ISSN No:-2456-2165

Impact of Organic and Inorganic Fertilizers on Microbial Populations at Various Altitudes of Nepal in Paddy Field Soil Systems

Saraswoti Kandel[1*]
[1]National Soil Science Research Centre
Nepal Agriculture Research Council (NARC)
Khumaltar, Lalitpur, Nepal

Pratiksha Sharma[2]
[2]National Plant Pathology Research Centre
National Agricultural Research Centre (NARC)
Khumaltar, Lalitpur, Nepal

Suraj Chaudhary[3]
[3]Central Department of Biotechnology
Tribhuvan University (TU)
Kritipur, Nepal

Abstract:- Soil microbes are important in processes such as soil aggregate formation, nitrogen cycling, compound breakdown, and other transformations. Understanding how microorganisms react to chemical and organic fertilizers is critical for long-term agricultural intensification. Soil samples were collected from three distinct ecological sites of Nepal including terai (Parwanipur), mid-hills (Pakhribas), and high hills (Jumla) during winter seasons for period of three years 2017, 2018, and 2019. Sandy loam soils of Agricultural Research Farm, NARC, were used to assess the effects of compost and inorganic fertilizers on microbial dynamics in the continuous rice wheat cropping system. The results showed that the farmyard manure-treated plot had the highest microbial population counts (fungal and bacterial) in all years, followed by the inorganically treated plot and the control at all locations. The Pakhribas location had the highest bacterial and fungal population across all treatments. A significant change in fungal population was observed between treatments and experimental sites. Across all treatments, the Jumla location had the lowest fungus population and Pakhribas had the highest.

Keywords:- *Altitude, Bacterial Population, Farmyard Manure, Fungal Population, NPK.*

I. INTRODUCTION

Agro ecosystems rely on soil microorganisms. They play critical roles in processes such as the formation of soil aggregates, the formation of soil humus, the cycling of nutrients, the breakdown of different compounds, and other transformations (Luo *et al.* 2015; Wu *et al.* 2011). The biomass, diversity, and abundance of soil microorganisms are potential indicators of soil quality, which are linked to soil productivity, crop development, and yield, and can reveal the biological fertility, health, and vitality of the soil(Bending *et al.* 2004). The most typical method of managing agricultural soils is fertilization. The main purpose of both organic and inorganic fertilizers is to boost crop yield (Luo *et al.* 2015). Increased grain production must rely heavily on chemical fertilizer inputs to overcome nutrient deficiencies if it is to meet the needs of an expanding population (Zhang *et al.* 2019). Thus, for sustainable agricultural intensification, it's crucial to understand how microbes react to chemical and organic fertilizers.

Numerous research has revealed that fertilization management has a significant impact on the biomass of soil microorganisms and community structure (Liu *et al.* 2021). The functionality and quality of soils may change as a result of repeated fertilization because it alters the fertility and availability of nutrients, which can modify the physical, chemical, and biological characteristics of the soil. Microbes are a very sensitive part of agricultural ecosystems. Management techniques, such as the addition of manure and mineral fertilizer, may promote the growth of some microbes while restricting the growth of others (Luo *et al.* 2015; de Vries *et al.* 2006). According to some research, long-term chemical fertilization boosted microbial biomass and activity without significantly altering bacterial community structure. However, another study found that it reduced the diversity of arbuscular mycorrhizal fungi (Su *et al.* 2015). According to a study, soil acidity caused by fertilizer N application reduced the diversity and/or the number of soil microorganisms. Generally, the use of chemical N fertilizer encouraged the growth of fungus (Mujiya and Supriyadi 1970; Zhang *et al.* 2019).

Previous research has demonstrated variations in soil microbial community structure, abundance, and enzyme activity along a number of elevational gradients (Siles *et al.* 2016; Singh *et al.* 2012; Wang *et al.* 2015). Abiotic (temperature, precipitation, air composition, etc.) and biotic components experience considerable environmental gradients as altitude rises (vegetation, biodiversity and composition). As a result, plant communities along an altitudinal gradient may differ in terms of soil properties and microbial populations (Ma *et al.*, 2004; Xu et *al.*, 2014). Due to the harsher environment at higher altitudes, there was a reported increase in the ratio of Gram-negative/Gram-positive bacteria, fungus as well as an increase in the relative fraction of culturable psychrophilic heterotrophic bacteria (Siles *et al.* 2016). Numerous researchers have noted a decline in microbial

ISSN No:-2456-2165

population size, bacterial and fungal diversity (Lipson 2007; Schinner and Gstraunthaler 1981), as well as microbial activity like respiration rate, microbial biomass, and metabolic quotient with altitude (Margesin *et al*. 2009; Niklińska and Klimek 2007; Schinner and Gstraunthaler 1981).

