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THE EFFECTS OF FERTILIZER ON SOIL FOR TEA PRODUCTION

Deepshikha*
Department of Chemistry, M.M. College, Modinagar, Ghaziabad, UP

Abstract

Human being use fertilizers to increase crop production and soil quality and use chemicals to restrain pests and diseases. The long-term use of chemical and fertilizers was assessed on tea crop and rhizosphere soil properties in tea orchards. The heavy metals and bacterial composition were analysed by spectroscopy in rhizosphere soils. The results of using fertilizers showed decreased Cu, Pb, As and Cd contents in rhizosphere soil and tea leaves along with increased amount of the amino acids of tea, the pH of the soil and microorganisms to improve tea quality.

Keywords: Fertilizer, Soil quality, Rhizosphere soil, Metals, pH and Microorganism.

Introduction

The sustainability of agricultural systems is an important global issue. Therefore, it is essential to overview the effects of fertilizers on crop yield to achieve a balance between benefits and harms in modern agricultural practices. There are potential benefits of organic fertilizer applications over artificial ones. It was observed long-term application of chemical fertilizers led to serious soil acidification, nutritional imbalance, and deterioration of the rhizosphere micro-ecological environment; further increased the activity of heavy metal ions in soil. One of the important agricultural products in mountains is tea cultivation. *Camellia sinensis* is a common tea plant in mountains due to richness in antioxidants, vitamins and amino acids, the amino acids, tea polyphenols and caffeine are determine both taste and quality of tea. It was observed that the long-term production of tea bushes caused soil degradation, substantial quality and low yield became key problems in the sustainable development of tea orchards (Li *et al*., 2017). Due to the growing demand of tea leaves and limited land availability, farmers used nitrogen fertilizers to increase crop production. But, use of fertilizers led to undesirable effects lie decline in tea quality, soil acidification, heavy metals pollution, soil compaction and changes in soil microbiome (Gao, 2001; Lan and Xia, 2008). The long-term tea cultivation with nitrogen fertilizers altered the bacterial composition of soil and significantly decreased soil pH and microbial metabolic activity and reduced beneficial bacteria (Arafat *et al*., 2017; Li *et al*., 2016). There are potential benefits of organic fertilizers and reported raise in soil microbial activities and improved crop growth and restrained pests and diseases (Zhang and Wei, 2012; Chang *et al*., 2010). Soil has a large number of microbial species as well as other organisms that together form a highly complex ecosystem. Microorganisms are essential for nutrient recycling, healthy plant development and decomposition of organic matter (Ahmad *et al*., 2007). But, the environmental conditions and cultivation practices affect the microbiome and change soil characteristics (Wang *et al*., 2011). The production of tea with bio-organic fertilizers has superior colour and taste over to tea treated with chemical fertilizers (Lin *et al*., 2010; Zhang and Wei, 2012). The use of organic fertilizers showed higher seedling biomass and significantly improved the soil fungal to bacterial ratio as well as soil enzyme activity (Sun *et al*., 2017; Xu *et al*., 2010). The use of organic fertilizer could alleviate soil acidification, resulting in increased plant yields (Li *et al*., 2018). The long-term effects of fertilization practices on biological properties of soils focused mainly on tea plant yield and changes in soil nutrients (Owuor *et al*., 2011; Yang *et al*., 2012; Cheng *et al*., 2015). In the current study we used Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and High Throughput Sequencing Technology to determine the influence of organic and chemical fertilizers on bacterial composition and heavy metals in the rhizosphere of tea orchards.

Experimental Methods

Soil sampling

The samples of soil were collected from the rhizosphere orchards, which were treated with organic fertilizer or chemical fertilizer. Other samples from non-rhizosphere soils were collected from the organic and chemical fertilizer treated tea orchards. The rhizosphere and non-rhizosphere soils of tea trees were taken from each experimental plot by a 5-point sampling method. For each sample, three replicates were performed. Tea leaves from the OrgS and NorS treatment groups were sampled in the fields. After sieving (2 mm mesh) to remove stones and plant residues, soil samples were stored at -80°C.

