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Original Research

Soil Environment Modulation by Varying Physicochemical Attributes Change the Population Dynamics of Fecal *Escherichia coli*

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Abstract

Present research reveals the effect of different soil environment modifications on population dynamic tendency of fecal *Escherichia coli* (*E. coli*). Various soil environments were created by varying the temperature regimes, relative water content, pH, C/N ratio and ammonium content. The result showed that population dynamics of *E. coli* with all treatments increased initially and then decreased with the passage of time. Maximum population of *E. coli* at higher temperature regime (37ºC and 45ºC) was observed on 8th day of incubation, whereas, at low temperature regime (0.6ºC and 25ºC), it reached the peak after 15 days of incubation, other treatments (soil relative water content, pH, C/N, ammonium content) reached the peak all after 8 days of incubation. At the end of the incubation period (60 days), maximum population of *E. coli* was observed in the soil treated with 0.6ºC, 80% of relative soil water content, pH 7.0, 135 mg-ammonium/kg and C/N of 25:1. The results indicated that 45ºC, 90% of relative water content, pH 9.0, 125 mg-ammonium/kg, C/N of 10:1 inhibited *E. coli* growth, whereas, 37ºC, 80% of relative soil water content, pH 7.0, 135 mg-ammonium/kg and C/N of 25:1 stimulated *E. coli* growth.

Keywords: *Escherichia coli*, soil environment factors, population dynamics

Introduction

Livestock and poultry breeding industry are major contributors in agricultural productivity in China. They play a major role to fulfill the food demands of ever-increasing population around the globe by providing protein rich diet. However it also produces a large amount of manure, it is reported that Chinese aquaculture industry generates 3.8 billion tons of manure every year. The livestock manure is considered as an excellent source of nutrients, which can increase the soil fertility by providing organic matter and essential nutrients. The soil fortification with livestock manure potentially improves soil physical and chemical properties, nutrient profiling and ionic contents which positively influence crop growth, quality and yield

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[1-2], contrarily, livestock manure contains a large number of pathogenic microbes, which may cause soil environmental pollution and may cause zoonotic diseases if applying to field without treatment [3-5]. Therefore, different countries and organizations have designed different standards for pathogenic microbes in manure before returning to the field. Italy has the most restrictive limit on the number of *E. coli* in wastewater, which is 10^3 cfu L^{-1}, in WHO, Cyprus, Portugal and Spain (10^4 cfu L^{-1}), and in Greece (25000 cfu L^{-1}). China and France have the least restrictive limit on *E. coli* (10^5 cfu L^{-1}). [6] The application of livestock manure in field condition may enhance the population of pathogenic microbes including *E. coli* even if it has been treated. Additionally, various types of soil environments and farmland eco-systems can also favours the growth and population dynamics of microbes in field conditions which may increase the ecological risks.

E. coli is associated with the gastrointestinal tract of warm-blooded animals and has been considered as one of the most important pathogenic microbes in livestock manure. Various studies showed differential growth responses of *E. coli* due to different soil environment conditions. It is reported that *E. coli* survives better at 37ºC [7-8], while some other studies find that temperature higher than 4ºC inhibit the *E. coli* population in soil [9]. In case of soil water content, some studies indicated that higher soil moisture content (100% field capacity) enhanced the survival rate of *E. coli* [10], while some other studies indicated that moisture content did not significantly affect the inactivation rate of *E. coli* [11]. Adequate levels of carbon and nitrogen positively correlated with growth of microorganisms, hence, suitable soil C/N ratio enhance the *E. coli* growth in soil medium. Most of studies claim that C/N ratio of 25:1 is beneficial for *E. coli* growth, and decreasing levels of C/N will lead to early autolysis of the bacteria, while increase will result in imbalance of bacterial metabolism, but Singh et al. [12] believe that C/N ratio of 16:1 supported longer survival for *E. coli*. Soil pH is one of the important factors affecting survival and growth of soil microorganism, pH 6~8 is considered optimal for most bacteria (such as *E. coli*), and the soil pH of 4~10 is viable, which means bacteria is inhibited or inactivating if pH is lower than the minimum value or higher than the maximum value. Williams et al. [13] reported that the survival rate of *E. coli* is highest in pH 7.0. Wang et al. [14] finds that in the acidic soils t_d values decreased as the pH decreased or become more acidic resulting shorter t_d values, while in the neutral or alkaline soils (pH≥6.45) they observed longer t_d values. It is also reported that varying levels of ammonium and nitrate also affect the population dynamics of *E.coli* in soil, an average level of 5.5 mg ammonia/kg of dry chicken droppings results in a 4.5-log decline typhimurium cells to the point of no recovery within 46 h [15], but Taabodi et al. [16] finds that *E. coli* can absorb ammonium as nutrient when concentrations of ammonium and nitrate are both 1.0 mg L^{-1}, in which utilization of ammonium is 3.35 times higher than nitrate.