The impact of altitude and organic/inorganic fertilizer on soil microorganisms in Nepal's agro ecosystems has received little attention. The goal of this study was to evaluate the variations in microbial abundance in paddy soil as influenced by repeated applications of both organic and inorganic fertilizer across different altitudes.

II. MATERIALS AND METHODS

A. Study site

For the study, three distinct ecological sites of Nepal including the terai (Parwanipur), midhills (Pakhribas), and high hills (Jumla) were chosen. The study was conducted for period of three years 2017, 2018 and 2019. Table 1 shows the ecoclimatic characteristics of study sites.

Table 1: Ecoclimatic features of Parwanipur, Pakhribas and Jumla study site

Parameter	**Sites**		
	Parwanipur	**Pakhribas**	**Jumla**
Latitude	27° 4'40.89"N	27° 2'51.35"N	29°16'21.69" N
Longitude	84°56'1.25" E	87°17'36.38" E	82°10'49.17" E
Elevation	95 m asl	1738m asl	2346m asl

B. Experimental design

Soil sample were collected from long term fertility trial of different ecological locations, covering terai (Parwanipur), mid hills (Pakhribas) and high hills (Jumla) after rice harvest and analyzed stastistically for microbial diversity and abundance at lab in NSSRC, Khumaltar, Lalitpur, Nepal. The effects of continual application of organic manure and NPK fertilizers in rice wheat rotation at Pakhribas and Parwanipur with each plot of a 12(4x3) m^2 size and in barley and rice rotation at Jumla with each plot of 10 (5x2) m2 sizes has been studied. A randomized complete block design with different treatments and three replicates was used to set up the experiment on each site. For this investigation, only three treatments were observed including control, NPK fertilizer (at the recommended dose), and farmyard manure (6 Mt/hac) for three-year period (2017, 2018 and 2019) on each different site during the paddy growing season.

C. Soil sampling

Following harvest, soil samples were collected aseptically from each experimental plot's (0–15 cm) soil depth. Random soil samples were taken from each plot and thoroughly mixed to create a uniform mixture. 250g of the soil samples were taken, and they were placed in storage at 4°C for further study.

D. Enumeration of microbial populations

The serial dilution plate count method was used to isolate and estimate the populations of bacteria and fungi. 10 g of soil sample was added to 90 mL of sterile water, and the mixture was stirred for a short duration. Then, sterile distilled water was used to execute a series of dilutions up to 10^{-6} in a test tube. Spread 0.1 mL of the diluted soil suspension from each serial dilution on the various media—nutrient agar (NA) for bacteria and potato dextrose agar (PDA) for fungus. The plates were incubated for 5-7 days at 25°C for fungi and for 1-2 days at 30°C for bacteria. Colony forming units were counted after incubation and expressed as $CFUg^{-1}$ of soil on a moisture-free basis.

E. Data analysis

The data were analyzed using GraphPad Prism 8.3.0 and Microsoft excel. Treatment means were compared using Tukey's test (ANOVA) at 5% levels of significance.

III. RESULTS

A. Microbial populations at experimental site of Parwanipur

There was a significant difference in the bacterial population between the control and treated plots (Farmyard manure and recommended dose of NPK). Figure 1 shows that the bacterial population was highest in the organically treated plot and lowest in the control plot throughout the study year. There was no significant difference in bacterial population in organic manure treatment across different experimental years. However, when compared to prior and subsequent years, the bacterial population was reduced in 2018 with the control and recommended dose of NPK treatment. Treatment with the recommended amount of NPK resulted in a higher bacterial population when compared to the control treatment. The fungal population in paddy fields showed similar trends, with the maximum and minimum values recorded in the organic and control plots, respectively (shown in fig 2). The fungal population in the organic plot differed significantly from that in the control plot and NPK treatment. Along the trial year, an increasing trend in fungus population was detected with organic and inorganic fertilizer treated plots. Variation was seen in control plots.

