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Effects of raised-bed planting for enhanced summer maize yield on rhizosphere soil microbial functional groups and enzyme activity in Henan Province, China

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ABSTRACT

Although raised-bed planting systems can enhance productivity of summer maize (*Zea mays*) relative to other systems employed in China, mechanisms for this remain unclear. Because of their potential to enhance maize yield, we examined effects of raise-bed planting on rhizosphere microbial functional groups and associated enzyme activities in 2006 and 2007 crop seasons. Results confirmed that raised-bed planting significantly and substantially increased maize growth. Microbial functional groups and enzyme activities varied during the maize growing season, with patterns often correlated with plant growth. For raised-bed planting, mean numbers of bacteria, fungi, and actinomycetes across all three sampling times in 2006 were 7.3×10^6, 1.6×10^4, 1.9×10^5 CFUs g^{-1} dry soil, respectively, 82, 44, and 43% higher than those in flat planting, respectively. In 2007, relative differences were 108, 40, and 34% higher than those in flat planting, respectively. Raised-bed planting yielded mean saccharase, urease, protease and phosphatase activities across sampling times in 2006 of 2.3 mg glucose g^{-1} h^{-1}, 0.8 mg NH_3-N g^{-1} h^{-1}, 10.5 mg glycine kg^{-1} h^{-1}, and 0.4 mg nitrophenol g^{-1} h^{-1}, 6, 18, 34, and 31% higher than those in flat planting, respectively. In 2007, relative differences were 10, 16, 35, and 27% higher than those in flat planting, respectively. Rhizosphere enzyme activities were significantly correlated with maize yields. These results suggest that raised-bed planting (1) may enhance maize productivity in part by increasing availability of essential crop nutrients by stimulating microbial activity, and (2) is potentially important in sustainable increasing China's supply of maize.

1. Introduction

China faces the challenge of developing sustainable agricultural practices to increase its currently inadequate supply of maize (Dong et al., 2005). Tillage-based planting methods have exhibited a positive effect on maize yield and show great promise in meeting this challenge. One such method—raised-bed planting—is a planting system proposed for maize production in irrigated areas of the Yellow River Valley, the largest maize growing region in China (Wang et al., 2004). This system comprises planting maize on the top of raised beds and incorporating plant residues from the previous crop into the soil, which are chopped and left in the field (Limon-Ortega et al., 2006).

Previous studies have shown that raised-bed planting reduces seed mortality rates, increases water- and nitrogen (N)-use efficiency, and improves soil quality. In addition, less labor is required for irrigation and fertilizer is better managed relative to conventional flat planting (Limon-Ortega et al., 2000, 2002). More important, raised-bed planting can reduce crop lodging (crops falling over from high winds and/or heavy rain), while increasing yield by permitting farmers to grow more and superior crops (Govaerts et al., 2006; Wang et al., 2009).

The rhizosphere—the zone of soil immediately surrounding plant roots—exerts a profound impact on soil fertility and plant production (Gomes et al., 2001; Marschner et al., 2004). Rhizosphere microorganisms can influence plant growth and health via nutrient mineralization and N_2 fixation (van Loon et al., 1998; Gomes et al., 2001). Enzyme activity in the rhizosphere also plays a critical role in organic matter decomposition and nutrient mineralization required for plant growth (Badalucco et al., 1996; Böhme and Böhme, 2006; Saha et al., 2008). Dynamics of rhizosphere microorganisms and associated soil enzyme activities are influenced both by soil characteristics and the type of crop management employed (Miller et al., 1989; Marschner et al., 2004; Saha et al.,

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2008). Although many of these effects have been studied in agroecosystems of China (Tao et al., 2009; Wang et al., 2011; Yuan et al., 2011), fewer studies have focused on their relevance to maize production.

The Huanghuaihai region lies within the Henan Province, China's third-ranking province in maize production, according to the China National Bureau of Statistics. Best management practices for sustainable high production of maize in this region include those that integrate soil – crop systems to maintain food production (Chen et al., 2011). Although understanding the connection between maize yield and soil processes is necessary for appropriate management (Ajwa et al., 1999), empirical data are lacking on the function of microbial communities in the rhizosphere of maize and on how these properties are impacted by planting systems in this region.

The purpose of this study was to quantify effects of raised-bed maize planting by comparing rhizosphere microbial functional groups and rhizosphere soil enzyme activities in raised-bed versus conventional flat planting. Based on earlier work, we expected the raised-bed method to increase maize growth relative to flat planting, and hypothesized that this would be related to increase in soil microbial numbers and enzyme activity.

2. Materials and methods

2.1. Study site and planting treatment

A field experiment was conducted during the maize growing seasons of 2006 and 2007 in the farm of Xun County Academy of Agricultural Science (116°41′E, 41°02′N; 72.3 m above mean sea level), Henan Province, China. Using FAO classification, these soils were Eutric Cambisol sandy loams (United Nations' classification). Mean daily temperature and precipitation in 2006, 2007, and from 1963 to 2007 during the period of maize growth (from June to October) were collected from the Xun County Meteorological Bureau, and are summarized in Fig. 1.

Two planting systems were established in the field: raised bed planting and flat planting. Plots of each treatment (planting system) were 13 m × 4 m, and replicated three times. Raised beds were formed by hand after winter wheat (*Triticum aestivium*) was harvested and crop residue incorporated into the soil, and prior to sowing of maize (variety Xundan 20). Soil between two rows was used to form the ridges (raised beds) and furrows in each plot, with ridges 50 cm wide/15 cm high and furrows 30 cm wide; maize was planted on the ridges. Each raised bed plot consisted of five beds and five furrows. Two rows of maize were planted on the top of each bed, with an interplant distance of 30.6 cm within each row. Thus, for each plot there were 10 rows of maize for each plot with a density of 5250 plants ha^{-1}.

The flat planting treatment followed traditional practices, with neither ridges nor furrows created. Planting of maize seed was identical to that for the raised bed treatment, i.e., one of two rows spaced 50 cm and another spaced 30 cm; interplant distance was 30.6 cm.