Hence, there are different perceptions among studies on the effect of varying physicochemical properties of soil on the survival rate and population dynamic change of *E. coli* in soil and conclusions are mostly based on a single environmental factor in soil. However, soil environment is based on multiple factors which create the soil-microbes-atmosphere-continuum, composed of multiple interconnected factors affecting the population dynamics of microbes. There are limited reports available about the synergistic influence of various physicochemical factors of soil on *E. coli* growth. Therefore, a comprehensive study is designed to evaluate the synergistic influence of various soil environments created by varying levels of temperature, relative water content, pH value and ammonium-N, nitrate-N and C/N ratios. The objective of this study is to investigate the influence of various types of soil environments on population dynamics of *E. coli* in soil, which can provide the theoretical basis for application of biogas slurry for soil fertility and crop cultivation in eco-friendly manner.

Experimental

Slurry and Soil

The experiment was conducted in Yantai Institute of China Agricultural University, Yantai city, Shandong Province (119°34′~121°57′ E, 36°16′~38°23′ N), from November 5th, 2020 to January 30th, 2021. Slurry (pig slurry) was obtained from a biogas plant in Muping district, Yantai, Shandong Province, and soil used in experiment was brown soil (sandy loam soil). Basic properties of soil and slurry used in this study are listed in Table 1.

Table 1. Characteristics of soil and slurry used in this study.

Material	Characteristics				
	Number of *E. coli* (cfu/L)	Organic Matter Content (%)	Ammonium-N Content (mg/kg)	Nitrate-N Content (mg/kg)	TS (%)
Soil	0.00±0.00	1.201±0.05	21.15±1.02	2.95±0.54	\
Slurry	3.05×10^7±2000	0.46±0.04	453.09±2.53	172.72±2.55	1.57±0.08

Methods

Soil column experiment was conducted in PVC tube (7.5 cm diameter, 25 cm depth). In this study five different factors (soil temperature, relative water content, pH value, ammonium-N content, C/N ratio) were set up to explore the effect of each factor, respectively. Soil was air-dried, ground and sieved (<2 mm), then taken into the column, each column was filled with 1000 g soil. After that every column was filled with 140 g slurry and water content was adjusted with sterile water. The top and bottom of column were sealed with high temperature resistant tissue sealing film.

Experiment Design

Soil Temperature Treatments

Four temperature treatments were set up at 0.6ºC, 25ºC, 37ºC and 45ºC, temperature was kept through constant temperature incubator.

Soil Water Content Treatments

Three relative soil water content treatments were set up at 60% (soil water content 21.8%), 80% (soil water content 29.1%), 90% (soil water content 34.5%).

Soil pH Treatments

Three soil pH treatments were set up at 5.0, 7.0, 9.0, soil pH was adjusted through 0.1 mol/L H_2SO_4 and NaOH.

Soil Ammonium-N Content Treatments

Three soil ammonium-N treatments were set up at 125 mg/kg, 135 mg/kg, 145 mg/kg (nitrate-N contents were 25 mg/kg, 15 mg/kg, 5 mg/kg, respectively).

Soil C/N Treatments

Three soil C/N treatments were set up at 10:1, 25:1, 40:1, soil C/N was adjusted through organic fertilizers.

All experiments were repeated three times. Adjusted soil temperature at 37ºC, relative water content to 60% (soil water content 21.8%), pH to 7.0, ammonium-N content to 135 mg/kg, nitrate-N content to 15 mg/kg and soil C/N to 25:1.