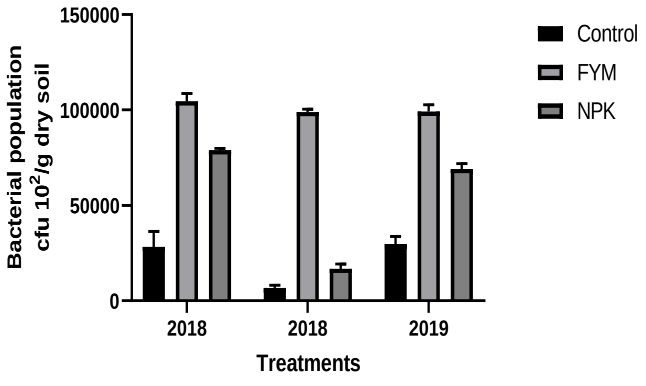

Fig 1: Effect of organic and inorganic fertilizers on bacterial population at experimental site of Parwanipur.

ISSN No:-2456-2165

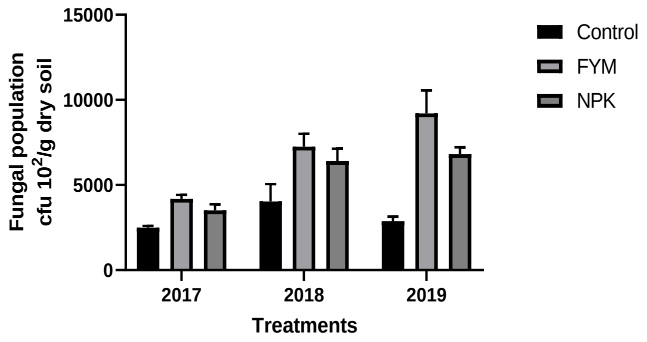

Fig 2: Effect of organic and inorganic fertilizers on fungal population at experimental site of Parwanipur.

B. *Microbial population at experimental site of Pakhribas*

There was a substantial difference in bacterial population between organically treated plots and other plots. Throughout the entire study period, the bacterial population was highest in the organically treated plots (shown in fig 3). During 2017 and 2019, the bacterial population was identical in control plots and suggested dose NPK treated plots. However, in 2018, there was some variance. In 2018, all treatments resulted in a decrease in the bacterial population. Variation in fungal population was detected at the Pakhribas experimental site with different treatments. During 2017 and 2018, a higher fungal population was reported in the control plot (shown in fig 4). In 2019, organically treated plots had the highest fungus population. Also in 2019, comparable findings were seen in both control and NPK-treated plots.

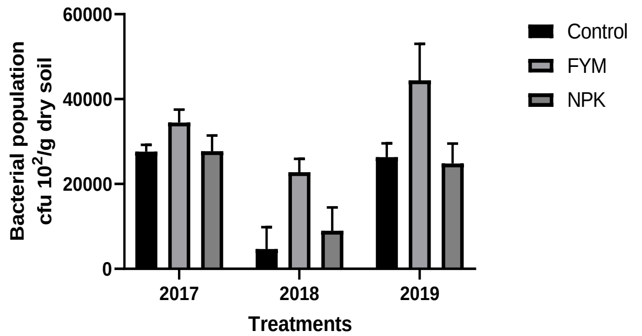

Fig 3: Effect of organic and inorganic fertilizers on bacterial population at experimental site of Pakhribas.

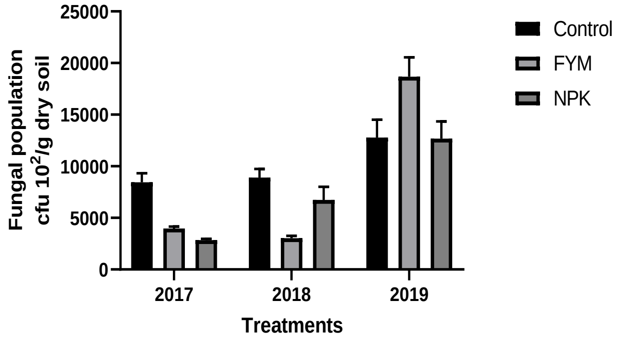

Fig 4: Effect of organic and inorganic fertilizers on fungal population at experimental site of Pakhribas.

C. *Microbial population at experimental site of Jumla*

The bacterial population showed substantial change across treatments. During 2017, 2018, and 2019, the bacterial population was maximum in organically treated plot and an increasing trend was also observed (shown in fig 5). Except for 2017, the control treatment had the lowest bacterial population. NPK-treated plots had a higher bacterial population than control plots. The fungus population varied depending on the treatment (shown in fig 6). Control therapy was observed with the highest fungal population in 2017. However, organically treated plots had the highest population in 2018 and 2019. During 2018 and 2019, a similar fungal population was reported in control plots and NPK treated plots. In 2019, there was a significant increase in the fungus population in organically treated fields.

