The pericarp of *Z. armatum* fruit is famous for its pungent and numbing flavor and is commonly used as a seasoning in Chinese cuisines. It has been used traditionally as an ethno-medicine for its antibacterial, anti-tumor, anti-inflammatory, and anti-oxidation properties (Nooreen et al., 2017). It is also one of the key tree species that has been extensively used under China's Grain for Green Project, due to its strong drought resistance and fast growth in areas that have suffered from serious land degradation (Piao, 2020).

Moreover, its pericarp can be sold to supplement the income of farmers. With the growth of economic and ecological benefits, it has become relatively important tree species in China (Cheng et al., 2015). At present, its cultivation is expanding in China with more than 0.12 million tons of dry pericarp production per year and an output value higher than $700 million (Zhou et al., 2020). However, its output and productivity are usually low, which is generally attributed to poor soil nutrient status caused by nutrients deficiency (Xu et al., 2019; Zhou et al., 2020).

Nitrogen (N), phosphorus (P) and potassium (K) are key limiting nutrients in poor soils and it is the exogenous supply of these nutrients that ultimately decides the yield and production tonnage (Bhattacharyya et al., 2008). Application of chemical fertilizer (CF) is a common management practice for addressing nutrients deficiencies. According to the FAO, global demand for CF is expected to surpass 200 million tons by the end of 2022, with China accounting for roughly 35% of global production (FAO, 2019; Zhang et al., 2021). CF has the high and fast nutrient release, which can quickly enhance available nutrients, crop growth and directly stimulate the microbial community (Zhou et al., 2020). However excessive CF application imposes problems such as productivity loss, changes in soil carbon (C) and total N stocks, environmental degradation (Bindraban et al., 2015; Shahid et al., 2017). These negative consequences are more evident in intensively managed plantations. In general, managed tree plantations have a negative nutrient balance i.e. more nutrients are lost from the system than are gained in response to conventional CF application (Mackensen and Fölster, 2000; Wang et al., 2016). According to Ye et al. (2020), about 50% of N and 90% of P from CF application escapes into the atmosphere and water sources, thereby causing the generation of greenhouse gases, soil salinization, acidification and eutrophication. Now, fertilizer management is not only a central issue in sustainable forest development, but also in the climate change mitigation debate across the globe (Doelman et al., 2019).

In poor soils, the success of nutrient management depends on the recovery of soil organic matter (SOM), which might be augmented through the application of farmyard manure (Bhattacharyya et al., 2008; Shahid et al., 2017). Previous studies have shown that the long-term application of organic manure (OM) improves soil C and total N stocks, microbial diversity, and maintains soil fertility. Moreover, as most of the OM is side product of agriculture farming and livestock, the preparation is cheaper and cost is low (Wang et al., 2019). Furthermore, OM utilization also reduces risks of environmental pollution (Shahid et al., 2017). However, in high-yielding systems, OM application alone might not meet plants' nutrient demands resulting in lower yields and thus a higher cost, therefore the use of CF cannot be entirely eliminated when considerable production is expected (Moe et al., 2019; Ye et al., 2020). Compound org-inorganic fertilization is one alternative that offers a chance for restoring soil fertility while reducing CF inputs by up to 50% without causing any yield losses (Zhang et al., 2021; Wang et al., 2019). Recently, OM incorporation to reduce CF fertilizer use has become popular because of enhanced crop growth, yields, soil microbial abundance and biomass, nutrient availability, and low environmental risks (Zhao et al., 2016; Han et al., 2016). Although the research about reducing the inputs of CF with OM supplementation has increased in recent years, there are few reports concerning intensively managed plantations (Mackensen and Fölster, 2000; Doelman et al., 2019). As a result, a thorough understanding of soil fertility and plant growth responses to the novel regime of reduced CF use combined with OM application is required for sustainable tree plantation management.

Fertilizer management modifies soil nutrient dynamics, pH, microbial biomass, community composition and extracellular enzyme activities, all of which are well-known indicators of soil fertility (Karami et al., 2012; Ye et al., 2020). Potentially mineralizable N i.e. ammonia and nitrate N (NH_4^+-N and NO_3^--N) can better reflect changes in soil fertility, are also highly sensitive to fertilizer application (Chen et al., 2018). Previous studies have established significant sensitivity of soil microbial communities and extracellular microbial enzyme activities to fertilizer management (Zhang et al., 2021). Aon and Colaneri (2001) regarded enzyme activities as "sensor indicators" in detecting changes in soil health and fertility. For instance, Chen et al. (2018) reported decreased P availability was owing to reduced phosphatase activity. Hu et al. (2014) reported significant changes in urease and invertase activities under fertilizer application. Thus assessing soil microbial, chemical and biochemical properties are useful indexes to evaluate the soil fertility and quality under different fertilization management (Bindraban et al., 2015; Kader et al., 2017). Furthermore, evaluating plant growth responses to various fertilizer regimes will help to establish the success of fertilizer management through plant-soil feedbacks. (Hu et al., 2014; Han et al., 2016).

Despite *Z. armatum*'s high ecological and economic importance, there have been no reports on its integrated nutrient management to improve production capacity, quality, soil fertility, and ecosystem services. Furthermore, the majority of previous studies on nutrient management for *Z. armatum* have focused solely on yield maximization, with little information on changes in soil bio-chemical properties and fertility.

It was proposed that conventional NPK treatment would directly increase the available soil nutrients, with some negative impacts on fertility. Sole OM application would provide substrate for the soil microorganisms, improve counts of soil culturable microbes, enzyme activities and soil pH. Whereas reduced rates of CF in combination with OM (MNPK) would improve and maintain soil fertility, without reducing plant growth. Therefore, this study investigated the effects of various fertilization strategies, including the conventional chemical, organic, and combinations of organic-inorganic fertilization on i) soil available nutrients, pH, extracellular enzymes activity and microbial count ii) to evaluate the effect of different fertilization methods on the growth of *Z. armatum* iii) to comprehensively evaluate soil fertility and plant growth in association to fertilization treatments to optimize the integrated nutrient management for enhanced growth rate along with conserving soil fertility of rapidly developing plantations on a sustained basis.

