Effect of nitrogen fertilization on maize yield responses to soil microbial activity and root length density in the North China Plain

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Abstract. A maize field experiment in the North China Plain was conducted to understand the effect of different N fertilizer rate on the yield of maize, using soil microbial activity and root length density (RLD) as performance parameters, due to their possibility to enhance productivity. The four N fertilizer rates were 0 (N0), 120 (N120), 210 (N210) and 300 (N300) kg N hm^{-2}. The results indicated that nitrogen (N) fertilizer had a significant influence not only on yield ($p<0.05$), but also on root length density ($p<0.05$) and soil microbial activity ($p<0.05$). In addition, the soil microbial activity and RLD were significantly related with maize yield. RLD differences were generally evident within the 100 cm soil layer, whereas there was no difference in the deeper soil under different N treatments. The most RLD concentrated in 0-60cm soil layer under N0, N120 and in 0-90cm soil layer under N210, N300. The microbial growth rate constant (k) was greater in N210 than other treatments. Generally, N fertilizer application can stimulate root growth and microbial activity, meanwhile, they can interact with each other, heighten the availability of N fertilizer in soil, thus enhanced yield of maize. According to our study, 210 kg N hm^{-2} was the optimum N fertilizer rate to achieve maximum yield and sustain the soil productivity.

1 Introduction

Maize (*Zea mays* L.) is one of the major crops in China, and occupies 17% of global maize production. The North China Plain is the largest agricultural production region in China ([45])accounts for more than 30% of China's maize production ([33]). Therefore, increasing maize yield in this region plays an important role in the national food security ([42]). Fertilization by especially nitrogen (N) inputs is a key management tool for maintaining crop productivity. Optimal N fertilizer applied into soil can increase nutrient of crop, soil fertility ([46]) and thus yield. However, high N fertilization is common in China ([8]) to ensure high yield, and local farmers have applied N fertilizer at a rate of 266 kg N hm^{-2} from 2010 to 2012 ([43]). Excessive N fertilizer would cause negative impacts on the environment ([10,16,37]) and in this region in particularly.

In agricultural production, the root systems are the primary part of the crop that determines the acquisition water and nutrients from the soil ([17]). Root length density (RLD) is an important parameter which plays a crucial role in crop growth, nutrient uptake and yield ([11]). For example, increasing maize root depth that the yield of maize increased from 6.0 to 7.8 t hm^{-2} ([3]) and increasing RLD of maize by 13.2% that can be raised grain yield by 17.3% and enhanced post-silking N uptake by 26.7% ([26]).

Soil micro-organisms in the agricultural soil environment have a great contribution. They are involved in the cycling of nitrogen, carbon, and sulfur ([22, 40]), decomposition of organic matter ([36]), and improvement of soil structure ([6]).They also serve as indicators of soil quality and fertility ([7]). In addition, N fertilizer can have a positive ([24]) or no effect ([39])on soil microbial activity. Furthermore, optimum N fertilizer was relative to soil N availability, improved soil microbial activity subsequently ([21]). Many of these studies of the soil microbial activity by micro-calorimetry focused on laboratory, field data have been rarely studied in maize soil.

It is essential to reduce excessive N fertilizer applied for development of sustainable agriculture in the North China Plain. Previous studies focus on improving the N use efficiency([8, 47]) and N management ([1]) based on biomass production and N accumulation in the soil. Few of these studies used root length density and soil microbial activity to study the efficiency of N and how these properties impacted yield of maize.

The primary objective of this study was to (1) investigate the effects of different N fertilizer rate on soil

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microbial activity through microcalorimetry, RLD and yield, (2) evaluate whether soil microbial activity, RLD and yield were correlated. We hypothesized that enhanced maize yield would be related to soil microbial activity and RLD through N fertilizer rate stimulation. To explore this effect, this work was based on a maize field experiment in the North China Plain.

2 Materials and methods

2.1 Experimental site description

The field experiment was conducted in a typical maize-wheat rotation cropping system at Baoding Farming Experimental Station ($38^0$5'N, $115^0$30'E), Hebei Province, China. This region has a temperate continental monsoon climate. The annual mean air temperature is 12℃. The average annual precipitation is 550mm, mainly falls from July to September. The soil type was a moisture loam containing soil organic matter 16.1 g kg^{-1}, total N 0.96 g kg^{-1}, Olsen-P 16.6 mg kg^{-1} and Olsen-K99.9mg kg^{-1} respectively in the top soil layer (0-20cm) before the experiment.