All plots received 225 kg ha^{-1} N fertilizer (urea) and 40 kg ha^{-1} P_2O_5 fertilizer for both years of study. During the first year of the study, maize was sowed on 8 June and harvested on 25 September 2006. In the second year, planting and harvesting was on 10 June and 30 September 2007, respectively.

Following harvest of wheat, but prior to implementation of planting treatments, soil samples were collected to determine nutrient content at four depths: 0–10, 10–20, 20–30, and 30–40 cm (Table 1). Soil bulk density of raised-bed and flat planting treatments was measured using the soil cores following maize harvest in each of 2006 and 2007 (Table 1).

2.2. Field sampling

To determine aboveground production, maize was sampled during three critical periods of crop growth: V6 stage (collar of the sixth leaf visible), R1 stage (silks visible), and R6 stage (physiological maturity). For the years of study, V6 stage was 2 July 2006 (24 days after sowing: DAS24) and 11 July 2007 (DAS31), R1 stage was 4 August 2006 (DAS57) and 11 August 2007 (DAS62), and R6 stage was 25 September 2006 (DAS109) and 30 September 2007 (DAS112). Three plants were sampled per plot at each time, and plant roots within a soil volume 30 cm × 30 cm × 20 cm were sampled on the same site. Microbial functional groups and enzyme determinations were done using rhizosphere soil, which was obtained by soil sampling and handling techniques (Tang et al., 2007). Rhizosphere soil was defined as the soil adhering to the roots and within the top 20 cm of the soil surface, after gently shaking the roots by hand (Baudoin et al., 2002).

2.3. Laboratory analyses

2.3.1. Microbial functional groups in the rhizosphere

Microbial functional groups were determined using the most probable number method, as described by previous studies (Alexander, 1982; Zhao and He, 2002). Briefly, 1 g of rhizosphere soil of each sample was shaken in 99 ml of water for 25 min (10^{-1} dilution), diffusing microbes thoroughly, after which consecutive 10-fold serial dilutions were obtained (until 10^{-8} dilution). Microbial functional groups determined during this process were bacteria, fungi, and actinomycetes. Bacteria culture medium was made of 3 g beef extract, 5 g peptone, 18 g agar nutrient, 5 g NaCl, and 1 L distilled water. Fungi culture medium was made of 1 g KNO_3, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.5 g K_2HPO_4, 20 g amylum, 0.5 g $MgSO_4{\cdot}7H_2O$, 18 g agar nutrient, 0.5 g NaCl, and 1 L H_2O (distilled, deionized). Actinomycetes were cultured on Gause's synthetic modified agar medium which was made of 10 g glucose, 5 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 18 g agar nutrient, 3.3 g rose Bengal, 3 g streptomycin. Three dilution rates were utilized for cloning bacteria (10^{-4}, 10^{-5}, and 10^{-6}), fungi (10^{-2}, 10^{-3}, and 10^{-4}), and actinomycetes (10^{-3}, 10^{-4}, and 10^{-5}), respectively; four replicate plates were made for each dilution rate. The plate counts for bacteria were conducted after aerobic incubation at 34 °C for 1 d. For fungi and actinomycetes, this was at 25 °C for 2 d and 25 °C for 6 d, respectively.

2.3.2. Rhizosphere soil enzyme activities

Rhizosphere soil enzyme activities were measured using modified techniques. Soil saccharase (SAC, EC 3.2.1.26) activity was measured with the method of 3,5-dinitro salicylic acid colorimetry using sucrose as the substrate (Wang et al., 2011; Ge et al., 2009). The amount of 3-amino-5-nitro salicylic acid released over 1 h was assayed colorimetrically at 508 nm, with activity was expressed as mg glucose g^{-1} dry soil. Soil urease activity was measured by indophenol colorimetry with urea as the substrate (Yao and Wu, 1998). Ammonium released over 1 h was assayed colorimetrically at 578 nm and expressed as mg NH_3-N g^{-1} dry soil. Soil protease activity was estimated as the mass (mg) of glycine kg^{-1} released from added white gelatin within a total incubation period of 1 h at 30 °C (Cai and Shen, 2005). Soil phosphatase activity was measured with disodium phenyl phosphate colorimetry (Ge et al., 2009), and the amount of phenol released over 1 h was assayed colorimetrically at 410 nm and enzyme activity was expressed as mg *p*-nitrophenol released g^{-1} dry soil. All determinations of enzymatic activities were performed in triplicate, with values reported as means.