*Corresponding author Email: apdeepshikha@gmail.com

Analysis of tea biochemical properties

The tea leaves were roasted, dried and ground into a fine powder testing. The amino acid content was measured using the ninhydrin colorimetric methods. Tea polyphenols and caffeine contents were determined using a Waters HPLC system (C18 column: Inertsil ODS-SP, 4.6 × 250 mm, 5 μm). The chromatographic conditions were as follows for tea polyphenols: mobile phase A: mixture solution (water: acetic acid: acetonitrile = 90:0.1:10, v/v/v); mobile phase B: acetonitrile; elution gradient: mobile phase B 0% (0 min)→0% (10 min)→10% (20 min)→0% (25 min)→0% (30 min); oven temperature: 40°C; detection wavelength: 280 nm; velocity: 1 mL/min. For caffeine: mobile phase A: water; mobile phase B: methanol; elution gradient: mobile phase B 65% (0 min)→65% (35 min); detection wavelength: 275 nm; velocity: 1 mL/min.

Analysis of soil chemical properties

Soil chemical properties analysis included pH, total nitrogen (TN), total phosphorus (TP), total potassium (TK), available nitrogen (AN), available phosphorus (AP), and available potassium (AK). Soil pH was determined using a glass electrode pH meter (1:2.5 soils to water suspensions). TN, TP, and TK were determined using Kjeldahl digestion, sodium carbonate fusion and NaOH melts flamer methods, respectively (Bao, 2000). AN was determined using the alkaline hydrolyzable method. AP was extracted with hydrochloric acid and ammonium fluoride, and contents were measured using the molybdenum blue method. AK was extracted with ammonium acetate and measured by flame photometry (Pansu and Gautheyrou, 2006).

Analysis of heavy soil metal

The microwave digestion system (Milestone ETHOS UP, Italy) was used to extract cuprum (Cu), plumbum (Pb), cadmium (Cd) and arsenic (As) from soil samples. The contents of these metals were determined by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer NexION 300X, U.S.A), using parameters listed in Table 1.

Genomic DNA purification and PCR amplification

Total soil DNA was extracted using the BioFast soil Genomic DNA Extraction kit (BioFlux, Hangzhou, China), following the manufacturer's instructions. For each soil sample, three independent DNA extractions were performed. DNA was diluted to a concentration of 1 ng/μL in sterile water. The variable regions 3 to 4 (V3–V4) were amplified with the specific primers 338F/806R (338F, 5'-ACTCCTACGGGAGGCAGCA-3'; 806R, 5'-GGACTACHVGGGTWTCTA AT-3'). The PCR reactions were conducted in a 50 μL mixture system, using TransStartFastpfu DNA Polymerase (TransGen Biotechnology, Beijing, U.S.A.). The PCR condition was initiated denaturation with 5 min at 95°C, followed by 35 cycles of 40s at 95°C, 40s at 58°C, 60s at 72°C and final elongation with 5 min at 72°C. PCR products were purified using the Qiagen Gel Extraction Kit (Qiagen, Germany) and subjected to sequencing on the Illumina HiSeq 2500 platform (Allwegene Technologies Co., Ltd., Beijing, China).

Statistical analysis

The FLASH method (Magoc and Salzberg, 2011) was used to merge paired-end reads. Following quality filtering and chimera removal (Edgar *et al*., 2011), the effective tags were used to perform OTU clustering and species annotation. Species annotation was performed using the Silva database (http://www.arb-silva.de) (Quast *et al*., 2013). For each remaining sequences, the RDP classifier (Version 2.2) algorithm (Wang *et al*., 2007) was used to annotate taxonomic information via the GreenGen database (DeSantis *et al*., 2006). Mothur version 1.31.2 (Schloss *et al*., 2009) was used to analyze the alpha diversities. Beta diversities were then calculated to analyze the complexity of species diversity. The Statistical Package for the Graph Pad Prism version 5.1 and the Data Processing System (DPS) version 7.05 were used for statistical analysis. Differences among the treatments were calculated and statistically analyzed using the analysis of variance (ANOVA) and the LSD multiple range tests ($p < 0.05$).