Materials and methods

Overview of the study area

The experimental site located in the Chengdu Campus of Sichuan Agricultural University (103°52'E, 30°42' N) is characterized by the subtropical humid monsoon climate. The average annual temperature is 16.2 °C, extreme maximum and minimum temperatures are 37.3 °C and 5.6 °C, respectively. The mean annual precipitation is 1012.4 mm, the frost-free period is 298 d and the mean annual sunshine duration is 1039.6 h.

Experimental design

The experimental soil was taken from the Chongzhou, Sichuan Province (103°67 E, 30°63 N), the region has a subtropical humid monsoon climate. After collection, air drying, removal of gravel and plant residues, the soil was thoroughly mixed and sieved using a 5 mm sieve. Soil samples were analyzed for physico-chemical properties. The soil according to Chinese Soil Genetic Classification, is characteristic purple soil (Zhong et al., 2019) with the following initial properties: sand 11.5%, silt 75.7%, clay 12.8%, field water capacity (FWC) 39.7%, average pH_{H2O} of 5.45, soil organic matter 5.97 g kg^{-1}, total N 0.94 g kg^{-1}, total P 0.41 g kg^{-1}, total K 18.67 g kg^{-1}, alkali-hydrolysable N (AHN) 56.92 mg kg^{-1}, available P (AP) 4.18 mg kg^{-1} and available K (AK) 93.95 mg kg^{-1}. Pest and disease-free seedlings of *Z. armatum* with an average height and root collar of about 20 cm and 2 mm, respectively, were used for the pot experiment.

A total of seven fertilization treatments were set up in this experiment, including control (without any fertilizer, CK), NK, NP, PK, NPK, OM, ½OM + ½NPK (MNPK). Each fertilization treatment was replicated three times, with five pots per replication containing one seedling each, for a total of fifteen pots in each treatment. The NK, NP and NPK treatments contained 300 kg N·ha^{-1} (urea); the PK, NP and NPK treatments contained 120 kg P_2O_5·ha^{-1} (calcium superphosphate); the PK, NK and NPK treatments contained 300 kg K_2O·ha^{-1} (potassium sulfate). The total amount of organic fertilizer applied in the OM treatment is based on its N content and the pot area, and the chemical fertilizer P and K were added to keep the P_2O and K_2O at same levels as the NPK treatment. The MNPK treatment contained one half of the organic C and nutrients in the OM treatment and one half of the nutrients in the NPK treatment. The organic fertilizer was prepared from chicken manure and its chemical properties are as follows: total organic C 406.2 g kg^{-1}; total N 19.09 g kg^{-1}; total K 13.76 g kg^{-1} and total P 0.47 g kg^{-1}. Air-dried soil (total 6.5 kg oven-dried weight basis equal to about 20 cm soil layer) was added up to 2/3 volume of polyethylene plastic basins (diameter and height is 20 cm and 25 cm, respectively). Another 1/3 volume of air-dried soil was fully mixed with the fertilizer to be added as topsoil (soil layer thickness is about 20 cm) and soil moisture was adjusted to 60% FWC. After transplanting seedlings on March 25th, 2019, pots were placed in the greenhouse shed which only sheltered from rain and did not control temperature and humidity.

Sampling and analysis

Seedlings height and root collar were measured at the harvest time (October 25th, 2019). To avoid heterogeneity and get one homogenized soil sample per replication, the potted soil from the five pots within one replication was poured out and thoroughly mixed after the seedlings were harvested. Subsamples of soil were then collected to further analyze the soil properties. The fresh soil was divided into two parts, one part of the fresh soil was used for the determination of inorganic N (NH_4^+-N and NO_3^--N) contents and culturable microbial (bacteria, fungi and actinomycetes) counts. The remaining soil was air-dried and used for determination of pH, available nutrients (alkali-hydrolysable N, available P and K) contents and enzyme activities (invertase, urease, acid phosphatase and catalase). NH_4^+-N was determined using the indophenols blue method and NO_3^--N using the cadmium reduction method (Gong et al., 2011). Total P was measured according to Parkinson and Allen (1975) and available P (AP) was measured according to Hassan et al. (2016). Available potassium

(AK) was extracted with ammonium acetate, and then determined by a flame photometry (Gong et al., 2011). Soil pH_{H2O} was determined in 1:2.5 (w/v) suspension of de-ionized water using a digital potentiometric pH meter (Hassan et al., 2018). Total N concentration in soil (g kg^{-1}) was determined by micro-Kjeldahl and AHN in the soil was determined by reduction-diffusion method as described by Hassan et al. (2017). The number of culturable bacteria, fungi and actinomycetes was determined by the dilution plate count technique on nutrient agar (Hassan et al., 2014; Gong et al., 2011). The acid phosphatase (APH), urease (URE), invertase (INV) and catalase (CAT) activities were determined by the procedures of Zhang et al. (2011) and Akhtar et al. (2019).

Statistics

Results are presented as means (n = 3) along with ± standard deviation. Results were calculated and statistically examined using an analysis of variance (ANOVA) and means separations were achieved by using Duncan's multiple range test (SSR) test at $P < 0.05$. All tests were performed using SPSS 17.0 software (SPSS Science, Chicago, USA). Pearson correlation coefficient was used to quantify the correlations among soil enzymes activities, available nutrients and soil culturable microorganisms, by using OriginPro 2019b 64Bit. For the comprehensive evaluation of soil fertility, the subordinate degree of selected indexes was calculated following Li et al. (2007). The indexes including: available nutrients (AHN, AP and AK), soil enzyme activities of APH, URE, INV, CAT and soil microbial (bacteria, fungi and actinomycetes) counts were used as soil fertility indicators, while the seedling height and root collar were used as plant growth indicators. The formula for each index's subordinate degree calculation is $X(u) = (X - X_{min})/(X_{max} - X_{min})$. The mean of the accumulated soil fertility (or plant growth) subordinate degrees for each index of each treatment is then calculated. The higher value represents higher soil fertility (or plant growth).

