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# INFLUENCE OF DIFFERENT FLUVO-AQUIC SOIL CONFIGURATIONS ON RHIZOSPHERE MICROORGANISMS AND THEIR ENZYME ACTIVITIES ON YIELD OF SUMMER MAIZE

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## Abstract

Pool experiments were carried out to study the effects of seven typical configurations (gritty soil (GS), silt soil (SS), sandy loam (SL), medium loam (ML), light clay (LC), loam in the upper (0 - 20 cm) soil layer and light clay in the lower (20 - 60 cm) soil layer (ULDC)) of fluvo-aquic soil on the activities of microorganisms in the rhizosphere of summer maize and yield. The yield of summer maize grown in ULDC was the highest at 10,415 kg/ha. There was a significant difference between the nutrient content of the plough layers of different soil configurations. The highest levels of organic matter, available nitrogen, phosphorus, and potassium were observed in ULDC, followed by ML, SL, LC, UCDS, SS, and GS. In addition, the different soil configurations ranked the same in terms of the number of rhizosphere microorganisms and the activities of protease and urease. ULDC, along with LC and ML in the top soil. These findings indicate that a fertile soil with ULDC preserves best fertility and moisture and is thus the ideal soil configuration for land consolidation and sustainable agriculture.

## Introduction

Soil configuration or soil profile pattern, which refers to the arrangement and combination of soil layers with different textures, is the most important feature of the soil profile. It has been found that clay soil is the most fertile, followed by loamy soil, and then sandy soil (Jindaluang *et al.* 2013, Kurunc *et al*. 2011, Min and Lee 2010). In addition, soil water retention differs between soil types. Sandy soil has a higher porosity, and weaker capillary action, and therefore has poor water storage and retention capacities (Su *et al.* 2005). In contrast, loamy and clay soils have stronger capillary action and can retain a large amount of water and nutrients (Li *et al.* 2013, Nath and G 2014).

The bioactive substances in soil, soil microorganisms and soil enzymes, directly participate in processes such as substance transformation, nutrient release, and nutrient fixation. Therefore, they are closely related to soil fertility and soil environmental quality. Compared with sand, clay and silt can maintain larger and more diverse microbial populations (REFS) because they have high organic contents and can therefore provide more energy and nutrients that support the growth of microorganisms (REFS) (Jung *et al.* 2008, Hu *et al.* 2014, Voroney 2007). Soil quality is a key factor influencing maize yield (Bronick and Lal 2005, Braimoh and Vlek 2006). Currently, most studies on soil configuration mainly focus on the classification of soil systems, utilisation of soil water, water-saving irrigation modes, diagnosis of soil conditions and evaluation of soil fertility. In addition, soil improvement and utilization and evaluation of the relationship between crop quality and soil fertility are also examined. However, there have been a few investigations on the activities of rhizosphere microorganisms in soil and their effect on yield.

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To provide a theoretical and technical basis for increasing the crop yield and sustainable development of summer maize by altering the soil configuration, the authors explored the influence of different soil configurations on the activities of microorganisms and soil protease and urease in the rhizosphere of summer maize and on yield.

## Materials and Methods

The experiment was carried out at the National Fluvo-Aquic Soil Monitoring Base, Henan Academy of Agricultural Sciences (HNAAS), located at 35.04°N and 113.68°E at an altitude of 63.40 m in 2011 and 2012. Meteorological data for the experimental site were downloaded from the China meteorological sharing service system (http://cdc.cma.gov.cn/) (Table 1). Maize was planted in ponds, each with an area of 1.0 m ×1.5 m and a depth of 0.8 m. Ponds were enclosed using cement boards with the bottom open. The soil was air-dried, sieved and divided into three soil particles. In this experiment, a total of seven soil configurations were tested: gritty soil (GS), silt soil (SS), sandy loam (SL), medium loam (ML), light clay (LC), loam in the upper (0 - 20 cm) soil layer and light clay in the lower (20-60 cm) soil layer (ULDC), and light clay in the upper layer and sandy loam in the lower layer (UCDS) (Table 2). The experiment was performed using a randomized block design, with each configuration replicated in three ponds. In each pond, 10 plant using with cv. Xianyu 335, were sown in two rows at a planting density of 100,050 plants/ha. The seeds of maize were sown on 11 June and harvested on 11 October. A protection zone, 2 m in width, was established around the test area for pond culture. Topdressing with 750 kg/ha of compound fertiliser (nitrogen (N), phosphorus (P), and potassium (K): 28:6:6) was applied between the rows in the field at the seedling stage. During the growth maize plants in all ponds were well irrigated using a water bag and weeded manually.