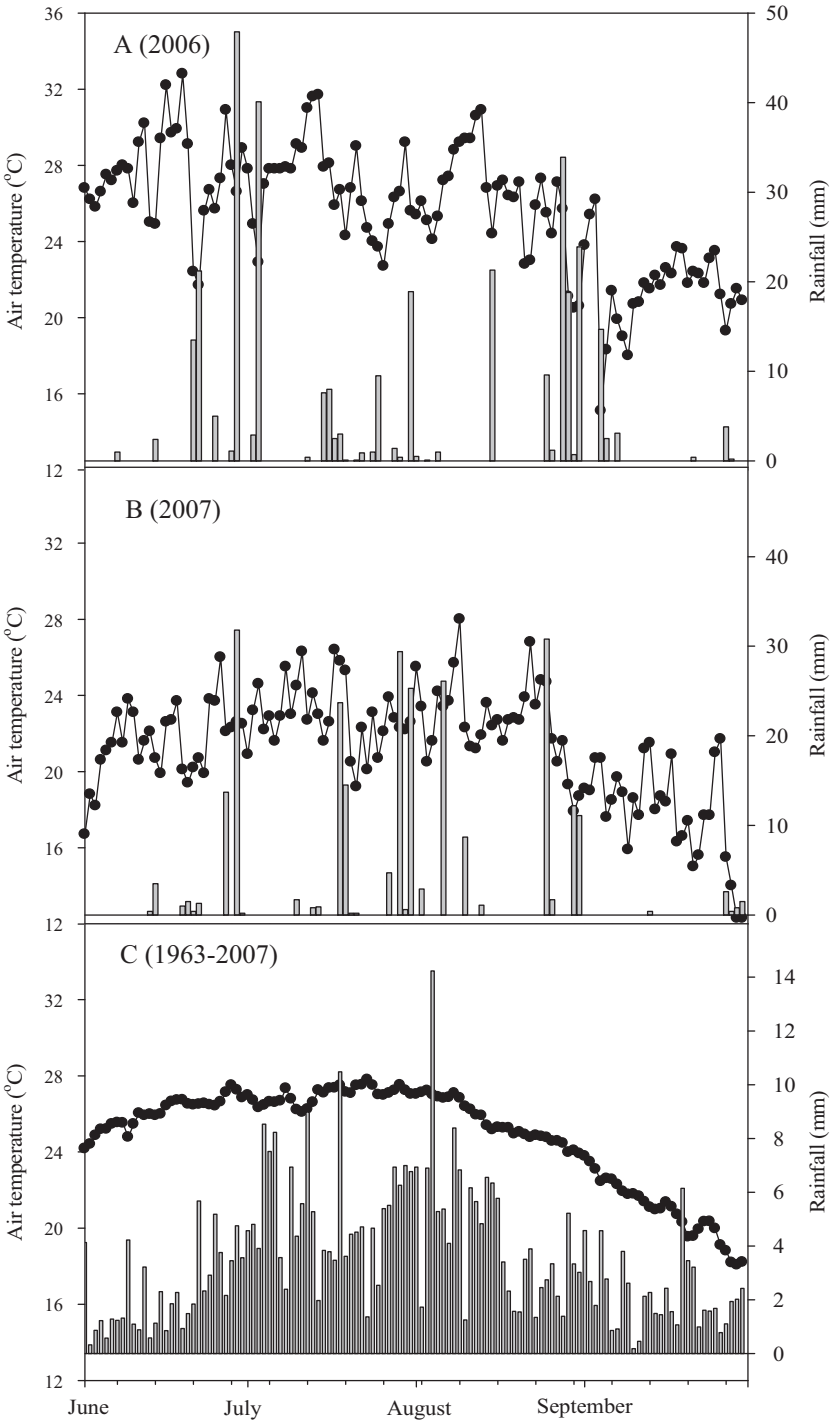

Fig. 1. Mean daily air temperature and mean daily rainfall in 2006 (A), 2007 (B) and 1963–2007 (C) during the periods of maize growth (June–October) in Xun County, Henan Province, China.

Table 1
Nutrient contents and bulk density of soils at the farm of Xun County Academy of Agricultural Science, Henan Province, China. *Note:* C, N, P, and K determinations were made on soil before planting, whereas bulk density was measured after the maize was harvested in 2006 and 2007. FP is flat planting and RBP is raised-bed planting.

Depth (cm)	Soil organic C ($g\,kg^{-1}$)	Alkaline-extractable N ($mg\,kg^{-1}$)	Olsen-extractable P ($mg\,kg^{-1}$)	NH_4OAc extractable K ($mg\,kg^{-1}$)	2006 Bulk density ($g\,cm^{-3}$)		2007 Bulk density ($g\,cm^{-3}$)	
					FP	RBP	FP	RBP
0–10	15.67 ± 0.79	73.79 ± 4.55	33.1 ± 2.87	99.31 ± 18.58	1.47	1.27	1.37	1.31
10–20	14.61 ± 1.69	56.21 ± 5.53	30.67 ± 1.39	84.81 ± 9.04	1.48	1.36	1.37	1.30
20–30	8.54 ± 1.16	42.66 ± 3.25	4.76 ± 0.37	66.22 ± 2.42	1.54	1.38	1.51	1.39
30–40	6.20 ± 0.26	28.9 ± 2.44	2.20 ± 0.26	63.70 ± 4.47	1.54	1.42	1.51	1.43

2.3.3. Soil properties

Soil organic carbon was analyzed using the H_2SO_4–$K_2Cr_2O_7$ oxidation method (Nelson and Sommers, 1982). Available soil nitrogen was measured with a micro-diffusion technique after alkaline hydrolysis. The Olsen method was used to determine available soil phosphorus (ISSCAS, 1978), whereas available soil potassium was measured in 1 mol L^{-1} NH_4OAc extracts by flame photometry (Wang et al., 2011). Soil water content was determined gravimetrically by drying at 105 °C.

2.4. Soil respiration

Soil net respiration rates were measured at the three critical sampling times during the maize growing season in 2006 and 2007 using a LI-8100 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA). Before measurement, two PVC soil collar chambers (11 cm diameter × 5 cm height) were inserted into the soil to approximately 2 cm in each plot, with the collar between rows of maize. Soil respiration rates were measured every 2 h from 6:00 to 18:00 on clear days. The rate of each chamber was calculated from mean daily values, with net respiration rate of each plot expressed as the mean of two chambers.

2.5. Statistical analyses

As for each planting treatment, one-way analysis of variance was employed to determine the difference of soil water content, soil respiration, rhizosphere soil microbial taxonomic group numbers, rhizosphere soil enzyme activities, and stem and leaf biomass over sampling times. Paired *t*-tests were used to determine differences ($p < 0.05$) of bacteria, fungi, actinomycetes, enzyme activities, and maize production between the treatments and between years. Difference of soil respiration rates between the two treatments was also analyzed with paired *t*-tests. Pearson product–moment correlation was used to quantify the relationship between microbial activity and soil water content, maize stem, leaf, and yield. Stepwise linear regression was used to analyze the relationship between rhizosphere microbial taxonomic group, rhizosphere soil enzyme activities and maize yield, and direct path confidences were also calculated. All of these statistical analyses were performed by SPSS 10.0 procedures.

