EFFECTS OF ARTIFICIALLY ALTERED SOIL STRUCTURE ON ^{15}N ABSORPTION AND UTILIZATION FOR MAIZE (*ZEA MAYS* L.) AT THE SEEDLING STAGE

LIU, X. – BATANDE, S. N. – ZHANG, X. W. – DOU, S.[*]

College of Resource and Environmental Science, Jilin Agricultural University, Changchun, Jilin Province 130118, China

[*]*Corresponding author*
e-mail: dousen1959@126.com

Abstract. Soil structure is an important parameter which governs soil and plant nitrogen (N) dynamics. N is essential in the development and growth of maize (*Zea mays* L.). In this field pot study carried out for four months, we investigated the effects of artificial soil structure alteration on N use efficiency (NUE) and N uptake by maize. Soil structure was artificially altered by (i) retaining soil with a blocky structure in a 2 mm sieve (CKU), and (ii) flooding the soil with 600 mL of distilled water (CKW). Unaltered soil was established as the control treatment (CK). Using a ^{15}N tracer method, soil ^{15}N retention, and ^{15}N utilization and absorption rate of maize during the seedling stage was studied. The results showed that plant height, dry matter weight, root status, chlorophyll contents, and ^{15}N utilization rate were significantly decreased by CKU and CKW. The ^{15}N utilization rates were 23.09%, 20.64% and 13.76%, under CK, CKU, and CKW, respectively. This indicated that after the structure of black soil was destroyed, maize growth declined, and ^{15}N absorption and utilization rates decreased at the seedling stage.
Keywords: *artificial altered, soil structure, maize seedling stage, ^{15}N utilization, ^{15}N residual*

Introduction

Soil structure controls many processes in the soil. Good soil structure plays an important role in agricultural production (Munkholm et al., 2003). Well-structured soils are high in organic matter and provide a continuous supply of nutrients to promote crop growth (Bimuller et al., 2016), improve soil fertility and increase crop yields (Liang et al., 2011). Contrarily, the soil with compact soil structure leads to deterioration of soil water and aeration conditions, which limits the growth of plant roots, thus hindering the effective use of nutrients and water by plants (Walia and Dick, 2018). It also reduces crop yield and causes serious nutrient leaching (Kavdır and Smucker, 2005). Panakoulia using principal component analysis identified clay content, bulk density, climatic conditions (precipitation and evapotranspiration), organic matter (OM) and its decomposition rates as the most important factors that controlled soil structure development (Panakoulia et al., 2017). However, most studies on soil structure have focused on the impact of a single influencing factor, in this study we focused on several.

Nitrogen (N) is one of the major limiting nutrients in agricultural production, and crop productivity depends largely on the high level of N applied to the soil (Alam et al., 2006). However, excessive application of N fertilizer can lead to serious ecological and environmental problems, as well as increase leaching of nitrate, resulting in eutrophication of surface water and pollution of underground water (Islam et al., 2007). The N in the soil is absorbed by crops mainly in inorganic forms (Pang, 2019), and the soil structure affects the transformation and residue of N (Bataung et al., 2012). Thusly, soil structure has a certain influence on the absorption and utilization of crop N.

Maize seedling stage (code 19 according to the BBCH scale (Lancashire et al., 1991)) is a vegetative growth period with long roots as the center, rapid root growth and slow above-ground growth (Chen, 2007). From the perspective of the whole growth period of maize, seedling stage is the critical period for nutrient demand, and its growth and development status can be used as an important indicator to judge whether the location of nutrient application is good or bad (Yu et al., 2017). At the same time, seedling growth is the basis of maize growth and yield in the future.

In recent years, there have been many studies on soil structure and, N absorption and utilization by crops, but most of them focus on a certain influencing factor of soil structure. Little research has been done on N absorption and utilization after soil structure is destroyed. Li showed that damaged soil structure significantly reduces the amount of organic N mineralization, and affects the cumulative porosity (Li, 2018). The uptake and utilization of crop N in destroyed soil structure has not been sufficiently studied. The ^{15}N tracer method can distinguish whether the N absorbed by the crop comes from the fertilizer or the soil, so as to determine the main N source of the crop (Zuo, 2012). In this paper, the ^{15}N tracer method was employed to study the use of N fertilizer in artificially damaged soil structure during maize seedling. This will provide a basis for the use and scientific application of N fertilizer in soil with damaged structure in the future.

Materials and methods

Site description

The soil selected in this study are from a maize (*Zea may* L) continuous monocropping field of Jilin Agricultural University, located in Jingyue district, Jilin province of China (N43°48'43.57", E125°23'38.50"). Maize monocropping is the main cropping system in the region. The area has an average temperature of 4.8°C, with an average annual sunshine duration of 2 688 h, and mean annual precipitation of 617 mm. About 65% of annual precipitation is concentrated in July and August (summer). The field is characterized by black soil, classified as Mollisol according to the World reference base classification system, with a loamy clay texture. The soil was amended with corn straw and constant fertilizer for three consecutive years from October 2014 to October 2017.

In June 4, 2018, undisturbed soil samples were randomly collected from different locations in the 0–20 cm soil depth using a stainless-steel soil sampler (5 cm in diameter). Collected soil samples were carefully mixed to form a composite, placed in a plastic box, and then immediately transported to the laboratory. Upon arriving in the laboratory, the soil was gently broken along natural break points and allowed to pass through a 10 mm sieve to remove stones and other impurities. Visible plant residues were removed from the sieved soil with forceps and the soil was then air-dried for 72 hours. The initial basic soil properties are shown in *Table 1*.