Results

Effects of different fertilization methods on soil pH and nutrients

The results showed that different fertilization treatments had significant ($P < 0.05$) effects on soil pH (Fig. 1). Compared with CK, the OM, MNPK and NPK treatments led to significant increase in soil pH. Specifically, under OM application the elevation of soil pH was the most significant up to 6.58 compared with 5.50 of CK (without any nutrient application). The effect of PK application on soil pH remained insignificant with respect to CK. While the NK and NP treatment lowered soil pH to 4.93 and 4.98, respectively.

The effect of different fertilization treatments on soil available nutrients, AHN, NH_4^+-N, NO_3^--N, AK and AP was significant ($P < 0.05$) compared to CK (Fig. 1). OM, NPK, NK and NP treatments significantly increased soil AHN content compared to PK and CK. Specifically OM treatment resulted in highest soil AHN content which was 2.3 times higher than CK. Combined application of organic manure and chemical fertilizer (MNPK) had 1.3 and 1.2 times lower AHN content than OM and NPK, respectively. The highest soil NH_4^+-N content was recorded for NPK treatment which is 2.5 times higher compared to CK. The intermediate NH_4^+-N content was observed in OM treated soil. There was no significant difference between soil NH_4^+-N content for MNPK and NK treatments ($P < 0.05$). While among all CF treatments, PK had the lowest soil NH_4^+-N content, which is also statistically insignificant relative to CK. The NK and MNPK application significantly increased soil NO_3^--N content, specifically for NK increase was up to 24.6 times higher than CK. There was no significant difference between NO_3^--N content of NP and NPK treatments. OM application had only a slightly positive effect on NO_3^--N content, while PK application remained insignificant with respect to CK. OM treatment led to the highest soil AP content which is significantly higher than AP contents of NPK, NP, PK, NK and CK ($P < 0.05$). The AP content for NK treatment was 1.1 times lower than CK. However, NK application resulted in significantly higher soil AK content compared to OM, MNPK and CK. OM treatment had 1.6 times lower soil AK content than NK treatment. There was no significant difference between soil AK content of PK and CK ($P < 0.05$) (Fig. 1).

Effects of different fertilization methods on soil enzymes activity

The soil enzymes URE, CAT, APH and INV activities were significantly affected by all fertilization treatments ($P < 0.05$). As shown in Fig. 2, OM treated soil exhibited the highest i.e. 2.8 times higher CAT activity compared to CK. MNPK and NPK treatment significantly increased soil CAT activity compared to CK. NK, NP and PK also promoted soil CAT activity but their effect was statistically insignificant with respect to CK. The highest soil URE activity was recorded for MNPK and increase was 1.6 times than CK. Soil URE activity for OM and NPK treatments was statistically similar but significantly higher than CK. NK and PK treatments also had statistically identical effects on

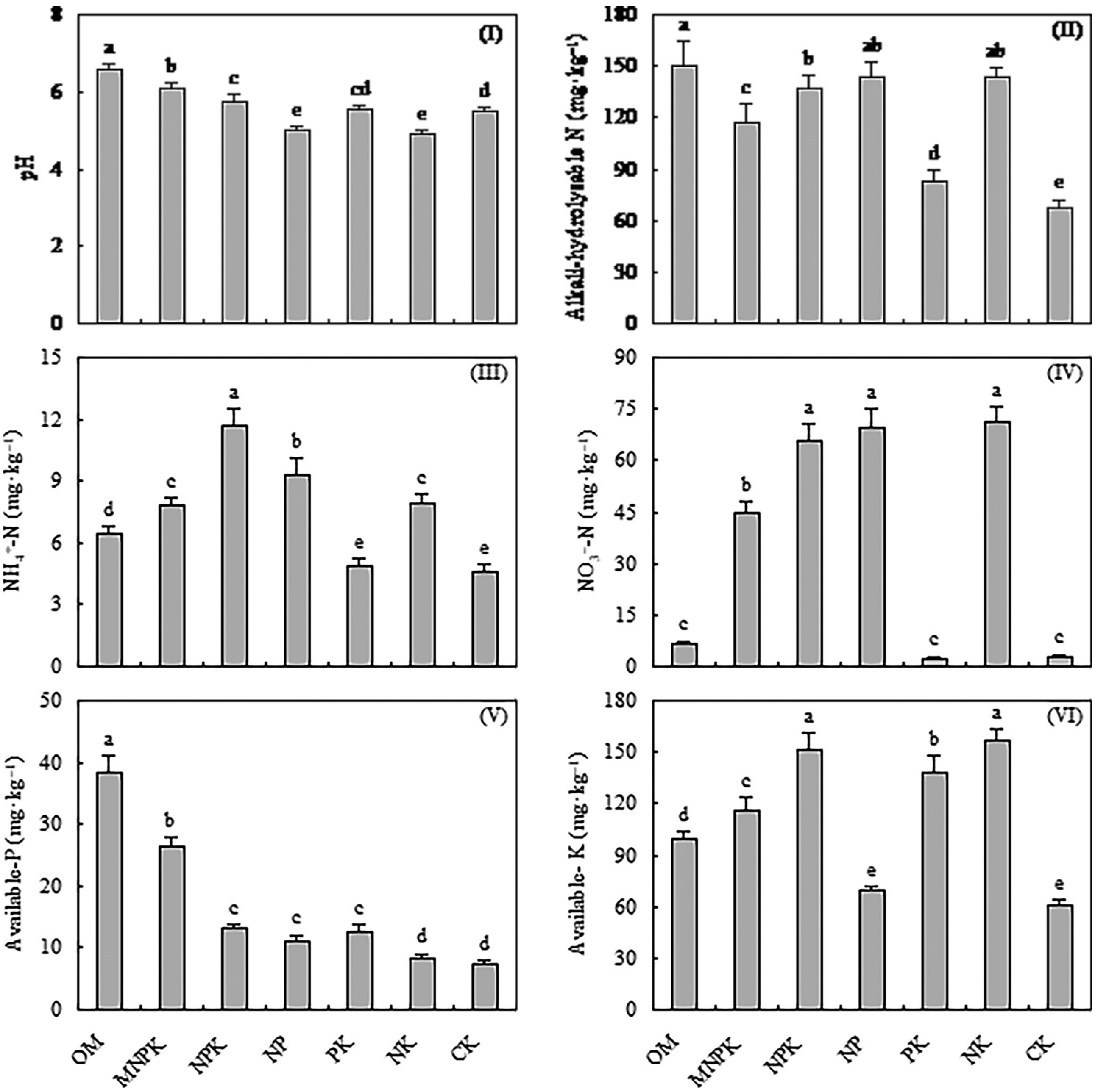

Fig. 1. Effects of different fertilization treatments on soil pH and available nutrients. Different small letters above the bars indicate significant differences within treatments (P < 0.05) and bars represent standard deviation (n = 3)