3. Results

3.1. Climatic factor, soil water content and soil respiration

In 2006 (Fig. 1A) and 2007 (Fig. 1B), mean daily temperature during the period of maize growth in the area ranged from 18 to 32 °C and 12 to 26 °C, and daily precipitation from 0 to 48 mm and 0 to 32 mm, respectively. Long-term (45 yr) mean daily temperature and daily precipitation ranged from 18 to 28 °C, and from 0 to 14 mm for precipitation (Fig. 1C). Soil water content over maize growth periods varied from 16.5 to 18% and 21.2 to 25.3% in 2006 and 2007, and the mean value in RBP was smaller than in FP (Fig. 2A and B). Soil respiration rates varied from 1 to 3.9 μmol m^{-2} s^{-1} in 2006, and 1.8 to 6.9 μmol m^{-2} s^{-1} in 2007, and the difference among sampling times for each planting treatment was significant in both 2006 and 2007 (Table 2). The mean value for raised-bed planting was larger than that for flat planting (Fig. 2C and D).

3.2. Microbial functional groups

Microbial functional groups showed similar temporal patterns among the two treatments (Fig. 3), and varied significantly with sample time (Table 2). In 2006, means for bacteria, fungi, and actinomycetes over the three sampling times were higher at DAS57 than at DAS24 and DAS109 (Fig. 3A, C, E). In 2007, means were higher at DAS62 than at DAS31 and DAS112 (Fig. 3B, D, F). In 2006, mean number of bacteria (7.3×10^6 CFUs g^{-1} dry soil), fungi (1.6×10^4 CFUs g^{-1} dry soil), and actinomycetes (1.9×10^5 CFUs g^{-1} dry soil) in the raised bed planting increased by 82, 44, and 43%, respectively, relative to flat planting (Fig. 3A, C, E); in 2007, the relative increase of bacteria (3.6×10^6 CFUs g^{-1} dry soil), fungi (1.3×10^4 g^{-1} CFUs dry soil), and actinomycetes (4.1×10^5 g^{-1} CFUs dry soil) was 108, 40, and 34% (Fig. 4B, D, F), respectively. Difference of microbial taxonomic between two years was analyzed by *t*-test (data was not shown), and bacteria and fungi decreased (53 and 17%, respectively) in 2007 relative to that in 2006, whereas actinomycetes increased (up to 116%) (Fig. 3).

3.3. Enzyme activities

Temporal patterns of enzyme activities were similar between 2006 and 2007, with highest activity at DAS57 in 2006 and at DAS62 in 2007 (Fig. 4). Mean saccharase activity (2.32 mg glucose g^{-1} h^{-1} and 2.27 mg glucose g^{-1} h^{-1}) in raised bed planting in 2006 and in 2007 was 5% greater than those in flat planting (2.19 mg glucose g^{-1} h^{-1} and 2.06 mg glucose g^{-1} h^{-1}), respectively. Similarly, activities of urease (0.83 mg NH_3-N g^{-1} h^{-1} and 1.24 mg NH_3-N g^{-1} h^{-1}), protease (10.48 mg glycine kg^{-1} h^{-1} and 13.87 mg glycine kg^{-1} h^{-1}), and phosphatase (0.38 mg nitrophenol g^{-1} h^{-1} and 0.62 mg nitrophenol g^{-1} h^{-1}) activities in 2006 and in 2007 were at least 16% greater in the raised bed planting than in the flat treatment.

3.4. Plant biomass and maize yield

Stem and leaf biomass varied significantly with sample time (Fig. 5, Table 2), and aboveground biomass (stem + leaf) of maize increased significantly from V6 stage to R1 stage, until R6 stage in both years (calculated from data shown in Fig. 5). Stem and leaf biomass were increased in raised bed planting relative to flat planting by 14 and 8% in 2006, and by 9 and 11% 1 in 2007, respectively. Leaf biomass in 2006 was 8% lower than those in 2007. Yields were higher in raised bed planting treatments than in flat treatments in 2006 (6%) and 2007 (12%), and the mean yield increased 18% in 2007 relative to 2006 (Table 3).

In 2006, seasonal variation of leaf biomass in the raised bed planting were significantly correlated with the variation of bacteria, whereas in 2007, temporal variations of fungi was significantly correlated with soil water content, stem and leaf biomass (Table 4). Mean maize yield of both planting methods was significantly correlated with actinomycetes ($r = 0.91$, $p = 0.01$, $n = 6$), saccharase ($r = 0.96$, $p = 0.001$, $n = 6$), and protease ($r = 0.86$, $p = 0.03$, $n = 6$) in 2006, and with bacterial ($r = 0.97$, $p = 0.001$, $n = 6$), actinomycetes ($r = 0.89$, $p = 0.02$, $n = 6$), and protease ($r = 0.89$, $p = 0.02$, $n = 6$) in 2007. Stepwise linear regression revealed that saccharase activity explained 90% variability for mean maize yield between the two planting methods in 2006. In 2007, bacteria, fungi density and protease activity explained 99% variability of mean maize yield, and their direct path coefficients were 1.06, −0.36 and 0.19, respectively.

4. Discussion

4.1. Dynamics of microbial functional groups and enzyme activities of the rhizosphere

Microbial functional groups and enzyme activities of the rhizosphere of maize exhibited distinct temporal changes during