Table 1. Initial basic soil properties

pH	Water content (%)	WSA (%)	BD (g cm^{-3})	SOM (g kg^{-1})	AH–N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Yield (kg ha^{-1})
6.55	26.78	76.85	1.08	19.61	106.1	24.3	116.1	11492

WSA, water stable aggregate-size fractions (sum of > 2 mm, 2 – 0.25 mm, 0.25 – 0.053 mm, and < 0.053 mm aggregates); BD, bulk density; SOM, soil organic matter; AH–N, alkaline hydrolysable nitrogen; P and K, available phosphorus and potassium, respectively; Yield, maize yield

Experimental design

The experiment was initiated in June 2018, designed as pot experiment, and included three treatments which were replicated three times and arranged in a completely randomized design. The pot (high-type polyethylene plastic bucket) had the bottom and barrel diameter of 24 cm, and height of 22 cm. The treatments were prepared by: (i) adding exactly 3 kg of soil (< 10 mm) into a plastic pot container, and designated as a control (CK), (ii) passing soil through a 2 mm sieve to retain the soil with blocky structure in the sieve, and 3 kg of the soil retained by the sieve was added in a plastic pot container (CKU), and (iii) another, 3 kg of soil (< 10 mm sieve) was added into a plastic pot container and then flooded with 600 mL of distilled water, henceforth referred as CKW (before planting, the soil remained submerged under water for 24 hours, until the water slowly evaporated, resulting to soil crusting on the surface after day 10). CKW represent waterlogging-prone soil. Each pot was planted with 2 identical corn seeds (xiang yu 998) in June 6, 2018 and one plant was left after seeding. Prior to planting, ^{15}N labeled urea (isotope content 5.15%) obtained from Shanghai institute of chemical engineering - Ministry of chemical industry was applied. Since, corn seedling (stage 19 in BBCH scale) was of interest in our study, only 0.22 g of ^{15}N labeled urea, 0.08 g of potassium dihydrogen phosphate and 0.08 g of potassium sulfate were incorporated into each pot (i.e., one third of the recommended fertilizer for the entire growth cycle). The pots were randomly placed in an open cart (100 cm high), and watered by 200 mL on weekly bases. In August 5, 2018, maize plant including roots, and 30 g of soil was collected from each pot, and designated as the first sowing data set (S1). The soil was air-dried and passed through a 2-mm sieve.

The experiment was repeated using the same pot soils used in S1 (i.e., CK, CKU and CKW treatments), but without fertilizer addition and altering soil structure further. Maize seed was planted in each pot in August 6, 2018, and watered on weekly basis. Maize plant including the roots were harvested in September 25, 2018 (at stage 19 according to the BBCH scale) from each pot. In the same day, 30 g of soil was collected from each pot. Collected maize plant and soil samples were designated as second sowing data set (S2).

Laboratory analysis

Plant analysis

The number of roots was determined by lying flat the roots systems of maize and counting them. Root volume and root dry quality were determined by drainage method and drying method, respectively. Maize plant height was obtained by measuring the maximum length from the soil surface to its natural extension at stage 19 of BBCH (Lancashire et al., 1991).

Weight dry matter was measured by oven-dry method, by oven drying above-ground maize parts at 105°C for 30 min, thereafter maize plant parts were dried at constant temperature (80°C) for 12 h. The oven-dried maize sample was allowed to cool to room temperature, and the weight change was recorded by a sensitive electric balance. Maize plant N, phosphorus (P) and potassium (K) contents were measured according to the method described Lu (1999). In short, 0.2 g of crushed and sieved (0.25 mm) above-ground maize plant was treated with concentrated H_2SO_4-H_2O_2 solution. The N and P were analyzed by a two-channel automatic flow analyzer. While, maize plant K content was determined by FP640 ASTM flame photometer (Lu, 1999). The chlorophyll content in maize plant leaves was determined following extraction with acetone and measured by

visible spectrophotometer (Model 722 spectrophotometer, Huanghua Faithful Instrument, China) according to the method describe in Lu (1999).

The δ^{15}N of corn plant sample was measured by the Isoprime100 Mass Spectrometer (Elementar Analysensysteme GmbH Inc. Germany), after corn plants were defoliated at 105°C for 30 min, and dried at 75°C for 48 h, thereafter weighed and grounded into powder.

Soil analysis: Physical and chemical properties

The soil from each data set (S1 and S2) was air-dried and allowed to pass through a 2–mm sieve, then analyzed for soil pH, moisture content, alkali-hydrolyzed N (AH–N), available P and K by referring to soil agricultural chemical analysis methods (Lu, 1999). Nitrate (NO_3^-–N) and ammonium (NH_4^+ –N) were extracted by 2 M KCl in a 1:5 soil-solution ratio and the extractant was determined by continuous flow analyzer (AA3 HR continuous flow analyzer). Soil organic carbon (SOC) was determined by potassium dichromate method (Nelson and Sommers, 1982), by treating 0.2 g of soil with $K_2Cr_2O_7$-H_2SO_4 solution and heating at 180°C for ~5 min. After cooling to room temperature, excess $K_2Cr_2O_7$ was titrated with 0.2 N $FeSO_4$ until the end point was reached.

Soil water stable aggregate-size (WSA) fractions were measured with a wet-sieving procedure (Cambardella and Elliot, 1993). This wet-sieving procedure resulted into four aggregate-size fractions: (i) large macro-aggregates (> 2 mm), (ii) small macro-aggregates (2-0.25 mm), (iii) micro-aggregates (0.25–0.053 mm) and (iv) silt/clay fraction (< 0.053 mm). All fractions were dried at 60°C. The δ^{15}N of air-dried and ground soil samples (100 mesh sieves) was measured with an isotope ratio mass spectrometer (IsoPrime100 Mass Spectrometer, Germany).