URE activity but increase was up to 1.2 and 1.3 times higher, respectively than CK. The APH activity was also recorded highest for OM treated soil and increase was 1.5 times compared to the APH activity of CK. The APH activity for MNPK treatment was 1.1 times lower than OM but 1.4 times higher over CK. There was no significant difference between APH activity of NP and NPK treatments. INV activity was also recorded highest for OM treated soils and increase was 1.5 times than CK. MNPK treatment improved soil INV activity by 1.1 times over CK. Improvement in soil INV activity in response to different fertilizer treatments was in the following order of OM > MNPK > NPK ≈ NP > PK > NK >CK.

Effects of different fertilization methods on the number of soils culturable microorganisms

The effects of different fertilization treatments on soil bacteria, fungi, actinomycetes and total microbial counts were significant ($P < 0.05$) compared to CK (Fig. 3). OM treated soil had the highest number of culturable bacteria, fungi and actinomycetes. OM application had 2.93 times, 1.58 times and 2.86 times higher counts of soil bacteria, fungi and actinomycetes, respectively than CK. OM treatment had 2.32 times and 2.96 times higher count of total microbes

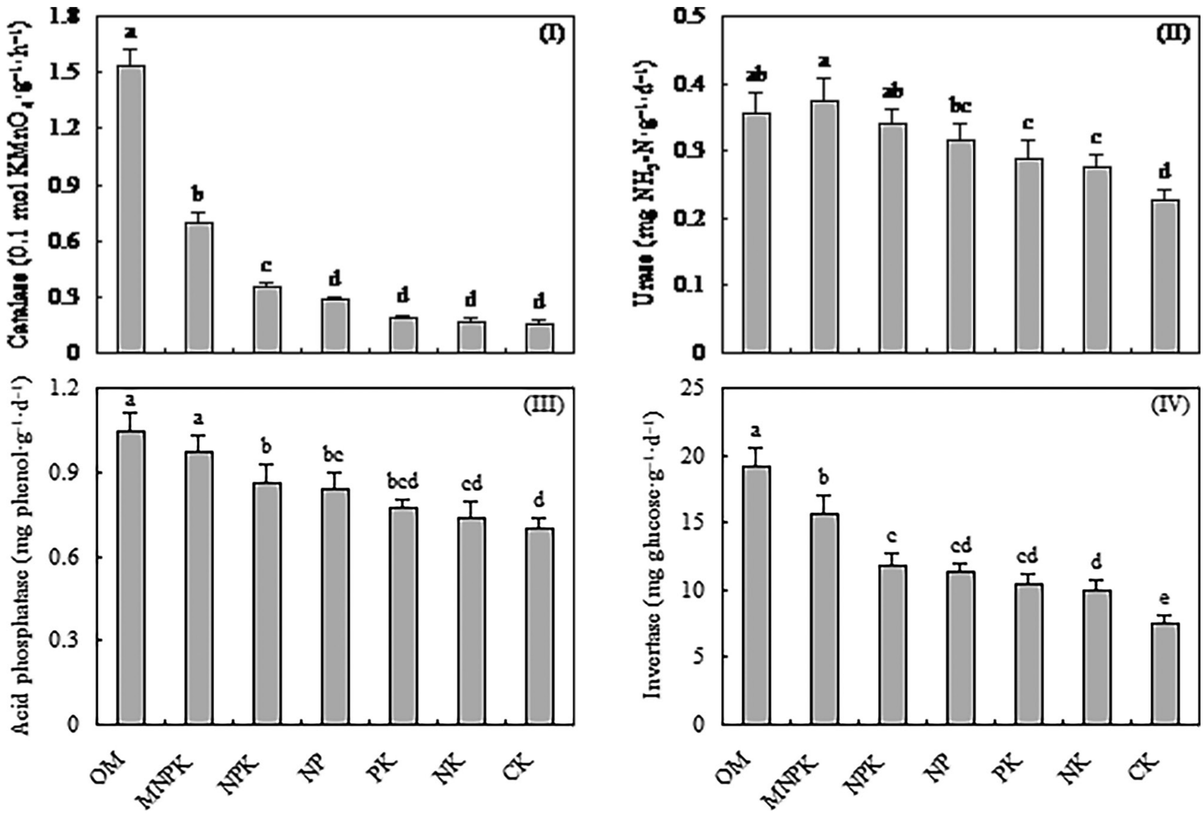

Fig. 2. Effects of different fertilization treatments on activities of extracellular soil enzymes. Different small letters above the bars indicate significant differences within treatments ($P < 0.05$) and bars represent standard deviation ($n = 3$)