**Table 1. Meteorological data of the experimental sites.**

| Time | Average temperature (ºC) | | Rainfall (mm) | | Sunshine time (hr) | |
|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 |
| Early June | 28.1 | 25.9 | 1.4 | 0 | 94.7 | 65.5 |
| Middle June | 27.3 | 28.8 | 0 | 0 | 71.3 | 106.8 |
| Late June | 27.4 | 26.5 | 21.9 | 33.3 | 67.2 | 46.3 |
| Early July | 28.1 | 26.9 | 42.3 | 58 | 54.5 | 44.1 |
| Middle July | 28.1 | 27.4 | 12.1 | 3.3 | 56.6 | 37.3 |
| Late July | 31.8 | 28.7 | 15.9 | 174.4 | 62.3 | 48 |
| Early August | 25.4 | 26.5 | 44.2 | 46.7 | 30.7 | 54.7 |
| Middle August | 26.6 | 24.9 | 12.6 | 25 | 20 | 36.1 |
| Late August | 26.8 | 23.9 | 19.1 | 0.2 | 66.2 | 76.5 |
| Early September | 20.7 | 22.9 | 18.5 | 20.1 | 10.5 | 41.7 |
| Middle September | 17.5 | 19.8 | 136.6 | 10.2 | 28.8 | 62.7 |
| Late September | 19.2 | 19.9 | 28.1 | 4.8 | 54.3 | 47.6 |

After harvest, the ears were air-dried. The ear length, diameter, bald tip length, number of grain rows, number of grains per row, 1000-kernel weight and yield were measured in the laboratory. Rhizosphere microorganisms and enzyme activities in soil samples were taken at a distance of 10 cm from the rows, and cores 0 - 20 cm deep were taken using an earth boring auger (made in Beijing New Landmark Soil Equipment Co., Ltd., Beijing). Samples obtained from five locations in each pond were taken at five stages of summer maize growth: pre-sowing, seedling, jointing, sulking, and maturity. After being uniformly mixed, these soil samples were put into sterile bags and immediately taken to the laboratory. The soil attached to the maize roots, namely, rhizospheric soil, was used to determine the number of soil microorganisms, while the remaining soil was naturally dried for measuring the activities of soil enzymes.

**Table 2. Tested soil mechanical composition.**

| DTSBC | Soil mechanical composition (%) | | |
|---|---|---|---|
| | < 0.002 mm | 0.02 - 2.00 mm | 0.002 - 0.02 mm |
| GS | 4.17 | 12.59 | 83.24 |
| SS | 8.21 | 14.52 | 77.27 |
| SL | 17.69 | 28.57 | 53.74 |
| ML | 10.57 | 18.95 | 70.46 |
| LC | 40.96 | 29.67 | 29.37 |
| ULDC | 50.84 | 27.67 | 21.49 |
| UCDS | 33.42 | 35.87 | 30.71 |

DTSBC, different types of the soil body configuration; GS, gritty soil; SS, silt soil; SL, sandy loam; ML, medium loam; LC, light clay; ULDC, upper loam and down clay; UCDS, upper clay and down sandy.

Fungi, bacteria, and actinomycetes contained in the soil were cultured in Thayer-Martin medium, beef-protein medium, and Gauze's No.1 medium, respectively, and their numbers were counted using the dilution-plate method (Alexander 1982). Proteinase activity was assayed as described by Yang (1988) with minor modifications. Urease activity was determined using the method described by Guan (1986) with minor modifications. Water and fertilizer loss were calculated based on the data obtained before sowing and after harvesting and were compared between different soil configurations. The statistical significance of the effect of soil configuration was analyzed by analysis of variance (ANOVA), and mean values were compared using the least significance difference (LSD) multiple comparison test in the SAS statistical package version 8.2 (SAS Institute 2001).