Results and Discussion

Tea and soil chemical characteristics

In this study, it was found that the use of organic fertilizer significantly increased the amino acids content of tea (Fig 1). The contents of polyphenol did not reach statistical significance in tea samples from fields treated with organic fertilizer compared to those treated with chemical fertilizer. In contrast, tea under long-term treatment with chemical fertilizer showed significantly higher contents of caffeine compared to tea with organic fertilizer. Columns with different letters are statistically different (LSD test, $P< 0.05$). Table 2 summarized the chemical properties of soil from tea orchards treated with either organic or chemical fertilizers. Contents of total nitrogen, total potassium, available nitrogen, available phosphorus and available potassium were similar between the two treatment groups ($P > 0.05$). However, soil pH level was significantly higher in the organic fertilizer treatment group compared to the chemical fertilizer treatment group (Table 2). OrgS and NorS refer to rhizosphere soils of organic fertilizer and chemical fertilizer treatments, respectively. CKOrgS and CKNorS refer to non-rhizosphere soils. Columns with different letters are statistically different (LSD test, $P < 0.05$).

Tea orchards with long-term organic or chemical fertilizer treatment showed significant differences in soil

chemical properties (Fig 2). Treatment with organic fertilizer resulted in significantly lower contents ($P < 0.05$) of cuprum (Cu), plumbum (Pb) and cadmium (Cd) in rhizosphere soils compared to the chemical fertilizer treatment group. A small decrease in arsenic (As) level was also detected in the organic fertilizer treatment group, but the difference was not statistically significant. Similar trends were observed in non-rhizosphere soil samples. Our results also showed that treatment with organic fertilizer significantly decreased contents of Cd, Pb and As in tea leaves (Fig 3). Columns with different letters are statistically different (LSD test, $P < 0.05$).

Alpha diversity indices of microbial community

A total of 544,096 effective clean tags with bacterial species annotation were obtained from 12 soil samples. Alpha diversity was calculated to determine the complexity of species diversity. We observed a significantly higher bacterial composition and Chao1 indices with samples from the organic fertilizer treatment group compared to samples from the chemical fertilizer treatment group. Long-term organic fertilizer treatment also had a positive effect on non-rhizosphere soil. Our results showed that chemical fertilizer significantly increased Shannon's diversity indices in rhizosphere soil in comparison to all treatments (Fig 4).

Beta diversity indices of microbial composition

We used weighted unifrac heat map, hierarchical clustering, and principal component analysis to identify differences in bacterial composition structure between the treatment groups (Fig 5). In comparison to CKNorS, higher distances were observed among the OrgS, CKOrgS and NorS samples. The PC1 and PC2 components of PCoA accounted for 45.93% and 26.65% of the total bacterial composition variations, respectively. We found that the bacterial composition of OrgS and CKOrgS soil samples belonged to the same group based on the principal component analysis. In contrast, the bacterial composition of NorS and CKNorS samples fell into two separate groups that were distinct from OrgS and CKOrgS samples.

Shifts in soil bacterial composition structure

In this study, the classified sequences were affiliated with 24 bacterial phyla among the treatment groups. The majority of the phyla were assigned to Actinobacteria, Chloroflexi, Proteobacteria, Acidobacteria, Gemmatimonadetes and Cyanobacteria (S1 Fig). Meanwhile, clear trends in variation at the phylum level were observed between the organic fertilizer and chemical fertilizer treatment groups. The number of OTUs exclusively found in OrgS and NorS samples were 78 (4.59%) and 88 (5.18%), respectively. The shared number of exclusive OTUs between OrgS and NorS were 1022 (60.19%). The shared number between OrgS and CKOrgS were 1109 (65.31%) and they dropped to 696 (41.10%) between NorS and CKNorS (Fig 6).