Statistical analysis and calculations

The data were analyzed by one-way analysis of variance using SPSS 22.0 (IBM Corporation, New York, USA). Significant differences among treatment means were evaluated using the least significant difference test at a $P < 0.05$. All figures were compiled using Origin 8.5 software.

The ^{15}N utilization rate (^{15}NUR, %) was calculated as follows:

$$^{15}NUR = Npf/Nf \times 100$$
$$Npf = NP \times Npff \qquad \text{(Eq.1)}$$
$$Npff = Npp/Nppf \times 100$$

where: N_{pf} is the amount of N absorbed by the plant in ^{15}N fertilizer (g pot^{-1}), N_f is the amount of applied ^{15}N fertilizer (g pot^{-1}), N_P is the amount of total N absorbed by plants (g pot^{-1}), N_{pff} is the percentage of total plant N from fertilizer (%), N_{pp} is the atomic percentage of ^{15}N fertilizer in plants exceeds, N_{ppf} is the atomic percentage of ^{15}N-labled fertilizer.

The ^{15}N retention rate (^{15}NRR, %) was calculated as follows:

$$^{15}NUR = Nsf/Nf \times 100$$
$$Nsf = Ns \times Nsff \qquad (Eq.2)$$
$$Nsff = Nss/Nssf \times 100$$

where: N_{sf} is the amount of N in the soil from ^{15}N fertilizer (g pot^{-1}), N_f is the amount of applied ^{15}N fertilizer (g pot^{-1}), N_s is the amount of soil total N (g pot^{-1}), N_{sff} is the percentage of total soil N from fertilizer (%), N_{ss} is the ^{15}N atoms of total N in the soil, N_{ssf} is the ^{15}N-labeled fertilizer atomic excess percentage.

The ^{15}N loss rate (^{15}NLR, %) was calculated as follows:

$$^{15}NLR = 100 - {}^{15}NUR - {}^{15}NUR \qquad (Eq.3)$$

Results and analysis

Changes in soil properties

Changes in soil aggregate-size distribution

The aggregate-size distribution of macro-aggregates (> 0.25 mm) across all treatments and sowing periods accounted for more than 60% of total soil weight. Of all aggregate-size fractions, the 2 – 0.25 mm aggregates were higher than that of other aggregate-size fractions in both data sets (*Table 2*). CKU and CKW treatments reduced 2 – 0.25 mm aggregate-size distribution by 11.67% and 17.84% compared to CK. But these treatments increased micro-aggregates (0.25 – 0.053 mm) and silt/clay fraction (< 0.053 mm). Compared with S1 results, the 2 – 0.25 mm and 0.25 – 0.053 mm soil aggregate-size fractions slightly increased in S2 across all treatments, whereas > 2 mm aggregates decreased slightly (*Table 2*).

*Table 2. Soil aggregate-size fractions (%) in soils' structure artificially altered*

Time	Treatment	>2 mm	2–0.25 mm	0.25–0.053 mm	<0.053 mm
S1	CK	21.25±0.08c	44.51±0.34a	25.77±0.12b	7.28±0.21c
	CKU	22.79±0.63b	39.86±0.48b	27.53±0.97a	8.21±0.29b
	CKW	23.97±0.62a	37.77±0.97c	25.47±0.66b	10.54±0.16a
S2	CK	20.78±0.50a	45.67±0.35a	26.48±0.71b	5.63±0.23c
	CKU	21.12±0.77a	41.38±0.51b	28.73±0.93a	6.22±0.21b
	CKW	21.78±0.57a	39.23±0.88c	27.11±0.64b	9.37±0.34a

Mean values ± SE. Means followed by the same letter in a column of a given sowing time across all treatments are not significantly different at p < 0.05. CK represents well-structured normal soil treated with corn straw return and constant fertilizer application for 3 consecutive years from October 2014 to October 2017. CKU is blocky soil structure retained by a 2 mm sieve; CKW represents a well-structed soil flooded with 600 mL of distilled water in CK. S1 represents the first sowing period; S2 represents the second sowing period

Soil organic carbon content changes

SOC content ranged from 11.42 to 14.02 g kg^{-1} across all treatments and data sets (*Fig. 1*). In both data sets, CK showed significantly ($P < 0.05$) high SOC contents than CKU and CKW. In fact, CKU decreased SOC by 8.23% in S1 and by 8.05 in S2 compared with CK. While, CKW reduced soil organic carbon by 15.22% and 15.79% in the S1 and

S2, respectively, compared with CK (*Fig. 1*). These results indicate that altering soil structure significantly encourages SOC losses.
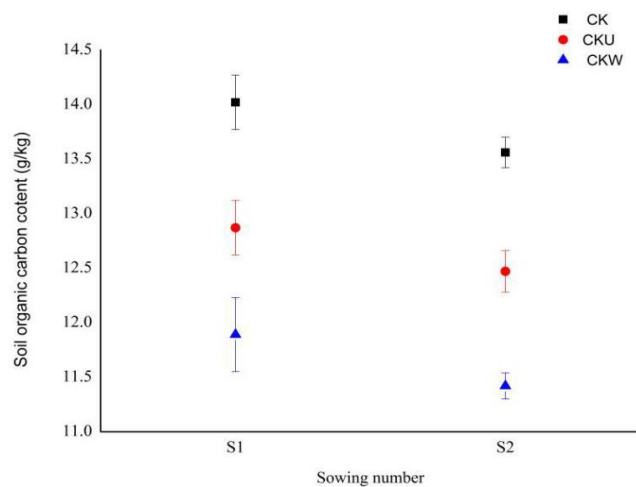
Figure 1. *Changes in organic carbon content after artificial soil destruction. Bars are standard error, and bars followed by the same letter for a given sowing time are not significantly different at p < 0.05. CK represents well-structured normal soil treated with corn straw return and constant fertilizer application for 3 consecutive years from October 2014 to October 2017. CKU is blocky soil structure retained by a 2 mm sieve; CKW represents a well-structed soil flooded with 600 mL of distilled water in CK. S1 represents the first sowing period; S2 represents the second sowing period*