## Results and Discussion

Summer maize yield and yield components differed between all soil configurations (Table 3). The variation in yield and 1000-grain weight (TGW) in different soil configurations were basically consistent. Maize yield and TGW in ULDC were the highest, followed by ML and the lowest in GS, and both traits significantly differed between ULDC and other all treatments except ML. The ranking of other yield components, ear length, ear diameter, number of grain rows, and number of grains in a row, was the same as for yield and TGW, but the differences in these traits between treatments were not significant.

Both before sowing and after harvesting, the nutrient contents in the plough layers of different soil configurations were quite different (Table 4). The highest levels of organic matter and available N, P, and K were found in ULDC both before sowing and after harvesting, followed by ML and the lowest was in GS. The levels of organic matter and available N, P, and K in all soil configurations decreased compared to the samples taken before sowing. The differences in available N between ULDC, SS and GS were significant.

**Table 3. Yield and yield components of summer maize under the different soil body configurations.**

| DTSBC | Ear length (cm) | Ear diameter (cm) | Bald tip length (cm) | Ear rows (row) | Row grains (grain) | Yield (kg/hm$^2$) | 1000-kernel weight (g) |
|---|---|---|---|---|---|---|---|
| GS | 61.33a | 23.33a | 8.00a | 15.07a | 22.07a | 7053.90dc | 303.38c |
| SS | 62.09a | 24.75a | 7.82a | 15.39a | 22.60a | 7259.83c | 318.24c |
| SL | 64.00a | 25.67a | 3.67c | 15.87a | 23.74a | 9419.24b | 353.78b |
| ML | 65.11a | 26.03a | 3.47c | 16.07a | 25.71a | 9976.42a | 359.68ab |
| LC | 62.67a | 25.00a | 5.33b | 16.13a | 24.00a | 8540.08b | 332.22c |
| ULDC | 69.67a | 27.67a | 3.17c | 16.53a | 25.95a | 10415.10a | 369.33a |
| UCDS | 62.00a | 24.67a | 7.67a | 15.73a | 24.27a | 7521.71c | 327.47c |

Different letters represented significance at $p < 5\%$ among 7 different types of the soil body configuration, same as follows.

**Table 4. Comparison of nutrient loss of different soil body configurations.**

| DTSBC | Original nutrients | | | | Residual nutrients | | | |
|---|---|---|---|---|---|---|---|---|
| | Organic matter (g/kg) | Hydrolytic N (mg/kg) | Available P (mg/kg) | Available K (mg/kg) | Organic matter (g/kg) | Hydrolytic N (mg/kg) | Available P (mg/kg) | Available K (mg/kg) |
| GS | 5.22d | 45.71c | 17.34c | 74.23c | 4.61d | 35.30c | 16.87c | 73.29c |
| SS | 5.57d | 46.26c | 17.56c | 75.43c | 4.98d | 36.81c | 17.08c | 74.46c |
| SL | 8.57b | 50.63b | 20.67ab | 79.59ab | 8.19b | 45.75b | 20.04ab | 78.33ab |
| ML | 8.74b | 51.72b | 20.39ab | 81.52a | 8.36b | 48.72b | 20.06ab | 80.85a |
| LC | 6.96c | 50.61b | 19.49b | 79.16b | 6.54c | 44.90b | 18.92b | 78.02b |
| ULDC | 9.52a | 56.50a | 21.26a | 84.47a | 9.27a | 55.46a | 20.91a | 83.78a |
| UCDS | 6.14cd | 50.11b | 19.46b | 79.08b | 5.48cd | 43.61b | 18.96b | 78.08b |

