EFFECTS OF LONG-TERM CROPPING OF TIBETAN BARLEY (*KUNLUN 14*) ON SOIL MICROBIAL DIVERSITY, ENZYMATIC ACTIVITIES, AND CROP PHENOTYPIC QUALITY

ZHAO, Y.[1,2#] – YAO, Y. H.[1,3,4,5#] – YAO, X. H.[1,3,4,5] – WU, K. L.[1,3,4,5*]

[1]*Qinghai University, Xining 810016, China*

[2]*College of Eco-Environmental Engineering, Qinghai University, Xining 810016, China*

[3]*Qinghai Academy of Agricultural and Forestry Science, No. 253 Ningda Road, Xining 810016, China*

[4]*Qinghai Key Laboratory of Hulless Barley Genetics and Breeding, Xining 810016, China*

[5]*Qinghai Subcenter of National Hulless Barley Improvement, Xining 810016, China*

[#]*Equal first authors*

*Corresponding author
e-mail: wklqaaf@sina.com, kunlunwu@yahoo.com

Abstract. There is limited information presenting the effect of long-term continuous cropping of Tibetan barley. The current study applied integrated high throughput sequencing and bioinformatics to determine the effect of long-term continuous cropping of Tibetan barley on the soil microbial, enzymatic characteristics and the plant agronomic traits. Results indicated that *Arthrobacter*, *Sphingomonas*, *Gaiella*, *Bacillus*, *Massilia*, *Knoellia*, *Mycobacterium* and *Pedobacter* were the dominant genera. The significant increase of these genera under increased cropping years was an indication of the constant depleted quality of the soil, promoting the survival of these bacterial genera generally associated with bioremediation of degraded soils. The microbial carbon and nitrogen biomass were significantly altered between the difference cropping years. There were statistically significant differences in the enzymatic activities among the cropping years (P < 0.05). Both the soil acid phosphatase enzymatic activity (10 µmol/day/gram) and sucrase enzymatic activity (80 µg/day/gram) were both highest after ten years of continuous cropping. The soil urease enzymatic activity was highest (750 µg/day/gram) at five years of continuous cropping while the soil polyphenol oxidase enzymatic activity was highest (440 µg/day/gram) at the control treatment. Moreover, there was a steady decline in all the agronomic traits (tillering number, number of kernels per ear, grain weight per plant, weight of a thousand seeds per plant, number of per plant and main ear length per plant) with increase in continuous cropping years, indicating the detrimental impact of long-term continuous cropping on highland barley phenotypic characteristics and yield quantity.
Keywords: *continuous cropping, crop quality, microbial community, nutrient deficiency, soil microbiology*

Introduction

Continuous cropping is an agricultural practice involving long-term cultivation of the same or allied plant species in the same soil or piece of land over a long period of continuous planting seasons (Krupenikov et al., 2011). Over the years, the common harmful effects of continuous cropping include nutrient deficiency in soil, emergence of plant diseases and insect pests and reduced organic matter contents. Measures such as crop rotation, application of organic fertilizers or use of microbial technologies have been recommended as potential solutions to these impacts (Rigon and Calonego, 2020). This has prompted most farmers to undertake rotational farming as a means of improving soil

quality and yield. Moreover, several related diseases may become aggravated with declined yield and quality under such continuous cultivation and management conditions (Gao et al., 2006). As an alternative, several studies have recommended the change of physicochemical properties of the soil, soil nutrient content, soil microbial community structure, soil enzyme activity, plant agronomic traits and physiological and biochemical indexes through rotational farming (Monaci et al., 2017). In some circumstances, continuous cropping systems under no-till (NT) systems has also been recognized as an important alternative to crop-fallow system. Intensified cropping systems have shown greater benefits than the crop-fallow systems with such benefits ranging from soil and water conservation, improved soil properties, increased soil organic carbon concentration (SOC) concentration and improved crop production (Tang et al., 2014). On the other hand, diverse crop rotations and continuous cropping systems has been confirmed to return more above- and below-ground biomass to soil than the continuous cropping systems with extended fallow periods. Additionally, annual return of crop residues under the NT systems has shown high potential in protecting the soil surface from water and wind erosion, reduce water evaporation, increase soil macro-aggregation, and enhance accumulation of carbon (Tang et al., 2014).