Changes in soil NH_4^+ –N and NO_3^- –N contents

The NH_4^+ –N ranged from 3.03 to 4.06 mg kg^{-1} and NO_3^-–N ranged from 22.71 to 30.69 mg kg^{-1} for both data sets. Soil NH_4^+ –N and NO_3^-–N contents from both sowing times showed a similar trend, which increased in this order CK > CKU > CKW (*Fig. 2*). Compared with CK, the CKU and CKW decreased NH_4^+ –N content by 13.87–14.98% and 7.98–8.47%, respectively, and decreased NO_3^-–N content by 24.48–25.51% and 18.75–19.17%, respectively. This indicated that soil NH_4^+ –N and NO_3^-–N contents changes with soil structure.

Soil ^{15}N total residual, total loss rate

Total soil ^{15}N residue rate appeared to follow this trend CK > CKU > CKW, while total ^{15}N loss rate followed this order CK < CKU < CKW (*Table 3*). The CKU and CKW treatments decreased total soil ^{15}N residual rate of 7.67% and 8.85% compared with CK, respectively. While, the total ^{15}N loss rate of CKU and CKW increased by 21.53% and 47.56% compared with CK. These results showed that altering soil structure decrease total ^{15}N residual rate and increases ^{15}N loss rate.

Plant growth

Root system in maize seedling stage

The number of roots, root volume and dry root matter of maize at seedling stage significantly decrease after CKU and CKW (*Table 4*). Compared with CK, the dry root of maize decreased by 26.83% under CKU and by 34.15% under CKW, in S1. In the S2,

CKW and CKU decreased the number of roots and dry root mass of maize at seedling stage (*Table 4*), suggesting that poor soil structure might impede root development.
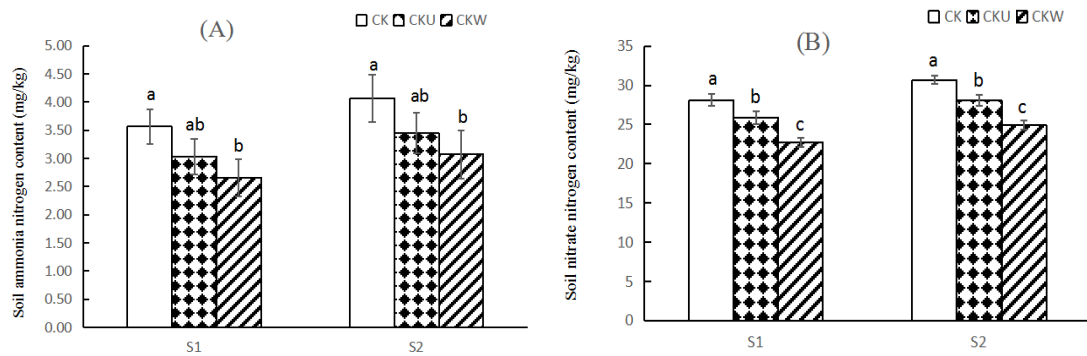
Figure 2. Changes in soil (A) ammonium nitrogen, and (B) nitrate nitrogen contents after the soil was artificially damaged. Bars are standard error, and bars followed by the same letter for a given sowing time are not significantly different at p < 0.05. CK represents well-structured normal soil treated with corn straw return and constant fertilizer application for 3 consecutive years from October 2014 to October 2017. CKU is blocky soil structure retained by a 2 mm sieve; CKW represents a well-structed soil flooded with 600 mL of distilled water in CK. S1 represents the first sowing period; S2 represents the second sowing period

Table 3. ^{15}N total residual, and total ^{15}N loss rate after artificial soil destruction

Treatment	Total ^{15}N residual rate (%)	Total ^{15}N loss rate (%)
CK	48.31±1.97a	28.60±1.62c
CKU	44.60±1.80b	34.76±1.03b
CKW	44.03±1.74b	42.21±2.21a

Mean values ± SE. Means followed by the same letter in a column are not significantly different at p < 0.05. CK represents well-structured normal soil treated with corn straw return and constant fertilizer application for 3 consecutive years from October 2014 to October 2017. CKU is blocky soil structure retained by a 2 mm sieve; CKW represents a well-structed soil flooded with 600 mL of distilled water in CK. S1 represents the first sowing period; S2 represents the second sowing period

Table 4. Root growth of maize at seedling stage after soil structure was artificially disturbed

Treatment	S1			S2		
	Root number (piece)	Root volume (cm³)	Dry root mass (g)	Root number (piece)	Root volume (cm³)	Dry root mass (g)
CK	8.33±0.58a	2.90±0.20a	0.27±0.02b	7.67±0.58a	2.63±0.15a	0.25±0.02a
CKU	6.33±0.58b	2.22±0.11b	0.20±0.01b	6.00±1.00b	1.94±0.06b	0.18±0.01b
CKW	5.67±0.58b	1.98±0.08b	0.18±0.01b	4.67±0.58b	1.81±0.06b	0.16±0.01c