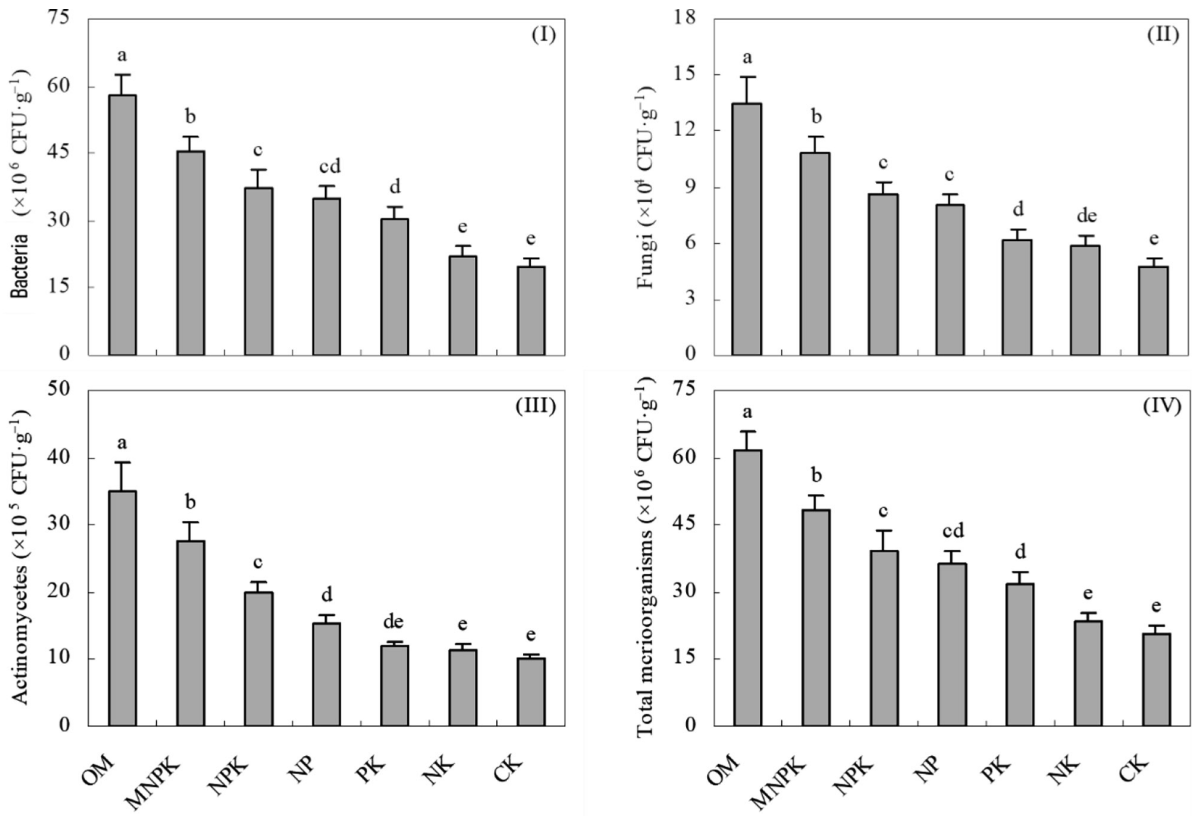

Fig. 3. Effects of different fertilization treatments on number of culturable soil micro-organisms. Different small letters above the bars indicate significant differences within treatments ($P < 0.05$) and bars represent standard deviation ($n = 3$)

than MNPK and CK, respectively. MNPK treatment exhibited second highest counts of soil culturable microorganisms. The counts of soil culturable microorganisms were statistically similar for NPK, NP and PK treatments but significant compared to CK ($P < 0.05$). However, the difference between NK and CK remained insignificant. The effect of different fertilization treatments on soil culturable microbes is in the order of OM > MNPK > NPK ≈ NP ≈ PK > NK ≈ CK.

Effect of different fertilization methods on plant height and root collar

The response of plant growth indicators i.e. plants height (*H*) and root collar (*D*) to the application of different fertilization treatments was recorded (Fig. 4). Fertilization significantly improved plant growth compared to CK ($P < 0.05$). As shown in Fig. 4, the highest *H* was recorded for MNPK and NPK treatments with an increase of 1.5 times and 1.4 times, respectively, compared to CK. Similarly, maximum *D* was recorded for MNPK, NPK and NP treatments. MNPK treatment specifically increased *D* 5.4 times compared to CK. Intermediate increase in seedling *H* (84.87 cm) and *D* (7.11 mm) was observed for OM treatment. The effect of NK application on *H* and *D* remained insignificant with respect to CK ($P < 0.05$).

Comprehensive evaluation of plant growth and soil fertility

Different fertilizer treatments affected soil fertility and plant growth to varying degrees (Fig. 5). The highest soil fertility comprehensive value was scored for OM and MNPK treated soils, the improvement was 18.3 times and 15.1 times respectively than CK.

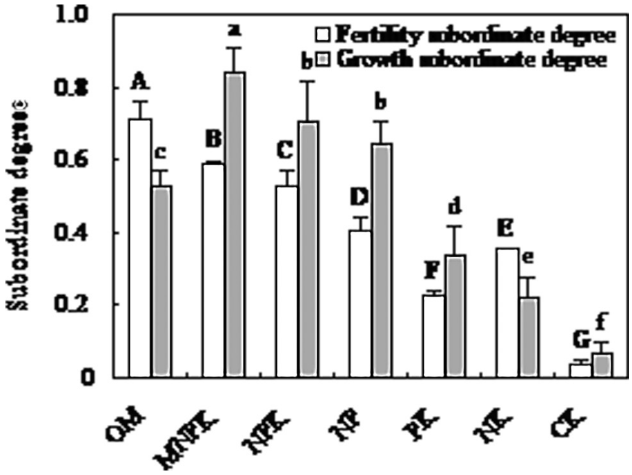

Fig. 5. Effects of different fertilization treatments on soil fertility and plant growth. Different letters above the bars indicate significant differences within treatments ($P < 0.05$) and bars represent standard deviation (n = 3)

The improvement in soil fertility in response to OM application was 1.2 times and 1.5 times in comparison to MNPK, NPK respectively. The order of soil fertility improvement with respect to different fertilization treatments was OM > MNPK > NPK > NP > NK > PK > CK. As shown in Fig. 5, MNPK treatment had the highest growth comprehensive value 12.1 times and NPK had second highest 10.3 times higher than CK, respectively. OM application improved seedling growth by 7.1 times than CK. The minimum growth improvement was observed for sole NK application relative to CK. The response of plant growth to different fertilization treatments is in the order of MNPK > NPK > NP > OM > PK > NK ≈ CK. The effect of fertilizer application on soil fertility and plant growth was significant ($P < 0.05$) and a positive correlation (0.771) was observed between soil fertility and plant growth. The highest growth