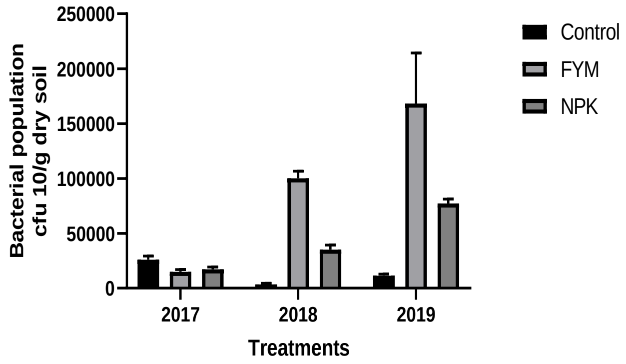

Fig 5: Effect of organic and inorganic fertilizers on bacterial population at experimental site of Jumla.

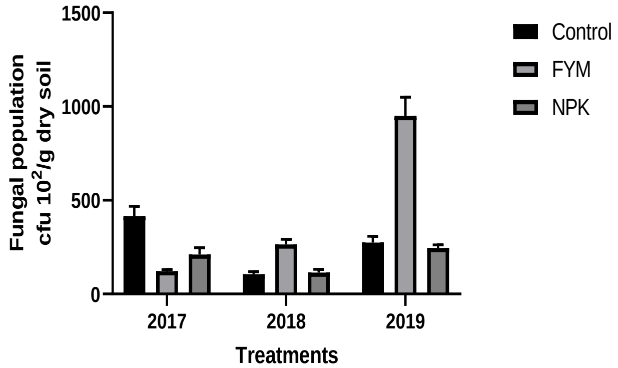

Fig 6: Effect of organic and inorganic fertilizers on fungal population at experimental site of Jumla.

The bacterial population varied throughout the three experimental sites: Parwanipur, Pakhribas, and Jumla. Bacterial populations were substantially lower in Jumla than in Pakhribas and Parwanipur. In all treatments, the Pakhribas study location had the highest bacterial population. The Pakhribas research site likewise had the highest fungus population. When compared to other study locations; the fungal population was lowest at Jumla. In all treatments, the bacterial and fungal populations were significantly greater in Parwanipur than in Jumla.

IV. DISCUSSION

The present experiment revealed that the soil microbial population increased in organically supplemented plots when compared to inorganic and control plots. This could be due to the introduction of organic amendments, which may have a significant impact on the size and activity of the microbial population. According to a research, organic amendments that increase the organic carbon and C/N ratio have a substantial impact on the structure of bacteria and eukaryotic communities (Marschner, Kandeler, and Marschner 2003). The manure soils also provided a lot of easily available C, which resulted in a more diversified and dynamic microbial ecology than in inorganically fertilized soil(Kirchner, Wollum, and King 1993). Joergensen *et al.* (2010) demonstrated that the long-term application of farmyard manure in conjunction with organic farming practices resulted in an increase in bacterial residue accumulation. When compared to mineral fertilizers, Murphy *et al.*, (2007) demonstrated that organic material such as compost or manure decomposes slowly in the soil and the continual release of nutrients can support the microbial biomass population for extended periods of time. Our findings are consistent with the findings of this investigation. In general, the amount and quality of organic material added to soils are the most important elements in influencing the number of various microbial groups and the activity of microorganisms involved in nutrient cycling (Diacono and Montemurro 2010).

Increased microbial abundance was observed with inorganic fertilization in all experimental sites Parwanipur, Pakhribas and Jumla. Kang *et al.*, (2005) showed an increase in bacterial population in response to chemical fertilizers, which might be attributable to the soil's improved nutritional status. Several studies have discovered that bacterial populations and abundance are relatively unresponsive to long-term inorganic fertilization (Wu *et al*. 2011). Long-term application of mineral fertilizers may enhance soil N and P content but reduce soil pH (Ge *et al*. 2010).The pH of the soil can have a big impact on microbial biomass. The decrease in microbial biomass can be detected with an increase or decrease in pH (Chen and He 2004).

At all three experimental sites, the bacterial population outnumbered the fungal population. When compared to the control soil, Parham *et al.* (2003) found that cattle dung application promoted the growth of bacteria but not fungus. This could be attributed in part to the restrictions of pH values in manure-treated soils, which were around 5.6, the highest of the treatments tested(Parham *et al.* 2003). Increased soil pH in the acidic range causes a shift in bacterial community dominance, whereas fungal communities are unaffected (Pennanen 2001).Variation in fungus species and populations might also be expected when the available substrate changes (Schinner and Gstraunthaler 1981).