Sampling and Examination

Soil samples were collected by inserting a cylindrical sampler (2 cm diameters, 25 cm high) vertically into soil column 20 cm from the center of soil column surface after 1, 2, 4, 8, 15, 30, 45 and 60 days of incubation, experimental units were separate units for each timepoint. The population of *E. coli* was estimated by the enumeration of typical colonies and were reported as colony-forming units per g of fresh soil (cfu g^{-1}) [17], soil ammonia concentration were determined through Kjeldahl distillation [18], nitrate-N content were measured using a high-resolution continuous-flow analyzer AutoAnalyzer3 [19], soil organic carbon (SOC) and total nitrogen (TN) content were determined by potassium dichromate oxidation method and Kjeldahl method, respectively [20]. Soil oxygen content was determined through gas detector (Qingdao Junyuan environmental protection equipment co. LTD, 20H17041).

Data Analysis

Main text paragraph. The effects of the treatments were tested by one-way analysis of variance (ANOVA). Means were compared between the treatments using the LSD (least significant difference) test at the 0.05 probability level.

Results and Discussion

Influence of Soil Temperature on Survival and Population of *E. coli*

Statistical analysis showed that compared with 0.6ºC treatment, counts of *E. coli* in soil at 25ºC, 37ºC and 45ºC were increased significantly after 8 days of incubation (P<0.05), which were increased by 39.92%, 85.02%, 19.96%, respectively (Table 2), after 60 days

Table 2. Influence of soil temperature on counts of *E. coli*

Temperature (ºC)	Counts of *E. coli* in soil (10^6 cfu/kg)							
	1 d	2 d	4 d	8 d	15 d	30 d	45 d	60 d
0.6	3.30ab	3.40bc	3.55c	4.12d	4.57c	3.54c	2.39b	1.12a
25	3.77a	4.05b	4.64b	5.76b	5.87b	4.17b	2.29b	0.46ab
37	3.86a	4.79a	6.48a	7.62a	7.30a	5.42a	3.22a	0.81a
45	3.54b	3.67bc	4.31b	4.94c	4.76c	3.10cd	1.39c	0.21ab

Note: Different letters in the table refer to statistically significant differences (P<0.05) among different treatments, the followed are as the same.

Table 3. Influence of soil water content on counts of *E. coli.*

Soil Water Content	Counts of *E. coli* in soil (10^6 cfu/kg)							
	1 d	2 d	4 d	8 d	15 d	30 d	45 d	60 d
21.8%	3.86a	4.79a	6.48ab	7.62b	7.30ab	5.42b	3.22a	0.81ab
29.1%	4.03a	4.88a	6.81a	8.20a	7.84a	5.96a	3.74a	1.20a
34.5%	3.69a	4.11ab	5.77b	6.18c	5.57c	3.78c	2.12b	0.33b

of incubation, count of *E. coli* at 0.6ºC was 1.44, 0.39, 4.35 times higher than which at 25ºC, 37ºC, 45°C, respectively, there was no significant difference among treatments (P>0.05). In terms of culture time, counts of *E. coli* in soil at 0.6ºC, 25ºC, 37ºC and 45ºC were all increased first and then decreased with the increasing time, the treatments of 37ºC and 45ºC peaked at 8th day of incubation, and 0.6ºC, 25ºC treatments peaked after 15 days of incubation. The maximum *E. coli* count was observed at 8 days of incubation at 37ºC treatment being the highest, followed by 25ºC, 45ºC, 0.6ºC.

Influence of Soil Water Content on Survival and Population of *E. coli*

Statistical analysis showed that compared with soil water content 34.5% treatment, counts of *E. coli* in soil at 21.8% and 29.1% were increased significantly after 8 days of incubation (P<0.05), which were increased by 23.26% and 32.68%, respectively (Table 3), after 60 days of incubation, count of *E. coli* at 29.1% treatment was 1.48 and 3.64 times higher at 21.8% as compared to 34.5%, significant difference (P<0.05) was found at 60th day. In terms of culture time, counts of *E. coli* in all treatments were increased first and then decreased with the increase in time, which peaked after 8 days of incubation with 29.1% treatment being the highest, followed by 21.8%, 34.5%.

Influence of Soil pH on Survival and Population of *E. coli*

Statistical analysis showed that compared with soil pH 9.0, counts of *E. coli* in soil at pH 5.0 and 7.0 were increased significantly after 8 days of incubation (P<0.05), which were increased by 37.98% and 78.16%, respectively. After 60 days of incubation, count of *E. coli* at soil pH 7.0 and 5.0 treatments were 1.72 and 4.26 times higher as compared to pH 9.0, there was no significant difference among treatments (P>0.05) (Fig. 1). In terms of culture time, counts of *E. coli* in all treatments were increased first and then decreased as time went by, which peaked after 8 days of incubation with soil pH 7.0 treatment being the highest, followed by soil pH 5.0, 9.0. Population of *E. coli* in all treatments decreased to less than 10^5 cfu/kg after 60 days of incubation.