2.2 Experimental design

The experiment was established in 2009 during the maize growing season. Summer maize (Zhengdan 958), a commonly used variety in North China Plain was planted after wheat harvest without tilling the soil. The row spacing was 60 cm and the density was 60000 plants hm^{-2}. The experiment comprised four treatments: 0 (N0), 120(N120), 210(N210), 300 kg N hm^{-2}(N300).Fertilizers used were urea (46%), phosphorus pentoxide(12%) and potassium sulfate(60%). Urea was applied three times during the growth stage, 40% of urea was applied as basal fertilizer, 40% of urea was applied at huge bellbottom stage, and 20% of urea was applied at tasselling. Total P and K fertilizer was applied both as basal fertilizer, which comprised 90 kg (P_2O_5)·hm^{-2} and 180kg (K_2O) ·hm^{-2}. Depending on rainfall period, the experiment was irrigated 60 mm during the early growing stage after planting. All the straw was chopped and returned to the soil after maize harvest.

2.3 Grain yield and root collection

After harvest in 2013, grain yield (kg hm^{-2}) was measured for all maize cobs in a 20m^2 area in each plot at moisture content of 14%. Root length density (RLD) (cm cm^{-3}) was calculated from the volume (cm^3) of the sampling core of the root length (cm) ([12]).

2.4 Soil sampling and analysis

Soil samples were taken after maize harvest at three depths: 0-30, 30-60, 60-90 cm. Fresh soil samples were passed immediately through a 2 mm sieve, and then air-dried for soil microbial activity analyses.

Soil microbial activity was determined by the microcalorimetry method ([13]). Soils (1.0g) were placed in ampoules (4.5ml) at 28℃ for several days. The heat flow of the samples was recorded continuously by the computer automatically. The heat generated by biological processed in living cells is directly recorded by microcalorimetry over time. Data were collected as different heat flow power-time until the heat signal returned to baseline indicating no further measurable metabolic activity.

2.5 Data analyses

The difference of yield of maize was determined by significant difference ($p< 0.05$). Data were analyzed using correlation analysis and analysis of variance (ANOVA) using the SPSS 18. RLD was analyzed and calculated using Win Rhizo Pro Vision 5.0a. Microbial activity analyzed using Origin 8.0.3. Correlation analysis was used to analyze the relationship between micro-calorimetric parameters, RLD, yield and N fertilizer. ANOVA was used to detect N fertilizer influencing soil parameters. Regression analysis was applied to calculate the optimum maize yield.

3 Results

3.1 Maize yield

Yields were affected by N fertilizer are shown in Table1. After four years of N fertilizer experiment, the yields of maize were significantly (p<0.05, Table 2) affected by N fertilizer.

Table 1. Yield of maize under different N fertilizer rate in the four seasons from 2010 to 2013.

Treat-ment	2010	2011	2012	2013
N0	8947±322a	7470±117b	6794±284c	6264±57c
N120	9014±238a	7603±146b	8954±159ab	9473±193ab
N210	9276±166a	9513±98a	9704±86a	9987±72a
N300	8999±286a	8729±201ab	9172±143ab	9627±a111b

Note: Maize yields of treatments followed by different letters are significantly different at p<0.05, ±: Standard Deviation.

The first maize yields in 2010 had no difference among N treatments. The second maize yields in 2011, the highest yield was observed in N210 which was significantly higher than N0 and N120, but it was no significant difference between N210 and N300. The maize yields in 2012 and 2013, the difference of yield varied with the rate of N fertilizer compared to the control, but there were no significant differences between N120 and N300. The results suggested that increased N fertilizer to 300kg hm^{-2} had no positive effect on yield. Generally, the yield of N0 from 2010 to 2013 always decreased and had the lowest yield which was significantly lower compared to other treatments in 2012 and 2013.

Table 2. The analysis of the Correlation between micro-calorimetric parameters, RLD, yield and N fertilizer and the analysis of variance.

	Nitrogen	Yield	RLD	Q_{total}	P_{max}	k
Correlation analysis						
Nitrogen	1					
Yield	0.770**	1				
RLD	0.899**	0.827**	1			
Q_{total}	0.038	0.063	-0.038	1		
P_{max}	0.255	0.230	0.299	-0.632*	1	
k	0.520	0.824**	0.612*	0.249	0.556	1
Analysis of variance						
Treatments		*	*	ns	ns	*

RLD: root length density, Q_{total}: the total thermal, P_{max}: peak height values, k: growth rate constant.
*: significant at P<0.05, **: significant at P<0.01, ns: not significant at P<0.05.