The relative abundance of these bacterial orders varied among the different soil samples. A comparison between OrgS and NorS showed that organic fertilizer treatment resulted in a significant increase in the relative abundance of Burkholderiales, Myxococcales, Streptomycetales, Nitrospirales, Ktedonobacterales, Acidobacteriales, Gemmatimonadales, Solibacterales and a decrease in Pseudonocardiales, Frankiales, Rhizobiales, Xanthomonadales (S1 Table and Fig 7). Heat map analysis of the top 20 most abundant genera within the hierarchical cluster showed clear variations in bacterial composition structure across the four groups of soil samples and these differences were statistically significant. Treatment with chemical fertilizer resulted in increased abundance of Acidothermus, Acidicaldus, Acidobacterium and decreased abundance of the potentially beneficial Nitrospira and Burkholderia in comparison with the organic fertilizer treatment group. No significant differences were detected in comparisons between OrgS and CKOrgS groups, as well as between NorS and CKNorS groups (Fig 8).

Effects of soil chemical properties on dominant genera

Redundancy analysis (RDA) was performed to study the relationship between soil chemical properties and abundance of dominant genera. The first two RDA components (RDA1 and RDA2) separated the organic fertilizer treated soils from the chemical fertilizer treated soils (Fig 9). The chemical fertilizer treated samples (NorS) were positively related to the cadmium (Cd), Cuprum (Cu) and plumbum (Pb).

The organic fertilizer treated samples (OrgS and CKOrgS) were positively related to a higher relative abundance of Catenulispora, Candidatus_Solibacter, Burkholderia-Paraburkholderia, Gemmatirosa, Nitrospira, Rhizomicrobium and negatively related to Acidobacterium, Acidothermus and Acidicaldus. Strong associations were found among total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), available nitrogen (AN), cadmium (Cd), Cuprum (Cu) and plumbum (Pb) with the abundance of Acidothermus, Acidobacterium and Acidicaldus. The abundance of Acidibacter, Catenulispora, Burkholderia-Paraburkholderia, Gemmatirosa, Nitrospira, Candidatus_Solibacter, Rhizomicrobium and Sorangium were found to be highly associated with soil pH.

Conclusion

It is concluded that long-term application of organic fertilizer treatment improved the rhizosphere environment in tea orchards and tea quality and decreased the level of heavy metals in rhizosphere soil. Moreover, soil pH and shift in microbiomes were related to fertilizers treatments. The organic fertilizer could shape microbial composition and recruit beneficial bacteria into the rhizosphere of tea. Further study can provide a promising strategy to tea orchards by treatment with organic fertilizers.

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Table 1: Parameters for the ICP-MS

As	Cd	Pb	Cu	Parameters
Radio -frequency power		1300 w		
Plasma velocity		13.00 L/min		
Auxiliary airflow velocity		1.40 L/min		
Flow rate of the carrier gas		0.95 L/min		
Mass-to-charge ratio (m/z)	63	208	111	75
Atomization device		MCN		
Atomizer chamber				

Table 2: Chemical properties of soils from tea orchards with different treatments

Soil chemical properties	CKOrgS	CKNorS	OrgS	NorS
Total nitrogen (TN) (g/kg)	1.06b	1.38ab	1.59ab	1.82a
Total phosphorus (TP) (g/kg)	0.27b	0.22b	0.36b	1.60a
Total potassium (TK) (g/kg)	6.16a	4.73a	6.42a	5.47a
Available nitrogen (AN) (g/kg)	0.08b	0.13ab	0.16ab	0.18a
Available phosphorus (AP) (g/kg)	0.01b	0.01b	0.03ab	0.08a
Available potassium (AK) (g/kg)	0.11a	0.10a	0.13a	0.13a
pH	4.24a	4.10ab	4.19a	4.00b