Mean values ± SE. Means followed by the same letter in a column across all treatments are not significantly different at p < 0.05. CK represents well-structured normal soil treated with corn straw return and constant fertilizer application for 3 consecutive years from October 2014 to October 2017. CKU is blocky soil structure retained by a 2mm sieve; CKW represents a well-structed soil flooded with 600 mL of distilled water in CK. S1 represents the first sowing period; S2 represents the second sowing period

Growth of maize plant at seedling stage

In both data sets, the CKU and CKW appeared to significantly hinder plant growth, shown by a decrease in maize plant height and dry weight (*Table 5*). Both maize height and dry weight in S1 and S2 data sets were significantly higher under CK compared with CKU and CKW, and these parameters appeared to decreased in this order CKW < CKU < CK (*Table* 5).

Table 5. Growth of maize at seedling stage after artificial soil structure destruction

Treatment	S1		S2	
	Height (cm)	**Weight (g)**	**Height (cm)**	**Weight (g)**
CK	80.50±0.87a	7.37±0.11a	50.00±2.00a	3.06±0.17a
CKU	73.77±0.71b	6.38±0.05b	44.50±1.95b	2.32±0.18b
CKW	70.03±0.95c	6.16±0.06c	42.53±1.86b	2.14±0.12b

Mean values ± SE. Means followed by the same letter in a column across all treatments are not significantly different at $p < 0.05$. CK represents well-structured normal soil treated with corn straw return and constant fertilizer application for 3 consecutive years from October 2014 to October 2017. CKU is blocky soil structure retained by a 2 mm sieve; CKW represents a well-structed soil flooded with 600 mL of distilled water in CK. S1 represents the first sowing period; S2 represents the second sowing period

Chlorophyll content in maize leaves at seedling stage

The content of chlorophyll a ranged from 1.21–1.61 mg g⁻¹, and chlorophyll b ranged from 0.18–0.35 mg g⁻¹ in S1 and S2 (*Table 6*). Only chlorophyll a appeared to be affected by soil structure, because it significantly decreased upon alteration in soil structure (*Table 6*). Whereas, chlorophyll b was not significantly affected by changes in soil structure in both sowing times (*Table 6*).

Table 6. Chlorophyll content in maize leaves at seedling stage after artificial destruction

Treatment	S1			S2		
	Chlorophyll a	**Chlorophyll b**	**Total chlorophyll**	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**
	mg g⁻¹					
CK	1.61±0.03a	0.35±0.04a	1.96±0.02a	1.44±0.06a	0.30±0.06a	1.74±0.04a
CKU	1.46±0.05b	0.30±0.05a	1.76±0.04b	1.27±0.04b	0.19±0.07a	1.46±0.04b
CKW	1.41±0.04b	0.29±0.05a	1.70±0.07b	1.21±0.02b	0.18±0.04a	1.39±0.03c

Mean values ± SE. Means followed by the same letter in a column across all treatments are not significantly different at $p < 0.05$. CK represents well-structured normal soil treated with corn straw return and constant fertilizer application for 3 consecutive years from October 2014 to October 2017. CKU is blocky soil structure retained by a 2 mm sieve; CKW represents a well-structed soil flooded with 600 mL of distilled water in CK. S1 represents the first sowing period; S2 represents the second sowing period

Nutrient content in maize seedling stage

Maize N, P, and K contents were significantly affected by changes in soil structure and ranged from 8.31 to 11.15 mg kg⁻¹, 3.32 to 4.90 mg kg⁻¹, and 2.49 to 4.69 mg kg⁻¹, respectively (*Table 7*). The contents of the same attributes were significantly ($P < 0.05$) higher under CK, followed by CKU, and then CKW. The CKW treatment showed the highest decrease in N, P, and K contents compared with CK, decreasing N by 20.71%, P

by 20.13%, and K by 39.05% in S1 data set. In the S2 data set, N, P and K contents of CKU decreased by 12.27%, 19.76%, and 32.44%, respectively, compared with CK, which means N, P and K contents in maize plant decreases after the soil structure is artificially changed.

Table 7. *Nutrient content of maize at seedling stage after artificial destruction*

Treatment	S1			S2		
	N	P	K	N	P	K
	mg kg^{-1}					
CK	11.15±0.70a	4.98±0.04a	4.69±0.10a	10.08±0.32a	4.62±0.05a	4.19±0.10a
CKU	9.66±0.70ab	4.37±0.12b	3.17±0.10b	8.84±0.27b	3.70±0.10b	2.83±0.21b
CKW	8.84±0.88b	3.98±0.05c	2.86±0.20c	8.31±0.21b	3.32±0.17c	2.49±0.21b

Mean values ± SE. Means followed by the same letter in a column are not significantly different at p < 0.05. N, P, and K represents nitrogen, phosphorus, and potassium, respectively. CK represents well-structured normal soil treated with corn straw return and constant fertilizer application for 3 consecutive years from October 2014 to October 2017. CKU is blocky soil structure retained by a 2mm sieve; CKW represents a well-structed soil flooded with 600 mL of distilled water in CK. S1 represents the first sowing period; S2 represents the second sowing period