Influence of Soil Ammonium-N and Nitrate-N on Survival of *E. coli*

Fig. 2 showed that compared with 125 mg-ammonium/kg, counts of *E. coli* in soil at 135 mg-ammonium/kg and 145 mg-ammonium/kg were increased significantly after 8 days of incubation (P<0.05), which were increased by 22.08% and 13.14%, respectively. After 60 days of incubation, count of *E. coli* in soil at 135 mg-ammonium/kg was 3.00, 2.03 times than which at 125 mg-ammonium/kg and 145 mg-ammonium/kg in turn, there was no significant difference among treatments (P>0.05). In terms of culture time, counts of *E. coli* in all treatments were increased first and then decreased as time went by, which peaked after 8 days of incubation with 135 mg-ammonium/kg treatment being the highest, followed by 145 mg-ammonium/kg, 125 mg-ammonium/kg. The population of *E. coli* in all treatments decreased to less than 10^5 cfu/kg after 60 days of incubation.

Influence of Soil C/N on Survival and Population of *E. coli*

Statistical analysis showed that compared with low C/N (10:1), counts of *E. coli* in soil at C/N 25:1 and 40:1 treatments were increased significantly after 8 days of incubation (P<0.05), which were increased by 11.88% and 5.12%, respectively. After 60 days of incubation, count of *E. coli* in soil at C/N of 25:1 was 3.49, 1.81 times than which at C/N 10:1 and 40:1 treatments in turn, there was no significant difference among treatments (P>0.05) (Fig. 3). In terms of culture time, counts of *E. coli* in all treatments were increased first and then decreased as time went by, which peaked after 8 days of incubation with C/N of 25:1 treatment being the highest, followed by C/N 40:1, 10:1. The population of *E. coli* in all treatments decreased to less than 10^5 cfu/kg after 60 days of incubation.

Discussion

The results showed that *E. coli* growth was stimulated in soil at 37ºC and inhibited when soil temperature was higher or lower than 37ºC (0.6ºC, 25ºC, 45ºC). It means 37ºC is optimum temperature for growth of *E. coli*, it might be suitable for enzyme activities in *E. coli*, which ultimately promoted

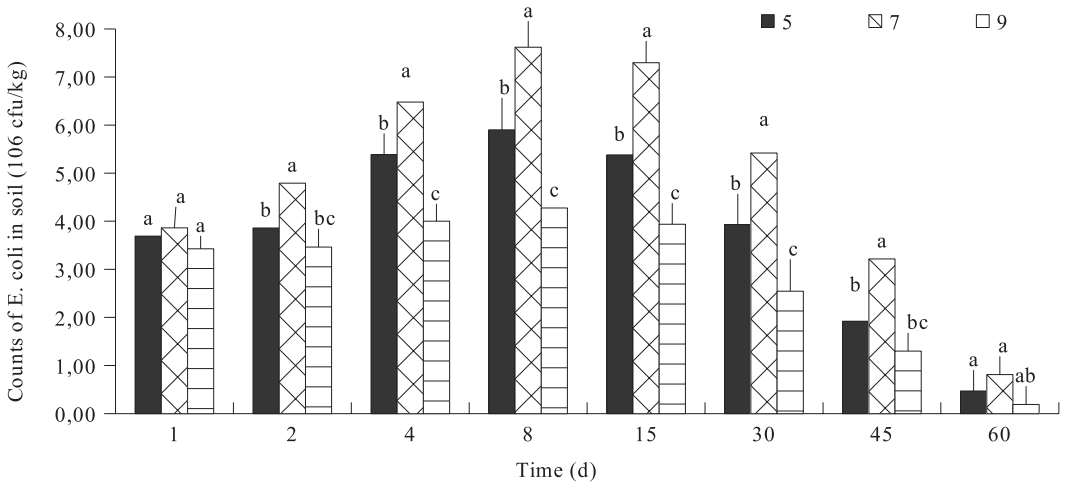

Fig. 1. Influence of soil pH on counts of *E. coli.*
Note: Different letters in the figure refer to statistically significant differences (P<0.05) among different treatments, the followed are as the same.