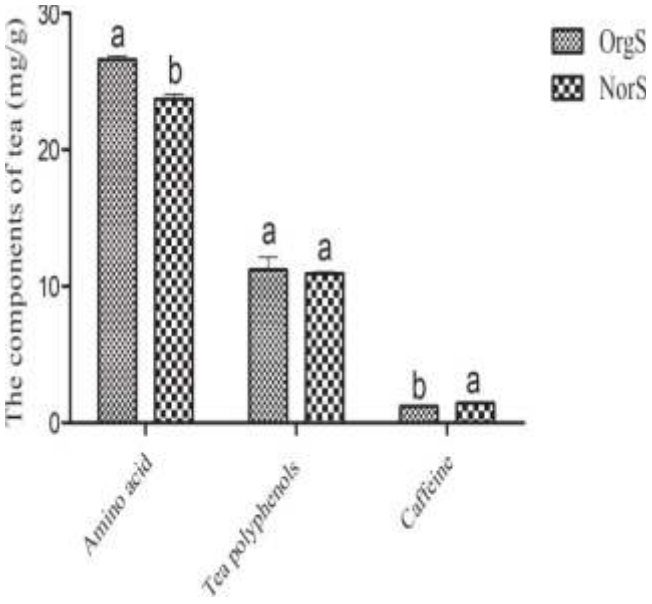

Fig. 1: Amino acids, polyphenols, and caffeine contents of tea under treatment with organic (OrgS) or chemical (NorS) fertilizer

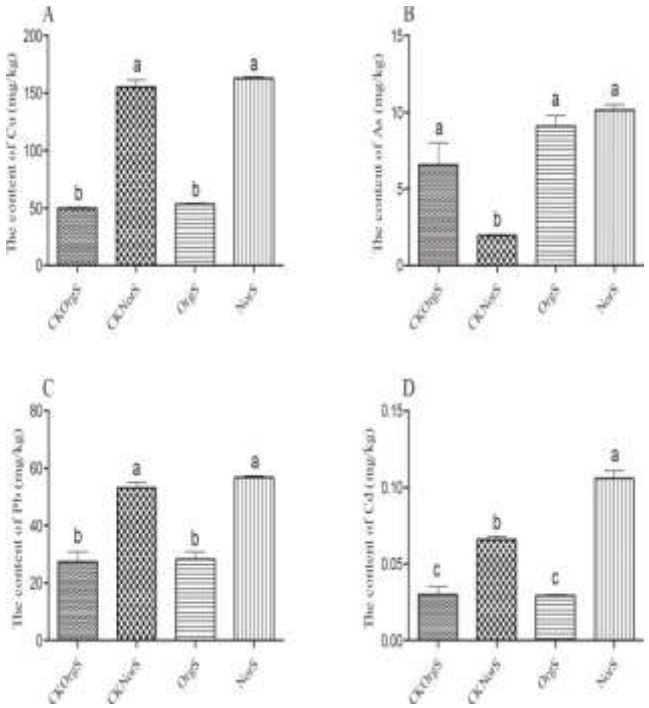

Fig. 2: Heavy metals content in non-rhizosphere and rhizosphere soil samples from tea orchards under organic or chemical fertilizer treatment. Columns with different letters are statistically different (LSD test, P< 0.05)