3.2 Root length density

Root length density (RLD) was found to be one of the most important parameters influencing grain yield ([23]). The effects of N fertilizer on RLD was investigated (Fig.1). Overall RLD in the 0-100cm soil layer was changed more obvious than in deeper soil layer.At N0, 49.8% in the 0-30 cm soil layer, 24.8% in the 30-60 cm soil layer, 9.6% in the 60-90 cm soil layer, 13.4% in the 90-120 cm soil layer 8.8%, in the 120-150 cm soil layer and 1.0% in the 150-180 cm soil layer, respectively, most RLD in the 0-60cm soil layer and about 50% RLD in the 0-30 cm soil layer. At N120, 58.6% in the 0-30 cm soil layer, 27.1% in the 30-60 cm soil layer, 1.6% in the 60-90 cm soil layer, 11.2% in the 90-120 cm soil layer, 0.8% in the 120-150 cm soil layer and 0.8% in the 150-180 cm soil layer, respectively, most RLD in the 0-60cm soil layer while 60% RLD in the 0-30 cm soil layer.At N210, 38.2% in the 0-30 cm soil layer, 26.7% in the 30-60 cm soil layer, 22.7% in the 60-90 cm soil layer, 5.9% in the 90-120 cm soil layer, 4.6% in the 120-150 cm soil layer and 1.8% in the 150-180 cm soil layer, respectively, most RLD in the 0-90cm soil layer, probably 50% RLD in the 30-90 cm soil layer.At N300, 38.8% in the 0-30 cm soil layer, 23.1% in the 30-60 cm soil layer, 18.6% in the 60-90 cm soil layer, 7.1% in the 90-120 cm soil layer, 8.8% in the 120-150 cm soil layer and 3.6% in the 150-180 cm soil layer, respectively, most RLD in the 0-90cm soil layer. Significant differences were found among the four treatments.

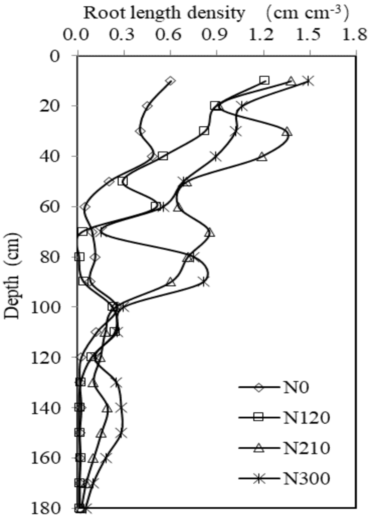

Fig.1 Root length in the 0-180 cm soil layer under different N fertilizer rate.

3.3 Microbial activity

The power-time curves under different N rates showed a huge difference (Fig. 2). The curves of surface soil layer presented a higher activity than deeper soil layer. The parameters of microcalorimetry can be observed in Table 3 and Fig.3. In the 0-30 cm soil layer, N210 had the highest P_{max}, k and the biggest Q_{total}. In the 30-60cm soil layer, N300 had a higher P_{max} and Q_{total} than other treatments. In the 60-90cm soil layer, the curves showed a flat curve with less k and P_{max}, all these indicated low microbial activity in deeper soil layer. It could be seen from these curves and parameter, the microbial growth was influenced by N fertilizer rate and soil layer.

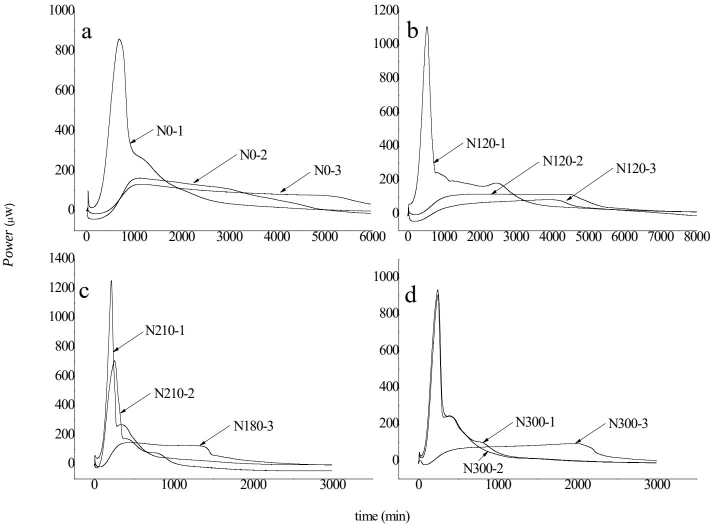

Fig.2 Power-time curves corresponding to different soil layer under different N fertilizer rate.