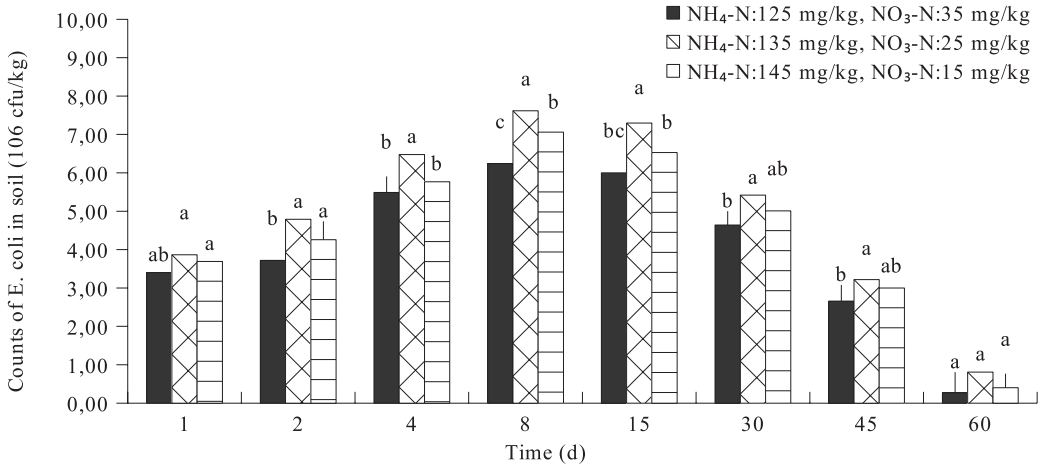

Fig. 2. Influence of soil ammonium-N and nitrate-N on counts of *E. coli.*

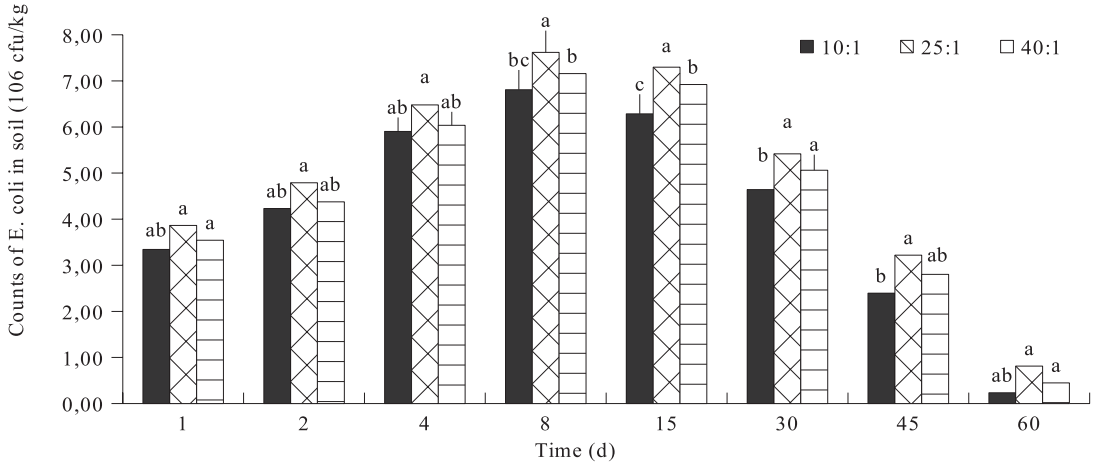

Fig. 3. Influence of soil C/N on counts of *E. coli.*

E. coli growth, and decrease of temperature resulted in *E. coli* transient growth inhibition and a cold shock response consisting of transient induction of several proteins, repression of heat shock proteins [21-22]. There was a dramatic increase in protein degradation at high temperature (>37ºC), which resulted in inhibited *E. coli* metabolism [23]. Our results showed that counts of *E. coli* in the soil at 0.6°C was higher than which at other temperatures after 60 days of incubation, it might be attributed to greater amount of protective enzymes and other proteins and also to a lower degree of permeability of the envelopes to toxic [24]. Other temperature treatment (25ºC) was conducive to soil nitrification, which led to increased soil nitrate-N content and inhibited *E. coli* growth [25-26], or stimulated formation of protein aggregates at elevated temperatures (45ºC) which led to complete inactivation of the protein [27].