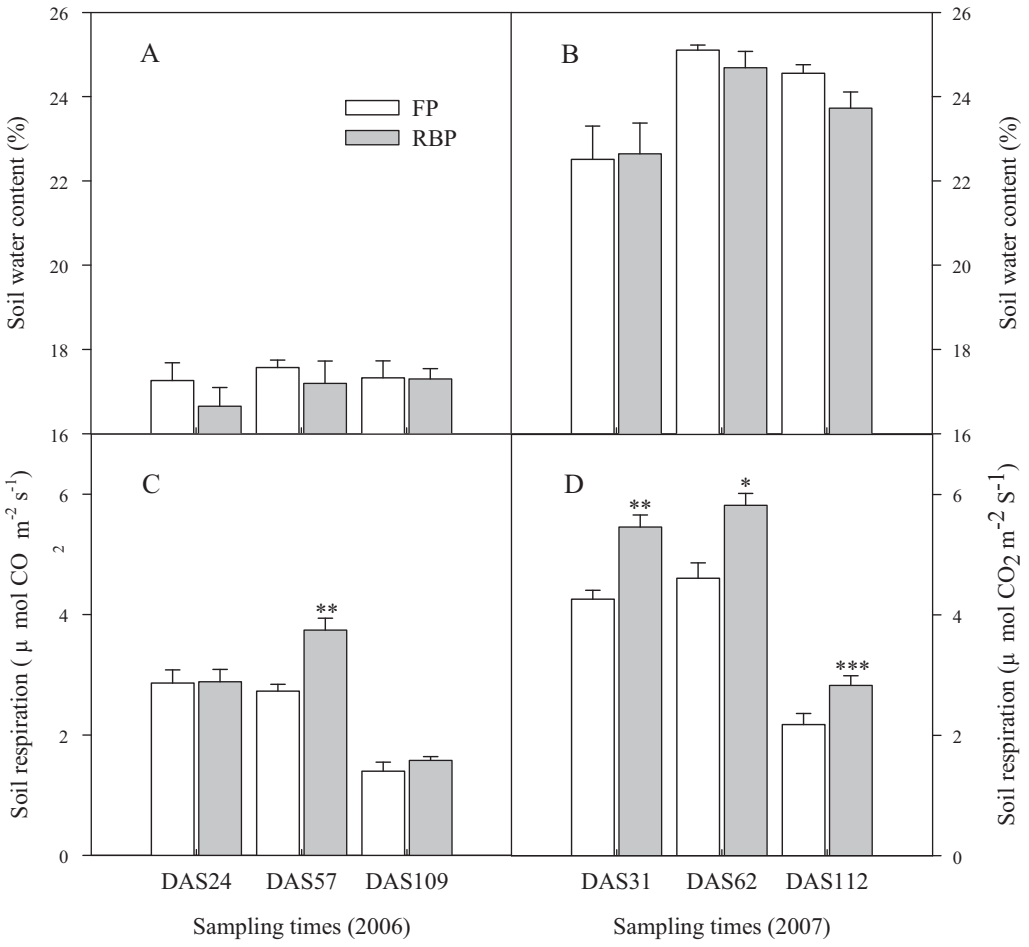

Fig. 2. Temporal variations of soil water content (A and B) and soil respiration (C and D) in the flat planting (FP) and raised bed planting (RBP) treatments during the periods of maize growth in 2006 and 2007. Values are means ($n = 3$), standard errors shown. *, ** and *** significance between two treatments at $p < 0.05$, 0.01, and 0.001 levels, respectively.

Table 2
F values produced by one-way analysis of variance used to determine differences of soil water content (SWC), soil respiration, rhizosphere soil microbial functional groups, rhizosphere soil enzyme activities, and maize stem and leaf productions (kg ha^{-1}) during the periods of maize growth in 2006 and 2007 for flat planting (FP) and raised-bed planting (RBP) treatments.

	2006		2007	
	FP	RBP	FP	RBP
SWC%	0.21	0.66	8.24*	3.78
Soil respiration	23.81***	23.06***	42.81***	77.47***
Bacteria (g^{-1})	18.18**	22.83**	3.13	4.78
Fungi (g^{-1})	6.17*	3.42	16.39**	16.85**
Actinomycetes (g^{-1})	61.12***	20.04**	16.66**	8.98*
Saccharase (mg glucose g^{-1} h^{-1})	13.73**	39.64***	4.43	0.32
Urease (mg NH_3-N g^{-1} h^{-1})	0.16	3.87	2.43	19.78**
Protease (mg glycine kg^{-1} h^{-1})	5.32*	14.48**	40.41***	86.98***
Phosphatase (mg nitrophenol g^{-1} h^{-1})	2.13	1.14	0.30	2.93
Stem (kg ha^{-1})	298.88***	211.81***	138.92***	373.70***
Leaf (kg ha^{-1})	257.69***	191.67***	261.99***	189.36***

* Significant difference among sampling times at $p < 0.05$.
** Significant difference among sampling times at $p < 0.01$.
*** Significant difference among sampling times at $p < 0.001$.

Table 3
Comparison of maize yield parameters between flat planting (FP) and raised-bed planting (RBP) systems in 2006 and 2007.

Year	Planting method	Grains per ear	1000-grain weight (g)	Yield (kg ha^{-1})
2006	FP	569.9 ± 9.8	268.5 ± 0.7	11293.2 ± 157.3
	RBP	573.3 ± 12.1	280.4 ± 0.6**	11961.9 ± 112.1*
2007	FP	508.6 ± 9.2	337.9 ± 2.4	12915.3 ± 154.0
	RBP	544.4 ± 10.0	359.0 ± 3.5**	14573.9 ± 226.2**

* Significant difference between two planting methods at $p < 0.05$.
** Significant difference between two planting methods at $p < 0.01$.