Microbial populations were plentiful in the Pawanipur and Pakhribas experimental sites, but somewhat lower at the Jumla location. Climate features in high-elevation mountain environments is frequently accompanied with increased environmental stress, such as low air pressure and cold climate, adverse nutritional circumstances, and altered field vegetation, all of which can influence microbial communities and activities(Ma *et al.* 2004).However, research has discovered that soil bacteria may not have tight elevational limits as long as the environment supplies some organic matter and at least brief intervals of water. At an elevation range of 900-1900m a.s.l., Djukic *et al.*(2010) discovered no consistent elevational trend in entire microbial community size, whereas fungal biomass decreased with increasing elevation. Our results are consistent with these findings.

Given that long-term agricultural usage of inorganic fertilizers unavoidably reduces microbial activity, Ge *et al.* (2010) urge that a combination of organic manure and inorganic fertilizers be considered based on the balance of crop demand and soil availability of accessible nutrients. Manna *et al.* (2007) demonstrated that a NPK fertilizer, either alone or in combination with manure application, had a positive effect on crop yields in a cereal-based cropping system, and that the combination of NPK fertilizer and manure had a significant impact on soil fertility improvement.

V. CONCLUSIONS

Organic and inorganic treatments had varied effects on the rhizosphere microbial community. When compared to inorganic treatment, the administration of farmyard manure resulted in a more pronounced growth of the microbial population. Furthermore, across multiple study sites at varying elevations, farmyard manure exhibited a higher microbial population than inorganic fertilizer. Thus, farmyard manure and inorganic fertilizers can be used alone or in combination for microbial abundance enhancement.

ACKNOWLEDGMENT

We would like to express our sincere gratitude to the National Soil Science Research Centre, NARC, Lalitpur, Nepal for their technical and financial support to have this manuscript.

REFERENCES

[1]. Bending, GD. (2004). "Microbial and Biochemical Soil Quality Indicators and Their Potential for Differentiating Areas under Contrasting Agricultural Management Regimes." *Soil Biology and Biochemistry 36*(11): 1785–92.

[2]. Chen GC, and He Z . (2004). "Effects of PH on Microbial Biomass -C and -P in Red Soils." In *The Red Soils of China*, Dordrecht: Springer Netherlands, 307–14. http://link.springer.com/10.1007/978-1-4020-2138-1_22.

[3]. Diacono M, and Montemurro F. (2010). "Long-Term Effects of Organic Amendments on Soil Fertility. A Review." *Agronomy for Sustainable Development 30*(2): 401–22.

[4]. Djukic I, Zehetner F, Mentler A, and Gerzabek MH.(2010). "Microbial Community Composition and Activity in Different Alpine Vegetation Zones." *Soil Biology and*