Change of utilization rate of ^{15}N in maize seedling stage

As shown from *Fig. 3*, the ^{15}N utilization rate exhibited similar trend in both S1 and S2 data sets, increasing in this order CK > CKU > CKW. The ^{15}N utilization rate of S1 was slightly higher than that in S2 across all treatments. Compared with CK, the ^{15}N utilization rate in CKU decreased by 11.51% and that in CKW decreased by 32.69%, in under S1. In S2 data set, the CKU and CKW decreased ^{15}N utilization rate by 9.56% and 49.91%, respectively *(Fig. 3)*. This indicate that soil altered by flooding (i.e., CKW treatment) significantly reduce the rate by maize utilizes ^{15}N during the seedling stage.
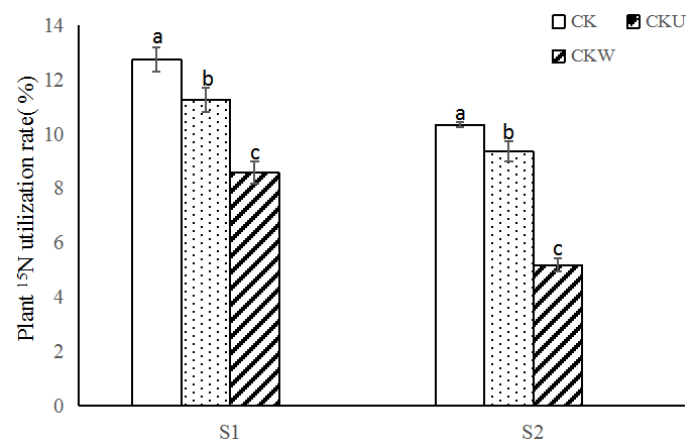
Figure 3. *The ^{15}N utilization rate after artificial soil structure destruction. Bars are standard error, and bars followed by the same letter for a given sowing time are not significantly different at p < 0.05. CK represents well-structured normal soil treated with corn straw return and constant fertilizer application for 3 consecutive years from October 2014 to October 2017. CKU is blocky soil structure retained by a 2mm sieve; CKW represents a well-structed soil flooded with 600 mL of distilled water in CK. S1 represents the first sowing period; S2 represents the second sowing period*

Discussion

Effects of artificially altered soil structure on soil properties

The present study demonstrated that soil flooding (i.e., CKW treatment) and altering soil structure (i.e., CKU treatment) significantly decreases $2 - 0.25$ mm aggregate-size fractions (*Table 2*), SOC content (*Fig. 1*) and soil mineral N (*Fig. 2*). These results indicate that altering soil structure and flooding affects soil mineral N, SOC content and soil physical properties, and is consistent with the results obtained by Harris (Harris et al., 2016) which showed that soils prone to flooding have low NO_3^- - N content and higher bulk density compared with not flooded soil. This may be because flooding lower N mineralization rates, and encourages volatilization of ammonia and denitrification losses (Bacon et al., 1986; Zhang and Wienhold, 2002). Whereas, structure disturbance experienced under CKU treatment may have increased accessibility of soil organic N and organic matter to soil micro-organisms for mineralization. Kristensen et al. (2000) also reported a decrease in soil mineral N and SOC contents when soil structure is disturbed.

Nitrogen fertilizer applied to the soil is converted into inorganic NO_3^-–N and NH_4^+–N, organic nitrogen (Caiyan et al., 2010). According to Mkhabela et al. (2008), NO_3^-–N and NH_4^+–N are the forms of inorganic N most greatly absorbed by the roots of maize. Therefore, the higher the availability of inorganic NO_3^-–N and NH_4^+–N in the soil and rhizosphere, and the utilization ability of the crop, the higher the N absorption by crops (Asibi et al., 2019). In the present study both CKW and CKU reduced soil NO_3^-–N and NH_4^+ –N (*Fig. 2*), resulting to losses of soil total ^{15}N and low soil total ^{15}N residual rate (*Table 3*). These results indicate that applied synthetic fertilizer N was not stabilized within the soil matrix, probably because flooding led to elevated nitrous (N_2O) production. Whereas, CKU may have influenced mineralization-immobilization turnover by increasing availability of protected N to microbes, and some of N stored in < 2 mm aggregates were lost due to sieving. Harris observed that waterlogging-prone soil can lead to elevated N_2O production, which reduces soil N (Harris et al., 2016). These results suggest that soils that had their structure disrupted have lower capacity for N retention and nutrient. Contrary to this, a study by Chilundo et al. (2018) showed that proper soil management practices reduce potential leaching losses of NO_3-N and NH_4-N, which in turn improves N retention. This is attested by results presented by our study which showed that non-destroyed soil structure (CK treatment) was more conducive in increasing NO_3-N and NH_4-N (*Fig. 2*), soil total ^{15}N residual rate, and significantly reduced soil total ^{15}N loss (*Table 3*).

Effects of artificial soil structure changes on plant characteristics

Root growth (*Table 3*), height and dry matter weight (*Table 4*) of maize at seedling stage appeared to cease after flooding and soil structural disturbance. These maize growth parameters (*Tables 3 and 4*) did not recover over the durations evaluated in this experiment. This could mean, when soil is flooded or the structure is disturbed it might take a relatively long time (> 6 months in the case of our study) to restore its inherent properties to support maize production. The decrease in roots system in flooded soil might be due to the scarcity or absence of oxygen at the root tip (Malik et al., 2002). Low chlorophyll content measured in flooded soil (CKW treatment) (*Table 5*), is attributable to chlorophyll destruction mediated by superoxide radicals formed under flooding stress (Wang et al., 2012). These results suggest that when the structure of the soil is altered, maize development is impaired, which could potentially affect crop yield.

Effects of artificial soil structure alteration on plant nutrients and N utilization efficiency

Compared with CK, the CKW and CKU treatments showed significantly lower leaf N, P and K content in both data sets (*Table 6*), and is consistent with the study by Ren, which reported a decrease in leaf N in waterlogged compared with non-waterlogged soil (Ren et al., 2017). This could be due to leaching of nutrients below the rooting depth of maize. Whereas, soil structure disturbance accelerates N mineralization of previously protected soil microbial biomass N (Kristensen et al., 2000), thus reducing available N for plant uptake.