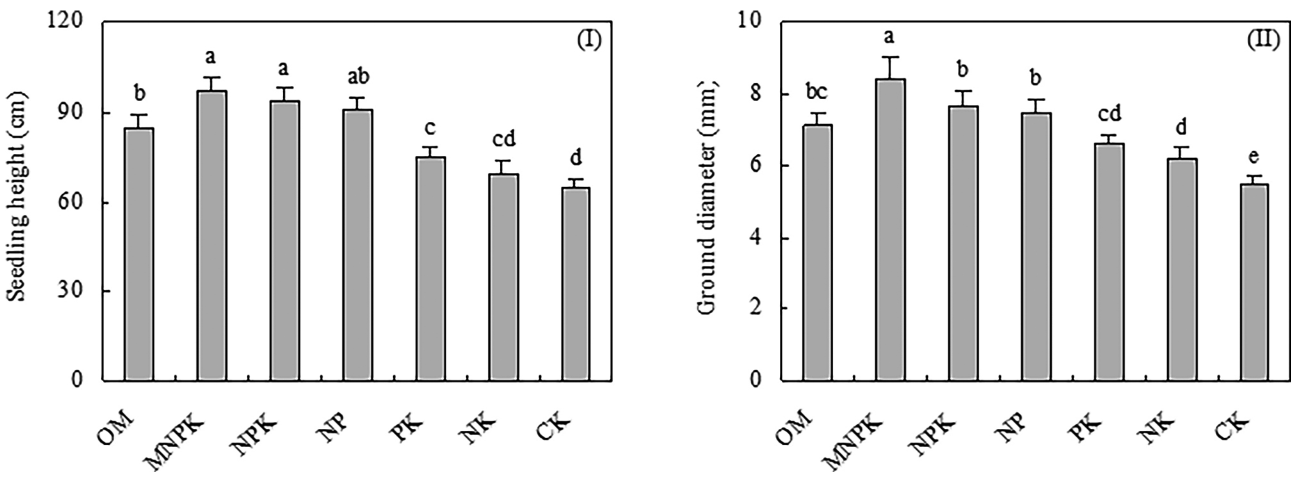

Fig. 4. Effect of different fertilization methods on plant height and ground diameter. Different letters above the bars indicate significant differences within treatments (P < 0.05) and bars represent standard deviation (n = 3)

comprehensive value was recorded for MNPK while the highest fertility comprehensive value was recorded for sole OM application.

Correlation analysis of soil enzymes activity, available nutrients and microbes

Correlation analysis illustrates that significant ($P < 0.05$) or extremely significant ($P < 0.01$) correlation exists among soil enzyme activities (URE, CAT, INV and APH), available nutrients (AHN, NH_4^+-N, NO_3^--N, AP and AK) and culturable soil microbes (Fungi, bacteria and actinomycetes) (Table 1). All available forms of soil N have a positive correlation with URE activity in an order of AHN > NH_4^+-N > NO_3^--N. Similarly, the availability of soil P has a significant correlation to APH activity. A strong positive correlation is also observed between AK and URE activity. Soil microbial count is extremely significantly correlated with URE activity and available N content ($P < 0.01$). URE, CAT, INV and APH activities are also positively correlated to bacteria, fungi and actinomycetes and to nutrient availability especially AP, AHN, NH_4^+-N and NO_3^--N ($P < 0.05$).

Discussion

Effects of different fertilization on soil pH and nutrients availability

In recent years, soil acidification has become a serious problem for modern agriculture in China due to increased use of acidifying nitrogenous fertilizers or incomplete cycling of N species in the soil (Guo et al., 2010). Whereas SOM neutralizes soil pH, and is known to be ultimate determinant of soil fertility. As reported by Lin et al. (2019), continuous CF application aggravated soil acidification in tea orchards on the contrary OM application improved soil pH towards neutral. Likewise, our hypothesis was also confirmed, indicating improvement in soil pH in response to OM and MNPK application (Fig. 1). However, OM brought highly significant improvement in soil pH from 5.48 (pH of soil samples prior to treatments) to 6.58. These results are in line with the findings of Palviainen et al. (2018), who reported increase in soil pH in response to biochar application. The application of sole NPK produced relatively higher soil pH compared to CK, as supported by findings of Dong et al. (2012). However, incorporation of OM into traditional chemical fertilizer brings more benefits to problem of soil acidification (Han et al., 2016; Wang et al., 2020), which further confirmed our proposed hypothesis. Wang et al. (2019) reported an increase in pH at the rate of 0.085 $year^{-1}$ after organic fertilizer was applied in acidic red soils. Because soil pH is also related to the parent material of soil formation, the degree of change in soil pH is complex (Zhao et al., 2016). However, application of poultry manure has been shown to increase soil pH. This could be attributed to increased availability of OM and calcium ions released into the soil solution during de-carboxylation of manure which is known to buffer soil pH (Agbede, 2010; Guo et al., 2010; Lin et al., 2019; Wang et al., 2019).

Alkali-hydrolysable N represents the potentially available organic-N, while NH_4^+-N and NO_3^--N are dominant inorganic N fractions which give obvious responses to even short-term management practices when total N shows no major changes (Drescher et al., 2020). Results of the present study indicated that the effects of different fertilization treatments on soil AHN, NH_4^+-N, NO_3^--N and AP was significant (P

Table 1. Pearson correlations coefficient (r) among soil available nutrients, extracellular enzyme activities and count of soil culturable microorganisms