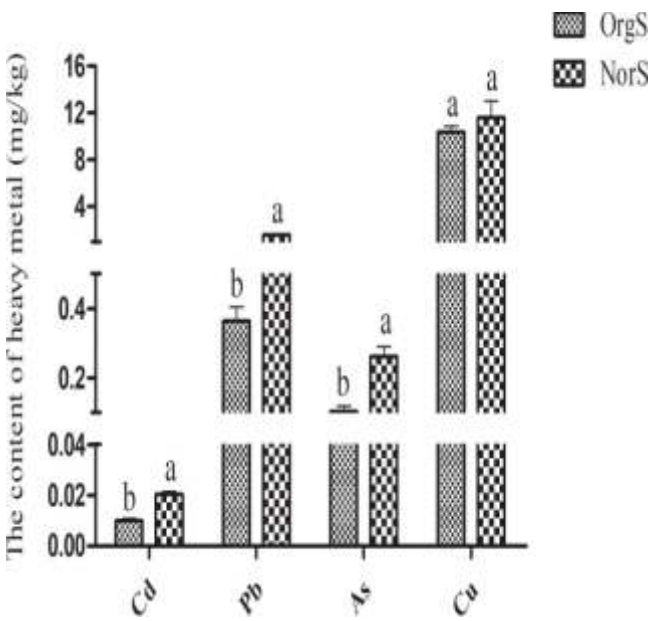

Fig. 3: Heavy metals content in tea leaf samples from tea orchards under organic or chemical fertilizer treatment. Columns with different letters are statistically different (LSD test, P< 0.05)

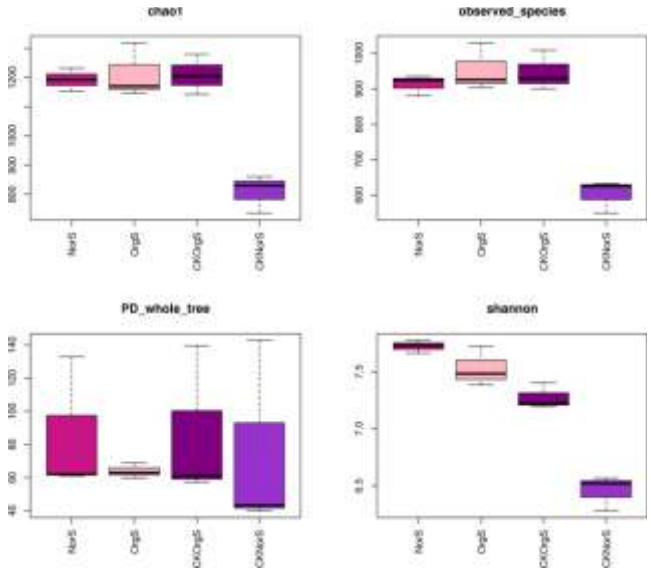

Fig. 4: Observed richness, OTUs and diversity of soil samples from organic and chemical fertilizer treatment groups

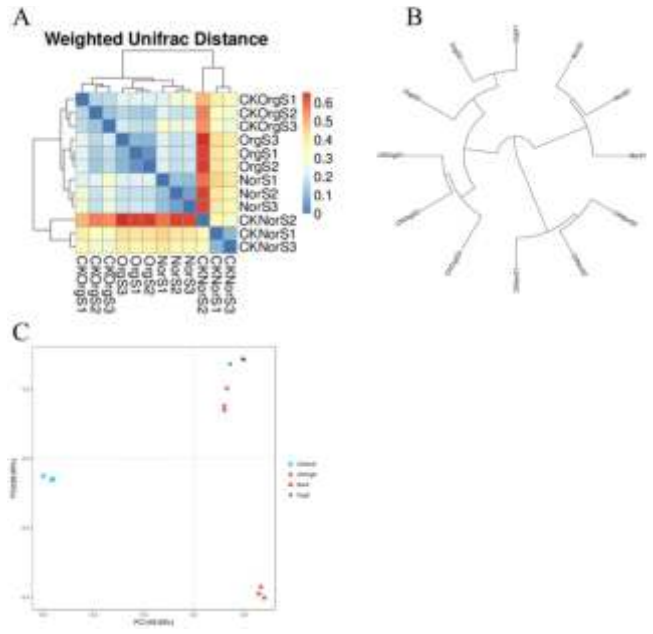

Fig. 5: Beta diversity analysis of microbial composition (A) Weighted unifrac heatmap; (B) Hierarchical clustering analysis; (C) Principal Component Analysis