Table 3. Growth rate constant (k) and correlation coefficients (r^2) of microbial growth

Treatment	Soil layer (cm)	k (min^{-1})×10^{-3}	r^2
N0	0-30	0.0052±0.00009	0.9973
	30-60	0.0013±0.00006	0.9952
	60-90	0.0015±0.00004	0.9908
N120	0-30	0.00915±0.00003	0.9998
	30-60	0.00912±0.00005	0.9908
	60-90	0.00708±0.00008	0.9934
N210	0-30	0.00965±0.00008	0.9998
	30-60	0.00925±0.00003	0.9906
	60-90	0.00828±0.00001	0.9904
N300	0-30	0.00926±0.00006	0.9985
	30-60	0.00868±0.00005	0.9986
	60-90	0.00392±0.00006	0.9992

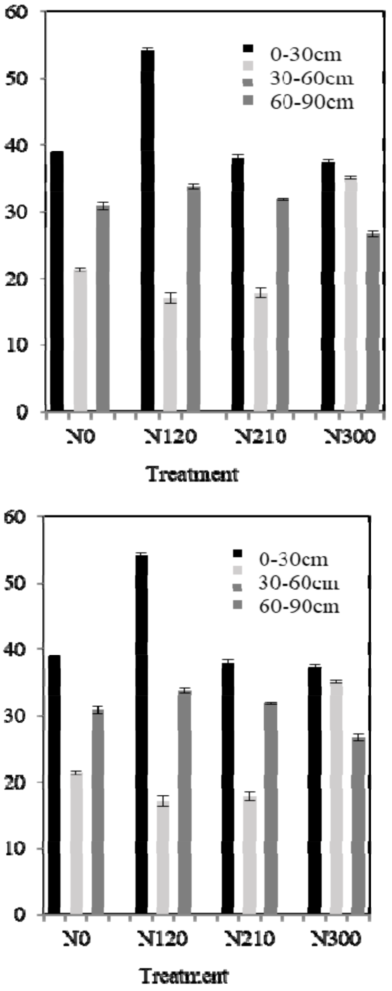

Fig.3. Microcalorimetric parameters from power–time curves. Error bars indicate the standard error of the mean.

3.4 Correlation analysis and Analysis of variance

N fertilizer application affected yield, RLD and k ($P<0.05$) according to analysis of variance. Meanwhile, correlation analysis showed that N fertilizer was positively correlated with yield, RLD and Q_{total}, P_{max}, k(Table2) as well, and there was a significant positively correlation ($P<0.01$) between yield and RLD, k. This is the reason why there was a perfect linear correlation between yield and RLD (Fig. 4).

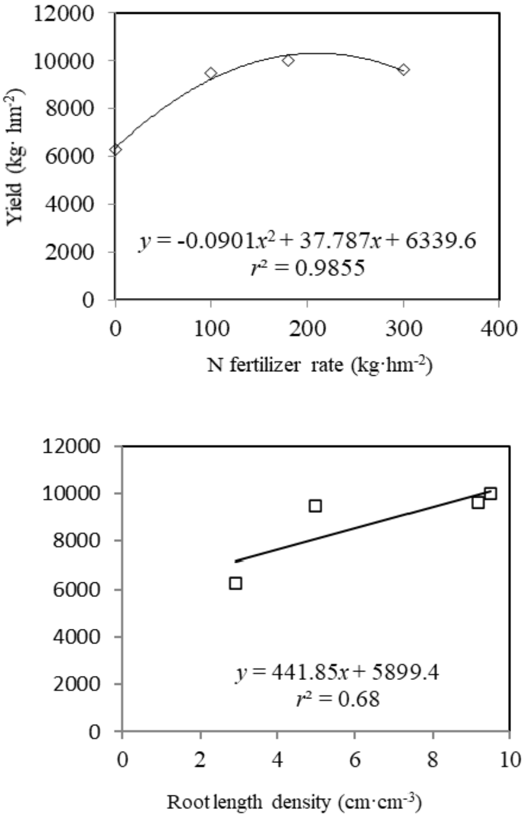

Fig.4. The relationship between N fertilizer rate and yield was expressed by fitting model and the relationship between root length density and yield was expressed by linear model (in the experiment of 2013).