	AHN	NH_4^+-N	NO_3^--N	AP	AK	pH	CAT	URE	APH	INV	Bac.	Fun.	Act.
NH_4^+-N	0.67**	1.00											
NO_3^--N	0.64**	0.83**	1.00										
AP	0.39	−0.06	−0.32*	1.00									
AK	0.27	0.33	0.33*	−0.06	1.00								
pH	0.07	−0.15	−0.51**	0.87**	−0.03	1.00							
CAT	0.39	0.06	−0.26	0.93**	0.08	0.86**	1.00						
URE	0.59**	0.46**	0.26	0.71**	0.18	0.57	0.64**	1.00					
APH	0.22	−0.04	−0.13	0.46*	−0.02	0.18	0.52**	0.12	1.00				
INV	0.54**	0.13	−0.11	0.95**	0.05	0.79**	0.87**	0.78**	0.26	1.00			
Bac.	0.49*	0.18	−0.13	0.92**	−0.01	0.77**	0.88**	0.74**	0.28	0.92**	1.00		
Fun.	0.59**	0.25	−0.04	0.93**	−0.02	0.76**	0.89**	0.81**	0.32	0.94**	0.97**	1.00	
Act.	0.49*	0.19	−0.12	0.96**	−0.02	0.85**	0.91**	0.79**	0.27	0.96**	0.93**	0.96**	1.00

AHN: Alkali-hydrolysable nitrogen, NH_4^+-N: ammonium-N, NO_3^--N: nitrate-N, AP: available-P, AK: available-K, CAT: catalase, URE: urease, APH: acid-phosphatase, INV: invertase, Bac: Soil bacteria, Fun: Soil fungi, Act: Soil actinomycetes. Values with "*" are significant at $P < 0.05$; "**" significant at $P < 0.01$ and positively correlated, values with "−" are negatively correlated.

< 0.05), whereas the effect on AK was insignificant (Fig. 1). Similarly, Lin et al. (2019) reported significant increases in the soil nutrient status at different degrees in response to application of different fertilization regimes. Cui et al. (2018) observed significantly higher levels of AHN, AP, and AK in MNPK treatment than in sole NPK or OM treatment, suggesting that combined organic-mineral fertilizers could be an alternative solution for sustainable fertility management. In the present study, it was proposed that OM application will sustain nutrient contents compared to sole NPK application. The results revealed soil AHN and AP content in OM treatment remained at a higher level compared to CF fertilizers (Fig. 1), and confirmed our hypothesis. Adams et al. (2020), Yang et al. (2020) and Dong et al. (2012) also found similar trends in fertilizer application and soil AP content. They linked it with demand-induced mobilization P from OM and the fact that slow release of nutrients from OM ensures nutrient availability overgrowth period.

Wang et al. (2020) also reported changes in AP, AK and available N (NH_4^+-N and NO_3^--N) contents under different fertilizer applications. NPK treatment had the highest soil NH_4^+-N content which is consistent with results of other studies (Hu et al., 2014; Zhao et al., 2016; Lin et al., 2019), and confirms our proposed hypothesis. Compared with NK, NP and NPK treatments, OM application showed lower soil NO_3^--N owing to comparatively slow OM turnover and N release rates suggesting that the application of OM can potentially decrease gaseous N_2O and N_2 through denitrification (Juan et al., 2008). The highest NO_3^--N content was observed in NK treatment which could be attributed to increased potential nitrification rates in response to N fertilization (Yang et al., 2020). However, in correlation to reduced growth rate of *Z. armatum* seedlings in NK treatment (Fig. 1) higher concentration of nitrate-N in soil could be due to reduced uptake by plants and then subsequent build up in soil (Adams et al., 2020). Although NO_3^--N is non-toxic in soil, owing to high leaching, groundwater pollution and N losses as potential greenhouse gases can give rise to high environmental risks, therefore the sole application of NK is not recommended. Yang et al. (2020) reported relatively higher NH_4^+-N content and soil nitrification potential in response to inorganic N fertilization, while higher AP and AK content were observed in co-application of OM and NPK. The soil treated with MNPK had lower levels of AHN, NH_4^+-N and NO_3^--N content (Fig. 1). Because co-application of 1/2OM+1/2NPK increases plant growth and subsequent N uptake and removal so that additional N does not build up in soil, this is also consistent with the findings of Adams et al. (2020).

Effects of different fertilization on soil enzymes activity

Catalase was first considered as the biological index of fertility in 1926 (Zhou 1987). Satefanic et al. (1981) proposed that a better biological index of fertility could be derived from the activities of catalase and dehydrogenase. Several researchers are agreed upon soil enzymatic activities are the candidate ''sensor'' and "indicators" of soil fertility, quality and health because they reflect the effects of soil properties, microbial status and pedological amendments (Skujins, 1978; Aon and Colaneri, 200; Hu et al., 2014). Results of present study are indicating significant alterations in soil enzyme activities of INV, URE, APH and CAT in response to fertilizer application. OM treated soils had 2.8 times higher CAT activity compared to CK. The proposed hypothesis that OM application improves soil biological properties was confirmed as OM treated soils exhibited highest CAT, INV and APH activity. Ouyang and Norton (2020) showed significantly increased soil enzyme activities in response to the continuous compost application. Unlike to highest activity recorded for OM application from current study Zhao et al. (2016), who reported highest enzyme activities for combined 1/2OM+1/2NPK application. The URE activity was significantly higher for MNPK than other treatments, which might be attributed to organic manure addition improved the SOM status and in turn enhanced the soil enzyme activity, which is similar to other studies (Juan et al., 2008). Hu et al. (2014) observed that activities of all enzymes were generally higher in the fertilized than in the unfertilized treatments, and the application of organic fertilizer produced higher activity than CF treatments. Zhao et al. (2016) also reported that soil INV, URE, APH and CAT activities differed considerably ($P < 0.05$) between different fertilization treatments. Urease hydrolyzes urea into available forms of soil N (Drescher et al., 2020) and as shown in Fig. 2 URE activity is positively related to AHN, NO_3^--N and NH_4^+-N. Similarly, activity of APH is positively correlated by AP content both found highest in OM treated soils compared to other treatments. Hu et al. (2014) investigated the activities of URE, CAT, APH and INV and found these were significantly correlated with each other and soil nutrients. Sekaran et al. (2018) also reported a significant correlation between enzyme activities and soil nutrient availability. Similar results were also reported in other studies (Thenabadu and Dharmakeerthi, 1996) indicating positive effects of organic manure incorporation on bio-chemical properties, nutrient availability and soil fertility.