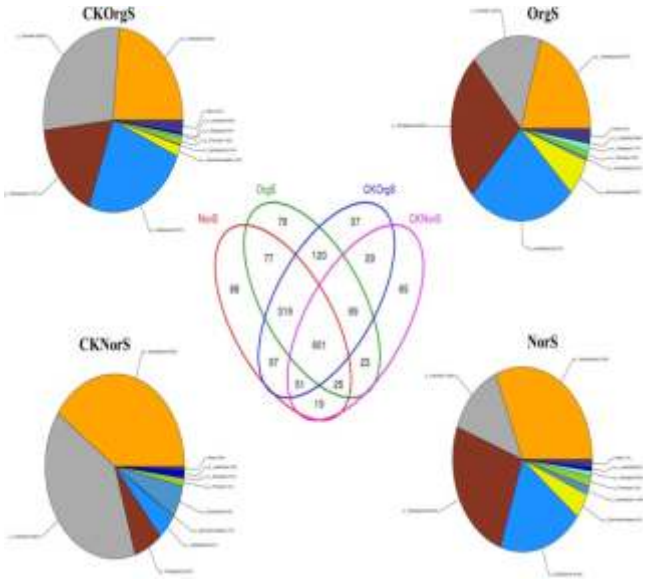

Fig. 6: Venn diagram and relative percentages of bacterial phyla in the four different soil samples

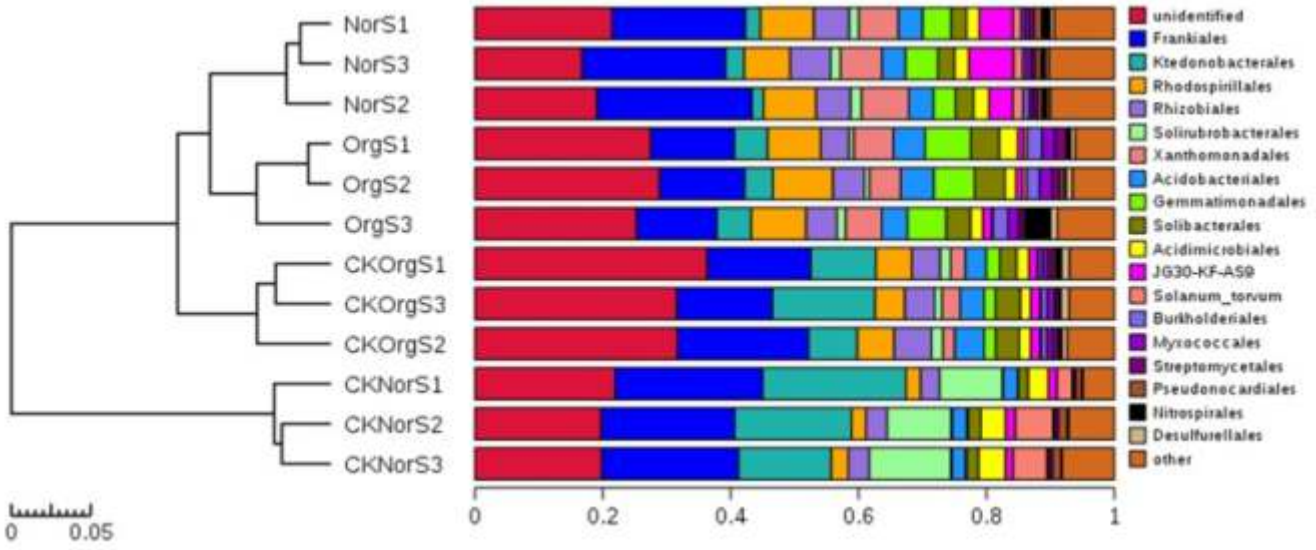

Fig. 7: Relative abundance of the top 20 bacterial orders in the four different soil samples