The number of soil bacteria in the rhizosphere of summer maize was continuously increasing after sowing and reaching a maximum at the silking stage. Thereafter, the number of bacteria dramatically decreased and at maturity was almost the same as that before sowing (Table 5a). There were much differences in the number of rhizosphere bacteria in different soil configurations. Before sowing, the number of bacteria was maximum in SL, followed by ULDC and GS had the lowest. For all soil configurations, the number of bacteria in the maize rhizosphere at the seedling, jointing and silking stages was significantly higher than that before sowing and observed in this order- ULDC > ML > SL > LC > UCDS > SS > GS. Before sowing, there were few fungi in the soil, with only a slight difference in the number of fungi between different soil configurations (Table 5b). SL had the highest quantity of fungi, followed by ULDC and GS. After sowing, the number of rhizosphere fungi increased rapidly by the seedling and jointing stages and reached a maximum at the silking stage, after which the number decreased. From sowing to the seedling stage, the number of rhizosphere fungi in ULDC increased the most (7.14-fold). The highest number of fungi in the maize rhizosphere from the seedling stage to the jointing stage and until the silking stage was observed for ULDC, followed by ML and GS had the fewer number. By the mature stage, the number of fungi decreased, and the highest and lowest reduction was observed for GS and ULDC, respectively.

**Table 5. Changes of soil rhizosphere bacteria number (5a), fungi number (5b), actinomyces number (5c) under different soil body configurations in summer maize.**

**(5a) Bacteria number (×$10^7$)**

| DTSBC | Pre-sowing | Seedling | Jointing | Silking | Maturity |
|---|---|---|---|---|---|
| GS | 1.29d | 4.09d | 7.05d | 8.36d | 1.56e |
| SS | 1.56cd | 4.21d | 7.67d | 8.99d | 1.89e |
| SL | 3.46a | 5.31b | 10.87bc | 15.62b | 4.62b |
| ML | 2.78a | 5.69ab | 12.84a | 17.68a | 4.78ab |
| LC | 2.53ab | 5.27bc | 9.46c | 13.14b | 3.04c |
| ULDC | 3.18a | 6.08a | 14.68a | 18.43a | 5.61a |
| UCDS | 1.95bc | 5.14c | 9.13cd | 10.59c | 2.36d |

**(5b) Fungi number (×$10^3$)**

| DTSBC | Pre-sowing | Seedling | Jointing | Silking | Maturity |
|---|---|---|---|---|---|
| GS | 2.09b | 8.26d | 22.47c | 32.56c | 14.45d |
| SS | 2.15b | 10.81cd | 25.45c | 34.22c | 16.27d |
| SL | 2.79a | 15.39ab | 36.41ab | 47.21b | 26.51ab |
| ML | 2.53a | 16.93a | 39.21a | 49.28ab | 28.64a |
| LC | 2.46a | 14.07b | 33.62b | 46.16b | 24.83bc |
| ULDC | 2.61a | 18.65a | 42.96a | 53.65a | 31.89a |
| UCDS | 2.38b | 12.55bc | 30.18b | 40.39b | 22.09c |

**(5c) Actinomyces number (×$10^5$)**

| DTSBC | Pre-sowing | Seedling | Jointing | Silking | Maturity |
|---|---|---|---|---|---|
| GS | 4.08c | 9.16a | 18.34d | 31.55d | 10.08d |
| SS | 4.29c | 10.3a | 19.57cd | 36.08d | 10.92d |
| SL | 8.91a | 16.08a | 30.27ab | 60.59b | 21.08bc |
| ML | 7.43a | 16.44a | 32.78a | 66.43b | 23.93b |
| LC | 7.19a | 15.87a | 27.41b | 55.39c | 18.22c |
| ULDC | 8.06a | 18.63a | 34.66a | 71.54a | 28.11a |
| UCDS | 6.32b | 13.51b | 24.08bc | 50.28c | 17.67c |

Table 5c showed, little difference in the number of actinomycetes among the seven soil configurations before sowing. At the seedling and jointing stages, the number of rhizosphere actinomycetes in all soil configurations observed between $14.28 \times 10^5$ and $26.73 \times 10^5$, reflects a

2.16 to 4.04-folds increase compared the number before sowing. At the seedling stage, ULDC had the largest number of rhizosphere actinomycetes than in ML, SL, LC, UCDS, SS and GS, respectively. At the silking stage, the maximum number of rhizosphere actinomycetes reached for all soil configurations, and ULDC had the largest number ($71.54 \times 10^5$). In contrast, the number of actinomycetes in different soil configurations at maturity all decreased, with ULDC having the least reduction in number.