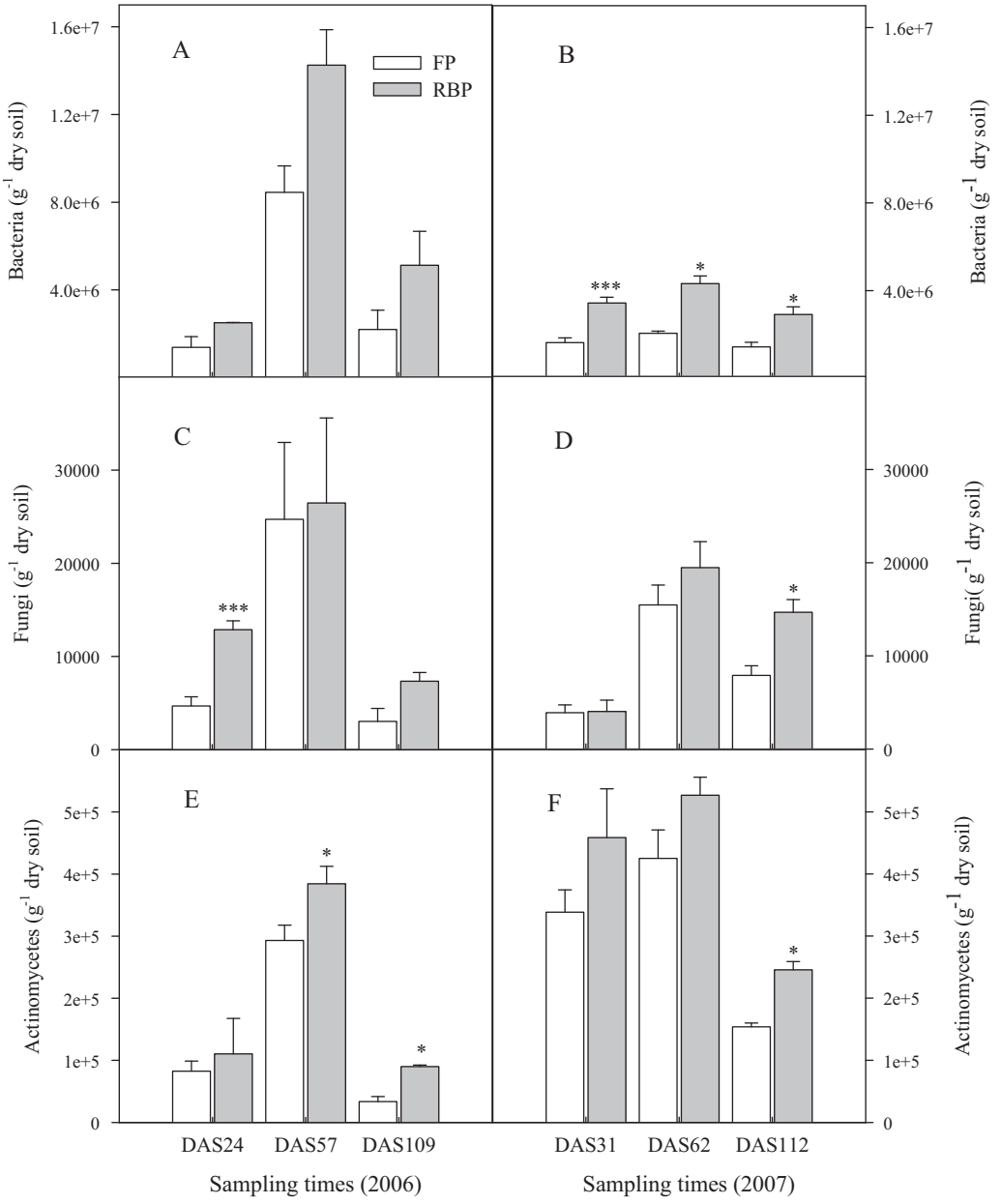

Fig. 3. Temporal variations of rhizosphere soil microbial functional groups in the flat planting (FP) and raised bed planting (RBP) treatments during the periods of maize growth. *, ** and *** significance between two treatments at $p < 0.05$, 0.01, and 0.001 levels, respectively. Values are means ($n = 3$), standard errors shown.

periods of growth. Highest densities and activities for both planting treatments were observed at R1 stage (August) in both years (Figs. 3 and 4). These results are consistent with previous studies that have shown soil microbes (Brant et al., 2006; Sardans et al., 2008) and enzyme activities to display distinct temporal variability (Watanabe and Hayano, 1996b; Zhou et al., 2005). The range of rhizosphere soil urease activity was similar to field values reported by Ge et al. (2009). Ranges of rhizosphere soil enzyme activities were similar to values reported for farmland in the Loess Plateau by Wang et al. (2011) and in other agro-ecosystems in China by Tao et al. (2009) and Yuan et al. (2011).

Temporal patterns of rhizosphere microbes are influenced greatly by the physical environment, such as soil moisture and temperature regimes (Watanabe and Hayano, 1996b; Kandeler et al., 2002; Ge et al., 2010). Over the growing period for maize, ambient air temperature and rainfall displayed distinct seasonal patterns (Fig. 1), which may alter soil microclimate and influence of microbial composition and function (Sardans et al., 2008).

4.2. Potential microbially mediated effects of planting methods on maize yield

Microbial functional groups and associated enzyme activities of the rhizosphere were higher in raised bed planting system than in flat planting over the periods of maize growth (Figs. 3 and 4). Mean yield of maize for both were 9% higher in raised-bed treatments than in flat-planting treatments. Correlations between yield and soil microbial functional groups (especially actinomycetes) and enzyme parameters suggest that this increase may have been caused, in part, by creation of more favorable microbial microhabitat during construction of the raised beds.