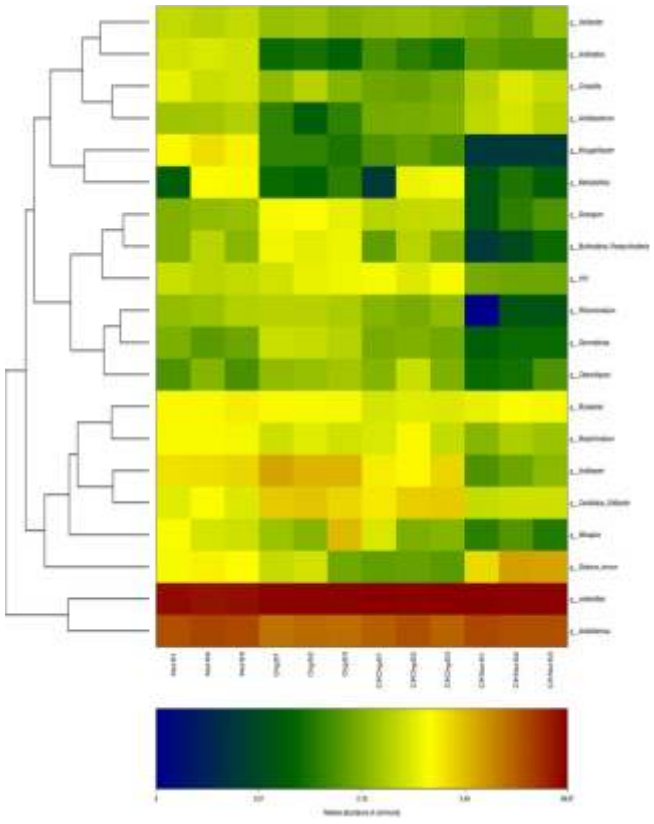

Fig. 8: Heat map analysis of the top 20 bacterial orders in the four different soil samples

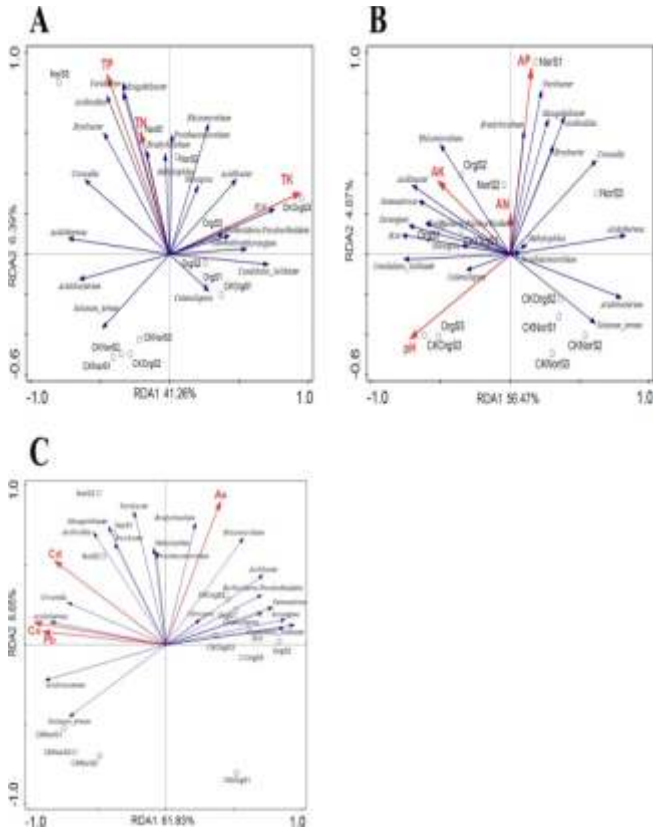

Fig. 9: Redundancy analysis (RDA) of the correlation between the most abundant genera of bacteria and soil physiochemical properties