The activities of protease and urease in rhizosphere soil were related to plant growth. Before sowing, the activities of both enzymes in each soil configuration were low. From the seedling stage to the jointing stage, the enzyme activities increased and reached the maximum level at the silking stage, after which the activities gradually decreased (Table 6). Before sowing, the activities of both protease and urease were highest in LC, followed by ULDC and SS had the lowest. At the seedling, jointing and silking stages, the largest change in protease and urease activities were observed for ULDC, followed by ML and the smallest change in GS. However, the variation in the activities of the two enzymes differed during growth. There was no significant difference in proteinase activity between different soil configurations before sowing, but a significant difference was observed at the jointing stage and at maturity. In contrast, the variation in urease activity was more moderate, and a significant difference between different soil configurations was observed at the silking stage. From pre-sowing to the silking stage, protease activity increased the most in ULDC (96.30%) while it increased the least in GS (by 20.34%). From the silking stage to maturity, rhizosome soil protease enzyme activity gradually decreased, with the highest reduction observed for GS, followed by SS and the fewer reduction in ULDC. The variation in urease activity between soil configurations was basically consistent with the variation in protease activity.

**Table 6. Activities of the soil rhizosphere protease and urease under different soil body configurations in summer maize (U/g).**

| DTSBC | Protease | | | | | Urease | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-sowing | Seedling | Jointing | Silking | Maturity | Pre-sowing | Seedling | Jointing | Silking | Maturity |
| GS | 0.59a | 0.62b | 0.64c | 0.71c | 0.52c | 0.33a | 0.39a | 0.48a | 0.61c | 0.36c |
| SS | 0.61a | 0.73b | 0.75c | 0.86c | 0.66c | 0.35a | 0.41a | 0.56a | 0.69c | 0.44bc |
| SL | 0.63a | 0.89b | 0.92b | 1.09b | 0.91b | 0.42a | 0.51a | 0.63a | 0.76b | 0.53b |
| ML | 0.75a | 1.12a | 1.18a | 1.3ab | 1.11a | 0.45a | 0.57a | 0.74a | 0.84a | 0.71a |
| LC | 0.86a | 1.01a | 1.09b | 1.14b | 0.95b | 0.51a | 0.54a | 0.71a | 0.8ab | 0.64a |
| ULDC | 0.81a | 1.19a | 1.32a | 1.59a | 1.38a | 0.49a | 0.59a | 0.81a | 0.97a | 0.8a |
| UCDS | 0.62a | 0.76b | 0.81bc | 1.01b | 0.82b | 0.41a | 0.46a | 0.61a | 0.72bc | 0.49b |

The activities of protease were represented with the milligrams of glycin and ammonium nitrogen produced by per gram dry soil per hour.

Previous studies have shown that soil texture influences the soil organic matter content and that the number of silt and clay particles is significantly positively correlated with the amount of organic matter (Jindaluang *et al*. 2013). Consistent with these previous studies, in this experiment, loamy and clay soil configurations (ULDC, SL, ML and LC) had remarkably higher organic matter content than sandy soil configurations (GS and SS). Previous investigations also indicated that the available nutrient content in soil is positively related to number of clay particles but negatively related to the number of sandy particles (Silver *et al*. 2000, Wang *et al*. 2000). The amount of nutrients in clay soil was the largest, followed by loamy soil and sandy soil (Heiniger *et al*. 2003, Zhang *et al*. 2012). In the present study, the available N, P and K in ULDC were significantly higher than in GS and SS. This is possibly because soil with a high clay and sandy particle content has abundant soil colloids, and therefore has a strong capacity to adsorb mineral ions (Min and Lee 2010). In addition, soil with a high clay and sandy particle content has high organic matter content.