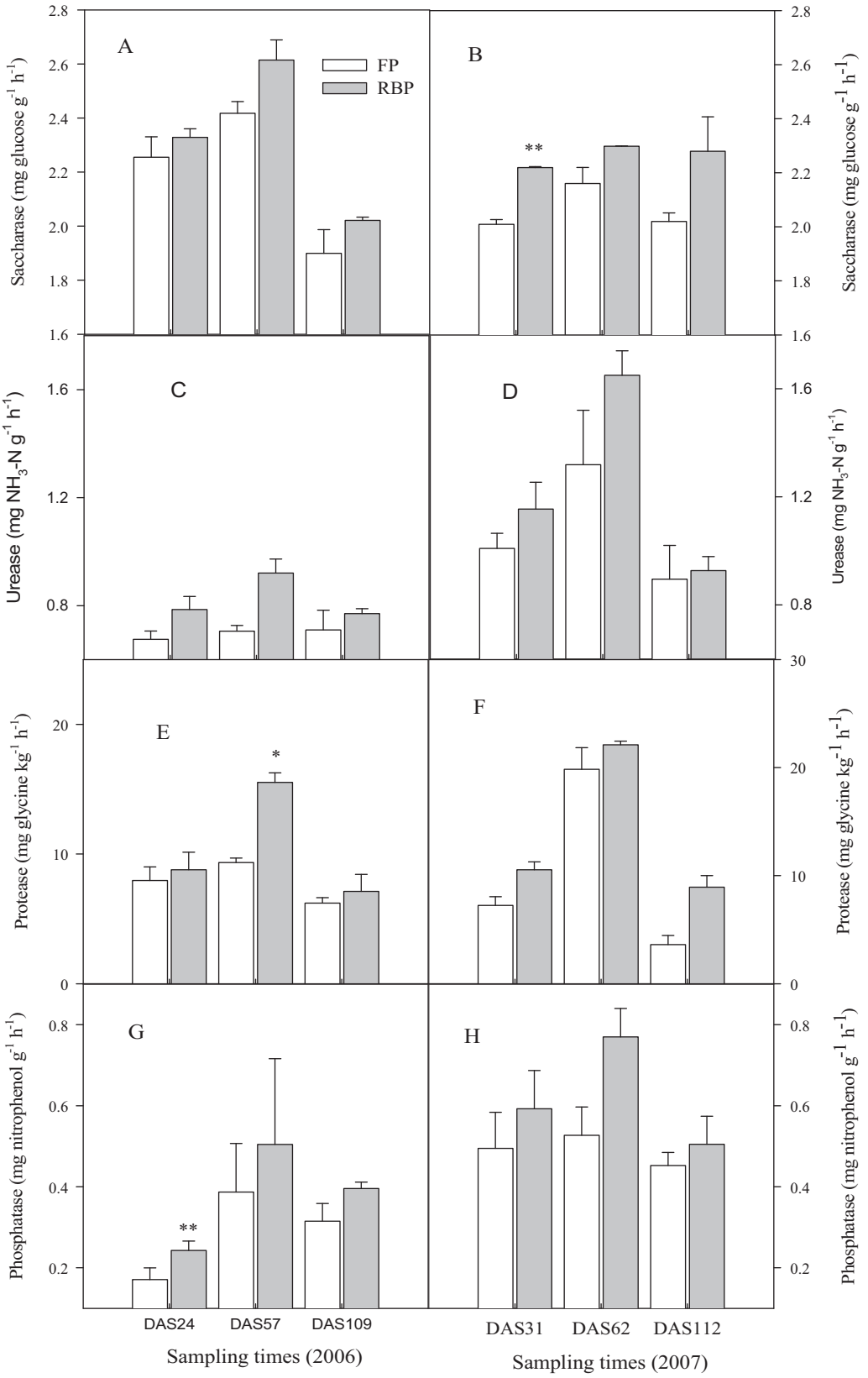

Fig. 4. Temporal variations of rhizosphere soil saccharase (mg g^{-1} h^{-1}), urease (mg g^{-1} h^{-1}), protease (mg kg^{-1} h^{-1}), and phosphatase (mg g^{-1} h^{-1}) enzymes activities in the flat planting (FP) and raised bed planting (RBP) treatments during the periods of maize growth. *, ** and *** significance between two treatments at $p < 0.05$, 0.01, and 0.001 levels, respectively. Values are means ($n = 3$), standard errors shown.

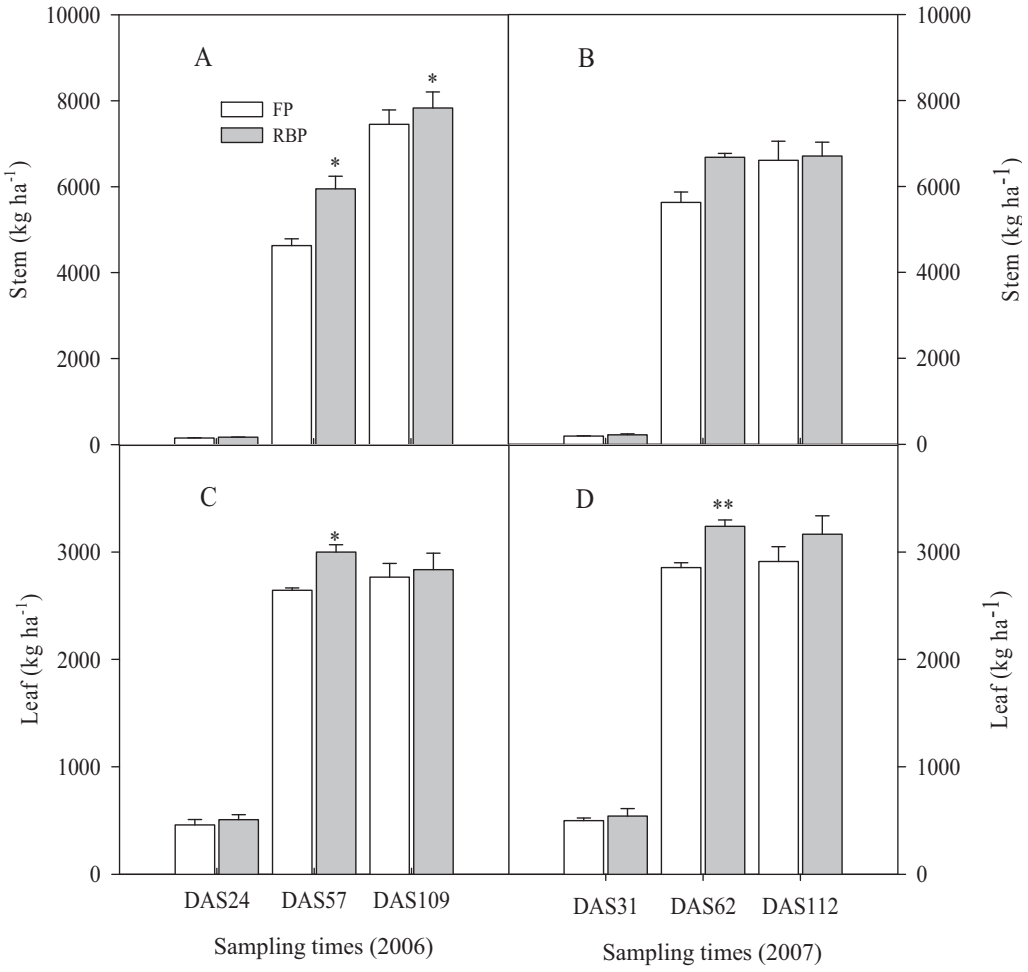

Fig. 5. Differences in the production of leaf and stems between flat planting (FP) and raised bed planting (RBP). *, ** and *** significance between two treatments at $p < 0.05$, 0.01, and 0.001 levels, respectively. Values are means ($n = 3$), standard errors shown.