Effects of different fertilization on the counts of culturable soil microorganisms

Soil microbes including bacteria, fungi, and actinomycetes play key roles in soil health and fertility. These biological components of soil usually respond more rapidly to changing soil conditions than either the chemical or physical soil properties (Sekaran et al., 2018). Sun et al. (2015) showed that bacterial abundance was increased by the application of NPK alone and the combined application of NPK and OM. Similarly, present study indicated that the effect of different fertilization treatments on bacteria, fungi, actinomycetes and total microbial count is significant in an order of OM > MNPK > NPK (Fig. 3), which further supports our proposed hypothesis. The highest microbial count in OM treated soil can be because of sufficient SOM availability which increases the pool of N and C sources for soil microorganisms' growth (Sekaran et al., 2018), a similar result was also reported by Wang et al. (2020). Zhang et al. (2021) however reported a stronger impact of CF on soil microbial communities compared to sole OM application and attributed it to the high NH_4^+-N concentration and available nutrients in CF treatments. Although several long-term studies have established a significantly positive impact of OM application on soil microbial attributes however short-term studies produce modest changes to microbial communities compared to CF (Zhang et al., 2021). Wang et al. (2020) reported that since changes of soil microbial communities are closely related to soil nutrient availability thus application of organic fertilizer improves soil physical properties, nutrient availability, microbial count and diversity and shortens the soil reclamation time. However, to elaborate and establish the effects of short-term OM application (particularly in combination with CF) on soil microbial community alterations further studies are required.

Effects of different fertilization on plant growth and soil fertility

The term soil fertility has ancient origins and has been consistently used over centuries to refer to the capability of soil to support plants, provisioning mineral nutrients, soil microbial attributes and extra-cellular enzyme activities (Adams et al., 2020). Different fertilizer treatments affected soil fertility to varying degrees as shown in (Fig. 5). Highest fertility comprehensive value was scored for sole OM (0.71) compared to CF, which is similar to previous studies (Hu et al., 2014; Xia et al., 2015; Zhao et al., 2016), and confirms our hypothesis of improved soil fertility in response to OM application. For instance, Lin et al. (2019) showed OM application improved levels of potentially available N in soil for plant uptake, microbial counts and improved soil health and fertility. Similarly, several previous studies (e.g. Juan et al., 2008; Agbede, 2010; Sekaran et al., 2018; Wang et al., 2020) have established benefits brought by OM application to soil fertility. Fertilization improved the seedling growth and comprehensive values are indicating that all fertilization treatments promoted the growth of seedlings compared to CK (Fig. 2) similar to other studies (Hasnain et al., 2020; Xu et al., 2020; Adekiya et al., 2020; Han et al., 2016; Alice et al. 2012). The seedlings growth is closely related to morphological indicators such as plant height and root collar (Hasnain et al., 2020; Xu et al., 2020). Results of this study showed highly positive impact of MNPK application on plant *H, D* and growth. Similarly, Alice et al. (2012) and Hasnain et al. (2020) also reported highest plant *H* for MNPK and suggested addition of manure to NPK perhaps increased the absorbing ability of the root systems for nutrients. For instance, Zhou et al. (2020) attributed improved plant growth to increased nutrient availability in nutrient poor soils such as the soils under *Z. armatum* plantations of Sichuan upon fertilization. N uptake promotes cytokinin production which enhances cell wall elasticity, number of meristematic cells, and cell growth while P availability is essential for the acquisition, storage, and use of energy (Razaq et al., 2017; Hu et al., 2014). MNPK treatment had the highest growth comprehensive value, as well as soil nutrient availability suggesting that MNPK application might be an optimum nutrient management for promoting seedling growth and soil fertility.

Synergistic relationship between plant growth and soil fertility

The soil fertility critically influences plant growth and development and the relationship between plant growth and fertility in managed systems is mediated by fertilizer application (Xia et al. 2015). The effects of fertilizer application on soil fertility and plant growth were significant and positive correlation (0.77) was found between soil fertility and plant growth indicating unavoidable dependency of plant growth on soil fertility (Lin et al., 2019; Razaq et al., 2017; Hu et al., 2014). OM application and incorporation to reduced rate of NPK improved soil fertility in terms of improved microbial properties, enzyme activities and nutrients availability (Table 1). A strongly positive correlation (0.77) is observed between plant growth and soil fertility comprehensive values. This implies that not only soil fertility improves plant growth but soil fertility is also directly regulated by plants growth. For instance, roots exudates impact rhizosphere microbial communities and extracellular enzymes which play decisive role in nutrients availability hence soil fertility (Han et al., 2014; Zhao et al., 2016). Although the highest growth comprehensive value was recorded for MNPK and highest fertility comprehensive value was recorded for sole OM application, however simultaneous consideration of both indexes established systems' comprehensive feedback to fertilizer management (Juan et al., 2008; Han et al., 2014; Ye et al., 2020). A better understanding of the interaction between applied fertilizer, plant growth and soil environment may improve nutrient management and ecosystems sustainability (Alice et al. 2012; Lin et al., 2019). Therefore, an accurate evaluation of soil fertility and plant growth can provide a theoretical foundation for managing continuous production and fertilization strategies for poor soils to function effectively as a component of a healthy ecosystem. However, findings of pot experiments cannot be accurately extended to field conditions. Furthermore, based on findings of pot experiments, a series of field trials are required to optimize integrated nutrient management of *Z. armatum*.

Conclusion

Conventional CF application improved plant growth nevertheless it also had negative impacts on soil fertility by aggravating soil acidification. OM application considerably improved nutrient status, soil pH, enzymes activities and count of culturable microorganisms. All these parameters are standard indices of soil fertility, thus improvement in these indices suggest OM application may potentially improve soil fertility. However, OM had only intermediate effects on plant growth, suggesting that OM alone will not be sufficient to meet the nutrients needs of rapidly growing plantations. On the other hand, OM application with reduced rates of NPK (i.e. MNPK) improved *Z. armatum* growth as well as soil chemical and biological properties. Therefore, it can be concluded that adding OM to balanced CF rates can improve plant growth and soil fertility on sustained basis. Moreover, OM incorporation should be evaluated further as an important component of an integrated nutrient management regime for higher yields and sustainable systems under field trials.

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