3.5 Yield and root length density in response to N fertilizer rate.

The correlation between N fertilizer and yield (Fig.4) can be expressed by the regression equation: $Y_{yield} = -0.0901X_N^2 + 37.787X_N + 6339.6$to calculate the optimum N fertilizer rate, the result showed that when N fertilizer rate exceeded 210 kg hm^{-2} there was no further yield. Compared with N300 and N120, N210 had higher RLD and microbial activity than other treatments which the two parameters also had a significantly positive ($p<0.05$) correlation between RLD and k (Table2) can mostly take up N from the soil and the correlation between root length density and yield (Fig. 4) both can be explained why N210 had a greater yield. In this view, the optimal range of N fertilizer rate in this region was ≤210 kg hm^{-2}.

4 Discussion

Root length density (RLD) and soil microbial activity in maize fields were differences under different N fertilizer and soil layers.Half RLD was found in the 0-60cm soil layer under N210 and N300, whereas half RLD was concentrated in the 0-30cm soil layer under N0 and

N120. These results are consistent with previous studies, a rainfed filed experiment in Samaru showed that about 64% of root length in the surface 0-15cm soil layer ([31]) and 80% of the total root length accumulated in the top 40cm ([34]) and RLD decreased with soil depths ([41, 44]). Maize RLD depended on nutrients which was reported by Peng ([32]).

With the soil deepen, the power-time curves show a huge difference (Fig. 2). Because microbial activity was significantly affected by soil depth ([18]), so we studied an experiment on the soil depth. In the 0-30 cm soil layer, N210 showed a stronger microbial activity with the highest $P_{\max}$, k and the biggest Q_{total}. In the 30-60cm soil layer, N300 showed a higher $P_{\max}$ and Q_{total}, perhaps due to apply large N fertilizer in the soil. In the 60-90cm soil layer, the curves showed a flat curve with less k and $P_{\max}$, this could be considered as poor soil microbial activity correspond with low heat ([28]).

Yield is an important criterion for evaluating the maize performance. The results showed that fertilizer is related to maize yield closely ([35]), especially depended on N fertilizer ([23, 49]). These findings were consistent with previous studies conducted on the N application which increased from 250 to 380 kg hm^{-2} while yield had no further increased ([20]), and by using a statistical method demonstrated reduced N fertilizer would not reduce yield ([5]), even better decrease the negative impact on the environment, also reported that higher N supply led to a non-significant increase in yield and changing the rate of N application had no a significant effect on the yield([25]).

Many studies have shown that maize yields are influenced by various factors, such as fertilizer ([3, 29]), water ([19]), tillage ([4]), agricultural management ([38]). In this study we suggest that high soil microbial activity and more RLD caused increased yield of maize. Certainly, the correlation between yield and soil microbial activity and RLD are consistent with this hypothesis (Table 2). Moreover, highest yield under 210kgN hm^{-2} was observed (Fig. 4). Because optimum N fertilizer management which can greatly sustain soil productivity, thus high crop yield ([48]).

Higher RLD development is related to high grain yield production([15]). Indeed, according to Table1, N210 had the highest yield. Correlations (Table 2) between yield and RLD suggest that 210 kg(N)·hm^{-2} in soil increased the root growth ([27]), more RLD may cause more water and nutrient could be used by crop. Microbial activity is related to RLD (Table 2) because root can generate root exudate that was known to have a multitude of functions in ecological interaction with the microbial soil activity ([2]), then high microbial activity can involve in the cycling of nitrogen to crop. Thus resulted in high yield ultimately.

Soil microbial activity as an biological indicator that evaluate the efficiency of soil fertility which directly influenced the yield of crop. For example, maize yield in Kano State positively correlated with soil organic C, and soil organic C accounted for 75% of the variation in maize yield ([9]). The microbial activity is related to soil organic matter decomposition while the soil cycles of soil C and N dominated by microbial activity are beneficial for the improvement in soil fertility ([6]), further increase the crop biomass and yield ([4]).

5 Conclusions

In conclusion, this study demonstrated that N fertilizer can increase maize yield by influenced soil microbial activity and root length density. High N fertilizer rate cannot be increased yield of maize, soil microbial activity and root length density. Compare to high N fertilizer rate, within this region the optimum rate of N fertilizer for great yield was$\leq$210 kg hm^{-2} while it kept the high maize yields.

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