The results of our study showed that, compared with CK maize ^{15}N absorption and utilization rate decreased significantly after soil structure was artificially altered (*Fig. 3*). This is because artificially altering soil structure significantly reduced soil aggregate-size distribution (*Table 2*) and SOC content (*Fig. 1*), soil factors which control the rates and products of N mineralization (Seyfried and Rao, 1988). Getahun showed that soil with high bulk density and lower porosity, deteriorates soil structure, thus affecting transport and absorption of nutrients (Getahun et al., 2018).

Kong et al. (2007) observed that soil N was reduced by changes in macroaggregates (> 0.25 mm). Therefore, a decline in > 0.25 mm aggregate-size fractions after CKW and CKU treatments in our study (*Table 2*), could have also contributed to lower ^{15}N utilization in maize (*Fig. 3*), because majority of protected soil organic N was lost when > 0.25 mm aggregates turned over (Kong et al., 2007). Xu et al. (2015) and Zhou et al. (2019) reported that increase in > 0.25 mm aggregate-size distribution could promote nutrient circulation, increase nutrient supply and absorption of maize roots.

Lal et al. (2007) showed that increasing SOC storage improves soil stability and porosity, and reduce soil erosion and nutrient leaching, which in turn encourages nutrient absorption by crops. A decrease in SOC content under CKW and CKU treatments compared with CK in our study (*Fig. 1*), might have contributed to a decrease in maize ^{15}N absorption and utilization rate (*Fig. 3*). Conversely, greater soil organic carbon content under CK, enhanced soil structure and fertility, as a result maize ^{15}N absorption and utilization rates was increased accordingly.

These results imply that ^{15}N absorption and utilization rate is dependent on several soil properties, which are exacerbated by poor soil structure. Therefore, poor soil structure might limit nutrient absorption/adsorption which will ultimately affects crop productivity.

Conclusion

After the soil structure was artificially altered, the growth and development of maize was hindered. Artificial altering soil structure decreased soil N and P content, and increased soil N losses. The ^{15}N utilization rate and uptake by maize at seedling stage was reduced in altered soil structure under both sowing times. The total ^{15}N absorption rate by maize under CK was 23.14%, the CKU and CKW were down to 20.63% and 13.77%, respectively, after soil structure is altered. Therefore, altering soil structure have adverse effects on soil nutrients and maize development. Through the study of this paper, further research on soil structure improvement measures to improve nitrogen use efficiency and increase crop yield will play an important role.

REFERENCES

[1]   Alam, M. M., Ladha, J. K., Rahman, Z., Khan, S. R., Harun-ur-Rashid, Khan, A. H., Buresh R. J. (2006): Nutrient management for increased productivity of rice–wheat cropping system in Bangladesh. – Field crops research 96: 374-386.

[2]   Asibi, A. E., Chai, Q., Coulter, J. A. (2019): Mechanisms of Nitrogen Use in Maize. – Agronomy 9: 775.

[3]   Bacon, P. E., McGarity, J. W., Hoult, E. H., Alter, D. (1986): Soil mineral nitrogen concentration within cycles of flood irrigation: Effect of rice stubble and fertilization management. – Soil Biol. Biochem 18(2): 173-178.

[4]   Bataung, M., Xianjin, Z., Guozheng, Y., Xianlong, Z. (2012): Review: Nitrogen assimilation in crop plants and its affecting factors. – Canadian Journal of Plant Science 92: 399-405.

[5]   Bimuller, C., Kreyling, O., Kölbl, A., Lützow, M., Kögel-Knabner, I. (2016): Carbon and nitrogen mineralization in hierarchically structured aggregates of different size. – Soil and Tillage Research 160: 23-33.

[6]   Caiyan, L., Jian, M., Xin, C., Xudong, Z., Yi, S., Bin, H. (2010): Effect of nitrogen fertilizer and maize straw incorporation on NH_4^+-^{15}N and NO_3-^{15}N accumulation in black soil of Northeast China among three consecutive cropping cycles. – Journal of soil science and plant nutrition 10: 444-453.

[7]   Cambardella, C. A., Elliott, E. T. (1993): Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. – Soil Science Society of America Journal 57: 1071-1076.

[8]   Chen, L. Q. (2007): How to manage the seedling stage of hybrid maize. – The seeds of science and technology 3: 33-35. (In Chinese).

[9]   Chilundo, M., Joel, A., Wesström, I., Brito, R., Messing, I. (2018): Influence of irrigation and fertilisation management on the seasonal distribution of water and nitrogen in a semi-arid loamy sandy soil. – Agricultural Water Management 199: 120-137.

[10]   Getahun, G. T., Kätterer, T., Munkholm, L. J., Parvage, M. M., Keller, T., Rychel, K., Kirchmann, H. (2018): Short-term effects of loosening and incorporation of straw slurry into the upper subsoil on soil physical properties and crop yield. – Soil and Tillage Research 184: 62-67.

[11]   Islam, Z., Bagchi, B., Hossain, M. (2007): Adoption of leaf color chart for nitrogen use efficiency in rice: Impact assessment of a farmer-participatory experiment in West Bengal, India. – Field Crops Research 103: 70-75.

[12]   Kavdır, Y., Smucker, A. J. M. (2005): Soil aggregate sequestration of cover crop root and shoot-derived nitrogen. – Plant and soil 272: 263-276.