Water content is one of the important factors of soil physical properties and has important effect on reproduction of soil microorganisms. The results showed that regardless of length of culture time, counts of *E. coli* in soil at relative water content of 80% treatment was always higher than which at 60% and 90% treatments. It illustrated that 80% relative soil water content improved *E. coli* metabolic activity, *E. coli* community-level activity decreases with decreasing soil moisture (60%) [28], soil oxygen content decreased as water content increased (90% of relative soil water content), which led to inhibited *E. coli* growth [29-30].

The results showed that counts of *E. coli* in soil was highest with soil pH 7.0 treatment (Fig. 1). It illustrated that soil with pH 7.0 provided a suitable acid-base environment for the propagation and growth of *E. coli* and improved its metabolic activity [31]. Counts of *E. coli* in soil decreased as soil pH increased (9.0) or decreased (5.0). That was because high adsorption capacities of Fe/Al (hydro) oxides with positive charges in acidic soils led to high inactivation for *E. coli* [32], and it was likely to be more negatively charged with increasing pH levels and could have led to the destruction *E. coli* at pH 9.0, meanwhile greater antimicrobial potential of humic acid contributed to inactivation for *E. coli* as it is more soluble at higher pH levels [33]. According to Fig. 1, alkaline soil (pH 9.0) have a stronger inhibitory effect on *E. coli* growth compared with acidic soil (pH 5.0). It might because soil with lower pH provided less substrate for soil nitrification and dampens the soil microbial activity of nitrification, which led to a decrease of soil nitrate-N content and the toxicity of nitrate-N to *E. coli* was weaken, in soil with high pH (9.0) nitrate-N content increased because of promoted nitrification, which strengthened inhibition on *E. coli* growth [34].

The results showed that there were different positive or negative effects of contents of ammonium-N and nitrate-N in soil on *E. coli* growth (Fig. 2). Counts of *E. coli* in soil showed a trend of increasing first and then decreasing after the application of slurry, it might because at initial stage of application of slurry ammonium-N content in soil was enough as the nitrogen source for *E. coli* growth, but contents of ammonium-N and nitrate-N was decreased and increased, respectively with extension of culture time caused by mineralization, immobilization, nitrification, which inhibited *E. coli* growth [25, 35-36]. Compared with 135 mg-ammonium/kg (25 mg-nitrate-N/kg), the decrease of counts of *E. coli* in soil at treatment of 125 mg-ammonium/kg (35 mg-nitrate-N/kg) was caused by the inhibitory effect of nitrate-N (35 mg/kg) on *E. coli*, and the decrease at treatment of 145 mg-ammonium/kg (15 mg-nitrate-N/kg) showed that high content of ammonium-N also led to inhibition of *E. coli* growth [37].

Statistical analysis showed that soil environment with C/N of 25:1 was beneficial for *E. coli* growth, soil environments with C/N 10:1 and 40:1 inhibited *E. coli* growth, and inhibitory effect of soil C/N of 10:1 was more significant than which of 40:1, which was the same as conclusions in previous study [38]. The reason resulted in such phenomenon was that environment with C/N of 25:1 was more suitable for level of microorganism metabolic activity [39], too high C/N (40:1) led to mineral N immobilization, which limited *E. coli* to utilize nitrogen as nutrients source [40], and low C/N increased the net nitrification rates, which increased soil nitrate-N content and led to inhibition on *E. coli* survival [40-42].

Conclusions

The influence of different soil environments on population dynamics of *E. coli* were determined in the study. The results showed that, after 60 days of incubation, the soil treated with 0.6ºC, 80% of relative soil water content, pH 7.0, 135 mg-ammonium/kg and C/N of 25:1 had the maximum of *E. coli*, and 45ºC, 90% of relative water content, pH 9.0, 125 mg-ammonium/kg, C/N of 10:1 inhibited *E. coli* growth. It also indicted that 37ºC, 80% of relative soil water content, pH 7.0, 135 mg-ammonium/kg and C/N of 25:1 stimulated *E. coli* growth, which led to the peak of coliforms occurring during cultivation. Also, interactions among soil environment factors on *E. coli* should be taken into comprehensive account.

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Conflict of Interest

The authors declare no conflict of interest.

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