Biochemistry *42*(2): 155–61. http://dx.doi.org/10.1016/j.soilbio.2009.10.006.
[5]. Ge, G. (2010). "Soil Biological Activity and Their Seasonal Variations in Response to Long-Term Application of Organic and Inorganic Fertilizers." *Plant and Soil 326*(1): 31–44.
[6]. Joergensen RG, Mäder P, and Fließbach A.(2010). "Long-Term Effects of Organic Farming on Fungal and Bacterial Residues in Relation to Microbial Energy Metabolism." *Biology and Fertility of Soils 46*(3): 303–7.
[7]. Kang GS, Beri V, Sidhu BS, and Rupela OP. (2005). "A New Index to Assess Soil Quality and Sustainability of Wheat-Based Cropping Systems." *Biology and Fertility of Soils 41*(6): 389–98.
[8]. Kirchner M J, Wollum AG, and King LD. (1993). "Soil Microbial Populations and Activities in Reduced Chemical Input Agroecosystems." *Soil Science Society of America Journal* 57(5): 1289–95. https://onlinelibrary.wiley.com/doi/10.2136/sssaj1993.03615995005700050021x.
[9]. Lipson DA. (2007). "Relationships between Temperature Responses and Bacterial Community Structure along Seasonal and Altitudinal Gradients." *FEMS Microbiology Ecology 59*(2): 418–27.
[10]. Liu S, Li P, Gan W, and Lu S. (2021). "Effect of Long- -Term Fertilization on Soil Microbial Activities and Metabolism in Paulownia Plantations." (July): 1–13.
[11]. Luo, P. (2015). "Influence of Long-Term Fertilization on Soil Microbial Biomass , Dehydrogenase Activity , and Bacterial and Fungal Community Structure in a Brown Soil of Northeast China." : 533–42.
[12]. Ma X, Chen T, Zhang G, and Wang R. (2004). "Microbial Community Structure along an Altitude Gradient in Three Different Localities." *Folia Microbiologica* *49(*2): 105–11. http://link.springer.com/10.1007/BF02931382.
[13]. Manna MC, Swarup A, Wanjari RH, and Ravankar HN. (2007). "Long-Term Effects of NPK Fertiliser and Manure on Soil Fertility and a Sorghum-Wheat Farming System." *Australian Journal of Experimental Agriculture 47*(6): 700–711.
[14]. Margesin R, Jud M., Tscherko D, and Schinner F. (2009). "Microbial Communities and Activities in Alpine and Subalpine Soils." *67*(2007): 208–18.
[15]. Marschner P, Kandeler E, and Marschner B. (2003). "Structure and Function of the Soil Microbial Community in a Long-Term Fertilizer Experiment." *Soil Biology and Biochemistry 35*(3): 453–61.
[16]. Mujiyati M, and Suriyadi S. (1970). "Effect of Manure and NPK to Increase Soil Bacterial Population of Azotobacter and Azospirillus in Chili (Capsicum Annum) Cultivation." *Nusantara Bioscience**1***(2): 59–64. https://smujo.id/nb/article/view/990.
[17]. Murphy DV. (2007). "Seasonal Dynamics of Carbon and Nitrogen Pools and Fluxes under Continuous Arable and Ley-Arable Rotations in a Temperate Environment." *European Journal of Soil Science 58*(6): 1410–24.
[18]. Niklińska M, and Klimek B. (2007). "Effect of Temperature on the Respiration Rate of Forest Soil Organic Layer along an Elevation Gradient in the Polish Carpathians." *Biology and Fertility of Soils 43*(5): 511–18.
[19]. Parham, JA. (2003). "Long-Term Cattle Manure Application in Soil. II. Effect on Soil Microbial Populations and Community Structure." *Biology and Fertility of Soils 38*(4): 209–15.
[20]. Pennanen T. (2001). "Microbial Communities in Boreal Coniferous Forest Humus Exposed to Heavy Metals and Changes in Soil PH - A Summary of the Use of Phospholipid Fatty Acids, Biolog® and 3H-Thymidine Incorporation Methods in Field Studies." *Geoderma 100*(1–2): 91–126.
[21]. Schinner F, and Gstraunthaler G. (1981). "Adaptation of Microbial Activities to the Environmental Conditions in Alpine Soils." *Oecologia 50*(1): 113–16.
[22]. Siles A, Cajthaml T, Minerbi S, and Margesin R. (2016). "Effect of Altitude and Season on Microbial Activity , Abundance and Community Structure in Alpine Forest Soils." (January): 1–12.
[23]. Singh, D. (2012). "A Hump-Backed Trend in Bacterial Diversity with Elevation on Mount Fuji, Japan." *Microbial Ecology 63*(2): 429–37.
[24]. Su, JQ. (2015). "Long-Term Balanced Fertilization Increases the Soil Microbial Functional Diversity in a Phosphorus-Limited Paddy Soil." *Molecular Ecology 24*(1): 136–50.
[25]. de Vries, Franciska T. (2006). "Fungal/Bacterial Ratios in Grasslands with Contrasting Nitrogen Management." *Soil Biology and Biochemistry 38*(8): 2092–2103.
[26]. Wang, JT. (2015). "Altitudinal Distribution Patterns of Soil Bacterial and Archaeal Communities Along Mt. Shegyla on the Tibetan Plateau." *Microbial Ecology 69*(1): 135–45.
[27]. Wu M, Qin H, Chen Z, and Wu J. (2011). "Effect of Long-Term Fertilization on Bacterial Composition in Rice Paddy Soil." : 397–405.
[28]. Xu, M. (2014). "European Journal of Soil Biology Soil Microbial Community Structure and Activity along a Montane Elevational Gradient on the Tibetan Plateau." *European Journal of Soil Biology 64*:6–14. http://dx.doi.org/10.1016/j.ejsobi.2014.06.002.
[29]. Zhang Y. (2019). "Effect of Different Fertilization Practices on Soil Microbial Community in a Wheat – Maize Rotation System."*Sustaiability 11*(15): 4088.