[13]   Kong, A. Y. Y., Fonte, S. J., van Kessel, C., Six, J. (2007): Soil aggregates control N cycling efficiency in long-term conventional and alternative cropping systems. – Nutr Cycl Agroecosyst 79: 45-58.

[14]   Kristensen, H. L., McCarty, G. W., Meisinger, J. J. (2000): Effects of Soil Structure Disturbance on Mineralization of Organic Soil Nitrogen. – Soil Sci. Soc. Am. J 64: 371-378.

[15]   Lal, R. (2007): Carbon sequestration. Philosophical Transactions of the Royal Society B. – Biological Sciences 363: 815-830.

[16]   Lancashire, P. D., Bleiholder, H., Van den Boom, T., Langelüddeke, P., Strauss, R., Weber, E., Witzenberger, A. (1991): A uniform decimal code for growth stages of crops and weeds. – Ann. App. Biol 119: 561-601.

[17]   Liang, A., McLaughlin, N. B., Xiaoping, Z., Shen, Y., Xiuhuan, S., Ruiqin, F. (2011): Short-term effects of tillage practices on soil aggregate fractions in a Chinese Mollisol. – Acta Agriculturae Scandinavica, Section B-Soil & Plant Science 61: 535-542.

[18] Li, Y. (2018): The Relationship between Soil Structure and Nitrogen Mineralization in Paddy soil. – Northeast Agricultural University 6-8. (In Chinese).

[19] Lu, R. K. (1999): Analytical methods for soil and agro-chemstry. – China Agricultural Science and Technology Publishing, Beijing.

[20] Malik, A., Colmer, T. D., Lambers, H., Setter, T. L., Schortemeyer, M. (2002): Short-term waterlogging has long-term effects on the growth and physiology of wheat. – New Phytologist 153: 225-236.

[21] Mkhabela, M. S., Madani, A., Gordon, R. (2008): Gaseous and leaching nitrogen losses from no-tillage and conventional tillage systems following surface application of cattle manure. – Soil and Tillage Research 98: 187-199.

[22] Munkholm, L. J., Schjønning, P., Rasmussen, K. J., Tanderup, K. (2003): Spatial and temporal effects of direct drilling on soil structure in the seedling environment. – Soil and Tillage Research 71(2): 163-173.

[23] Nelson, D. W., Sommers, L. E. (1982): Total carbon, organic carbon, and organic matter. – In: Page, A. L., Miller, R. H., Keeney, D. R. (eds.) Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Madison: American Society of Agronomy and Soil Science Society of America WI, pp. 539-579.

[24] Panakoulia, S. K., Nikolaidis, N. P., Paranychianakis, N. V., Menon, J. M., Schiefer, G. J., Lair, P., Krám, S., Banwart, A. (2017): Factors Controlling Soil Structure Dynamics and Carbon Sequestration Across Different Climatic and Lithological Conditions. – Advances in Agronomy 142: 241-276.

[25] Pang, F. Q. (2019): Technology of maize seedling management. – Rational crop: 18. (In Chinese).

[26] Ren, B., Dong, S., Zhao, B., Liu, P., Zhang, J. (2017): Responses of Nitrogen Metabolism, Uptake and Translocation of Maize to Waterlogging at Different Growth Stages. – Front. Plant Sci. 8: 1216.

[27] Harris, R. H., Armstrong, R. D., Wallace, A. J., Belyaeva, O. N. (2016): Effect of nitrogen fertiliser management on soil mineral nitrogen, nitrous oxide losses, yield and nitrogen uptake of wheat growing in waterlogging-prone soils of south-eastern Australia. – Soil Research 54: 619-633.

[28] Seyfried, M. S., Rao, P. S. C. (1988): Kinetics of nitrogen mineralization in Costa Rican soils: Model evaluation and pretreatment effects. – Plant Soil 106: 159-169.

[29] Walia, M. K., Dick, W. A. (2018): Selected soil physical properties and aggregate-associated carbon and nitrogen as influenced by gypsum, crop residue, and glucose. – Geoderma 320: 67-73.

[30] Wang, X., Liu, T., Li, C., Chen, H. (2012): Effects of soil flooding on photosynthesis and growth of *Zea mays* L. seedlings under different light intensities. – Afr. J. Biotechnol 11: 7676-7685.

[31] Xu, S., Zhang, L., McLaughlin, N. B., Junzhen, M., Qin, C., Jinghui, L. (2015): Effect of synthetic and natural water absorbing soil amendment soil physical properties under potato production in a semi-arid region. – Soil and Tillage Research 148: 31-39.

[32] Yu, H. Y., Qian, X. G., Luo, X. Q., Zhang, S. S., Ouyang, J. M., Zhang, Z. M. (2017): Effects of nitrogen fertilizer application depth on plant growth and nutrient uptake in maize seedling stage. – Jiangsu agricultural science 45: 44-48. (In Chinese).

[33] Zhang, R., Wienhold, B. J. (2002): The effect of soil moisture on mineral nitrogen, soil electrical conductivity, and pH. – Nutrient Cycling in Agroecosystems 63: 251-254.

[34] Zhou, L., Monreal, C. M., Xu, S. T., McLaughlin, N. B., Zhang, H. Y., Hao, G. C., Liu, J. H. (2019): Effect of bentonite-humic acid application on the improvement of soil structure and maize yield in a sandy soil of a semi-arid region. – Geoderma 338: 269-280.

[35] Zuo, H. J. (2012): Study on fertilizer Nitrogen direction and utilization rate based on high yield ^{15}N. – Chinese Academy of Agricultural Sciences. In Chinese.