Indeed, raised bed planting resulted in the improvement of soil physical properties such as bulk density, especially in the rooting zone (Table 1). Other studies have shown that the bedding process can increase soil aggregate formation and maintain optimal ratios of solid, liquid, and gaseous phase in agricultural soils (Limon-Ortega et al., 2006; Govaerts et al., 2007). Raised-bed planting also optimizes water holding capacity and conductivity of soil solutions, increasing bacterial counts relative to flat planting via enhanced aeration/porosity of soil (Hemmat and Eskandari, 2004; Patiño-Zúñiga et al., 2009). Higher soil respiration in the raised bed planting treatments also indicated that this method improved soil microbial activities. All of these improvement in soil microclimate also stimulates microbial decomposition of organic matter and mineralization of organic N (Govaerts et al., 2007; Verachtert et al., 2009), which, although not measured in this study, can further increase the size of microbial populations (Pennanen et al., 1999; Bradley et al., 2006). Indeed, the general pattern of higher bacteria/fungi ratios in raised-bed planting relative to flat planting (calculated from data shown in Fig. 4) is indicative of improved availability of N (Gilliam et al., 2011).

Enzyme activities in the rhizosphere are affected by abiotic conditions (e.g., temperature, moisture, aeration), the biochemical makeup of organic matter, and its distribution with soil depth (Zhou et al., 2005; Sardans et al., 2008; Wang et al., 2011). Microbially mediated increases in enzyme activities, along with additions of organic matter (Browman and Tabatabai, 1978), further improve the rhizosphere for plant growth (Badalucco et al., 1996; Wang et al., 2011). Because raised-bed planting incorporates crop residue into the soil, organic matter is more readily available to soil microorganisms (Kristensen et al., 2003), further contributing to the higher enzyme activities observed in raised-bed versus flat planting (Fig. 4).

We suggest that higher activity of enzymes in the rhizosphere of maize may have contributed to the increase of plant production and yield in the raised bed planting. Certainly, the direct path coefficient of protease activity with maize yield is consistent with this contention. Previous studies found that protease activity is correlated with bacterial number and rates of mineralization of organic N (Watanabe and Hayano, 1996a; Saviozzi et al., 2002), and organic P is mineralized to inorganic P by phosphatase (Tabatabai and Bremner, 1969). Saccharase activity is also related to available sugars that can readily be metabolized in the rhizosphere, and urease activity is strongly indicative of enhanced nitrogen transformation in the soil (Edwards et al., 2006; Xing et al., 2010).

Close-coupling between plant uptake of soil nutrients and rhizosphere enzyme activities suggests that higher activity rates may have increased the amount of available nutrients taken up by the plant (Kroehler and Linkins, 1988; Johnson et al., 2005). Indeed, plant production is closely associated with the supply of nutrients, such as N and P (Gilliam, 2006, 2007), in the soil. This has been observed in other studies (Limon-Ortega et al., 2000, 2002; Marschner et al., 2004), and is consistent with the observation that maize leaf and stem biomass in raised-bed planting were higher than those in flat planting (Fig. 5).

Table 4
Pearson's coefficient of (r) correlation between rhizosphere microbial functional groups and enzyme activities with soil water content (SWC) (%), maize stem, and leaf biomass in flat (FP) and raised-bed (RBP) planting. Three sampling times of three replication indicated $n = 9$ for all comparisons. Significant correlations ($P < 0.05$) are indicated in bold type.

			Bacterial (CFUs g^{-1} of soil)	Fungi (CFUs g^{-1} of soil)	Actinomycetes (CFUs g^{-1} of soil)	Saccharase (mg glucose g^{-1} h^{-1})	Urease (mg NH_3-N g^{-1} h^{-1})	Protease (mg glycine kg^{-1} h^{-1})	Phosphatase (mg nitrophenol g^{-1} h^{-1})
	SWC	r	0.21	0.11	0.18	−0.25	−0.06	0.002	−0.04
FP in 2006	Stem	r	0.21	0.07	−0.06	−0.52	0.15	−0.36	0.52
	Leaf	r	0.50	0.31	0.29	−0.26	0.24	−0.08	0.57
	SWC	r	0.34	0.46	−0.08	0.13	0.16	0.01	−0.36
RBP in 2006	Stem	r	0.43	0.04	0.20	−0.24	0.14	0.08	0.37
	Leaf	r	**0.66***	0.21	0.45	0.04	0.34	0.35	0.49
	SWC	r	0.07	**0.69***	−0.13	0.40	0.16	0.39	0.28
FP in 2007	Stem	r	0.11	0.62	−0.32	0.33	0.12	0.17	−0.10
	Leaf	r	0.18	**0.69***	−0.20	0.40	0.21	0.27	−0.06
	SWC	r	0.57	**0.68***	−0.05	0.25	0.45	0.59	−0.11
RBP in 2007	Stem	r	0.13	**0.88*****	−0.25	0.33	0.18	0.38	0.09
	Leaf	r	0.13	**0.91*****	−0.26	0.32	0.21	0.41	0.11

* Significance at $p < 0.05$.
** Significance at $p < 0.01$.
*** Significance at $p < 0.001$.

In conclusion, data from this study confirm that raised-bed tillage can increase yield of maize. Indeed, it is notable that all measures of plant productivity, including leaf, stem, and crop yield, were significantly higher for raised-bed than for flat planting (Fig. 5, Table 3). Furthermore, results for planting-mediated differences in microbial functional groups and enzyme activities suggest that this increase in maize productivity and yield may in part arise via stimulation of microbial activities. Thus, it is a reasonable hypothesis that enhanced soil conditions for increased microbial community function contributes to increased yield of maize with raised-bed planting. Clearly, further work is needed to test this hypothesis and to elucidate mechanistic relationships between raised bed-mediated improvements in soil microenvironments and increases in maize yield. Findings of this study certainly suggest that raised-bed planting represents a potentially important contribution to meeting China's challenge to sustainable increase its supply of corn.

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