Among the seven tested soil configurations, ULDC had the highest content of soil organic matter and available nutrients and also lost the least amount of nutrients during the growing season. Thus, ULDC was able to maintain and supply fertility. The loam soil in ULDC not only has a high nutrient content in the upper soil layer, but also has a suitable ratio of small and large soil pores, which are not usually found in clay soil. It was predicted that the suitable soil pores in ULDC could also offer sufficient oxygen for root respiration and microbial activities (Bouckaert *et al*. 2013, Bronick and Lal 2005) and that the clay in the lower layer of ULDC could preserve moisture and fertility, and therefore prevent the loss of water and fertilizer. In this way, the soil structure like ULDC provides more moisture and nutrients for crop growth (Min and Lee 2010, Li *et al*. 2013). Thus, ULDC is the ideal soil configuration for the growth and development of crops.

The number of microorganisms and the amount of enzymatic activity in rhizospheric soil showed seasonal changes. The highest numbers of microorganisms (bacteria, fungi and actinomycetes) and the highest urease and protease activities were observed at the sulking stage, and there were no significant differences between soil configurations. Previous studies suggested that the activities of both soil microorganisms and soil enzymes vary with time (Sun *et al*. 2016, Sugihara *et al*. 2010), and these changes are influenced to some extent by the physical soil environment, such as changes in soil moisture and temperature (Brockett *et al*. 2012, Moche *et al*. 2015). In this experiment, the clay and silt content significantly affected the number of soil microorganisms. The number of bacteria, fungi, and actinomycetes was highest in ULDC, which also had the most abundant organic matter and fertility-retention capacities. ULDC could therefore, provide sufficient nutrients, which are beneficial for microorganism growth. Soil proteinase activity is related to the number of soil bacteria and the rate of mineralisation of organic N, whereas soil urease activity is closely associated with the transformation of urea in soil (Xing *et al*. 2010). In addition, the activities of these enzymes are closely related to soil texture. The activities of proteinase and urease in ULDC, SL, ML and LC were higher than in GS and SS because loamy or clay soils provide sufficient nutrients for the growth and metabolism of soil microorganisms. Similar to the number of soil microorganisms, ULDC had the highest activities of proteinase and urease among the seven soil configurations tested.

Soil texture influences the motion of air, water, and heat in soil, and also affects the transformation of soil nutrients. Its structural composition is of great significance in facilitating crop growth and improving yield (Hassink 1992, Arvidsson 1998). In the present study, different soil configurations had different effects on maize yield and yield components. Soil configurations significantly influenced the bare tip length, number of grains per ear and TGW, and thereby affected the grain yield. Maize yield and yield components vary with soil texture because differences in soil physico-chemical and biological properties result in different capacities for water- and fertility-retention (Arvidsson 1998). Like the present findings, previous studies have found that soil structure affects yield. Arora *et al*. (2011) found that soybean seed yield was higher in sandy loam than loamy sand soil reflecting the effects of water availability. He *et al*. (2014) reported that spring wheat grain yield was higher in clay soil than in silt loam soil in most dry and wet years. Soil nutrients, moisture, rhizosphere microorganisms and enzymatic activities influence the yield and formation of maize grains. Therefore, farming in fertile soil configurations with fertility-retention is significant when aiming for a high yield of maize (Xin 2015).

Maize yield was clearly affected by soil configuration, with the highest yield observed for loam in the ULDC, followed by ML, and the lowest in GS. Maize yield was significantly correlated with soil nutrient content, the number of microorganisms and the level of enzymatic activity. There were significant differences between the nutrient content and nutrient-holding capacity of the plough soil layers of different soil configurations. The number of rhizosphere microorganisms and activities of soil proteases and ureases in each soil configuration first increased, reaching a maximum at the silking stage, and then decreased. From the silking stage to the dough stage, all indices tended to decrease; however, at all growth stages, these indices were highest in ULDC, followed by ML, SL, LC, UCDS, SS and GS. Based on these results, the ULDC configuration is the ideal soil configuration for achieving a high maize yield. The present findings also illustrate the importance of soil configuration to sustainable food production and land consolidation.

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