Recently, studies have pointed out that continuous cropping can be sustained for decades but requires careful management for good soil nutrient conditions and microbial ecology. Indeed, the changes in bacterial community diversity under continuous cropping of different vegetables have been shown to lead to the disorder of the functions of bacterial community, resulting to disrupted ecological balance in the soil and consequently lowering the crop quality (Lyu et al., 2020). For example, breaking continuous potato cropping with legumes improves the soil microbial communities, enzyme activities and tuber yield through phytoremediation. Indeed, phytoremediation of organic contaminants in the soil has majorly been related to the action of multiple enzymes in the plant rhizosphere soil (Liu et al., 2015). Despite all these underlying effects of continuous cropping on both the soil and quality of yield parameters, there is no sufficient information on the effects of long term (up to ten years) continuous cropping of Tibetan barley on the soil microbial and enzymatic dynamics as well as the crop phenotypic characteristic. Therefore, the goal of this study was to experimentally determine the effects of long-term continuous cropping of the Tibetan barley on soil microbial and enzymatic properties and the plant's phenotypic traits. The study was undertaken through integrated high throughput sequencing and bioinformatics as well as soil enzymatic activities and yield quantities measurements.

Materials and methods

Site description

The study was undertaken on an experimental field in Qinghai province, China located at 37°21' N and 101°43' E, within an altitude of 2,987 m. It has Qinghai-Tibet plateau climatic features with a cold, warm, and humid climate with annual sunshine hours range of between 2260 and 2740 h. The annual average temperature is 8.0 °C with a daily temperature range of 11.6–17.5 °C. The annual rainfall is between 530 and 560 mm, annual evaporation of between 1130 and 1340 mm (Zhang et al., 2000). The regional soil type is Kastanozems based on the FAO classification (Yan et al., 2018).

Experimental design and sample collection

The experiment was set up in the experimental field in Qinghai province from August 10th the year 2020. The experimental farmland was a fallow plot covered with green grass for period of more than 5 years prior to the start of each experimental treatment and control. The treatments and control (Non cropping soil) were designed in quadruplicate measuring 5 m by 5 m, and marked as indicated in *Table 1*. The study involved one genotype of Tibetan barley (*Kunlun 14*). Each treatment field was subjected to conventional management and the soil and plant sampling was done during the flowering period of the Tibetan barley at Zadoks growth scale of 69. The conventional management involved planting at 15 cm by 15 cm for row spacing, periodic mechanical weeding and irrigation whenever it was necessary, and application of diammonium phosphate fertilizer at a rate of 7.5 kg per acre annually, which continued for the entire experimental period.

Table 1. Experimental arrangement design

Treatments	Plots per treatment			
Control	CK-1	CK-2	CK-3	CK-4
2 years treatment	CC2-1	CC2-2	CC2-3	CC2-4
5 years treatment	CC5-1	CC5-2	CC5-3	CC5-4
10 years treatment	CC10-1	CC10-2	CC10-3	CC10-4

The soil was sampled in triplicate from the root area at 22.5 cm depth where the root zone was evenly distributed, and five plants were randomly sampled from each quadruplicate plots, giving a total of 80 plant samples. The soil was fully mixed, loaded into a self-sealed bag, put into the ice box, quickly brought back to the laboratory under -80 °C refrigerator and ferried to the laboratory for further analysis.

DNA extraction and PCR amplification and bio-information analysis process

The isolation of DNA followed the protocol presented in Han et al. (2017). The paired end (PE) reads obtained by Miseq sequencing were first stitched according to the overlap relationship, and the sequence quality was controlled and filtered. The samples were distinguished and OTU clustering and species taxonomy analysis carried out. These included the Diversity Index Analysis, based on the OTU clustering results. The detection of sequencing depth, based on taxonomic information was carried out at various classification levels of the statistical division of the community structure analysis. On the basis of the above analysis, a series of in-depth statistical and visual analysis was undertaken on the multi-sample community composition and system development information, such as multi-analysis and difference differentiation test.

Taxonomic analysis and species composition and variance analysis

The OTU Taxonomics Comprehensive Information Table, which combines the OTU analysis results with the taxonomic information, and the rank-abundance curve was used to analyze the abundance and uniformity of species OTU. This involved Pan/Core Species Analysis, Alpha Diversity Analysis and Dilution curve analysis as described in Willis (2019) and Costa et al. (2020). To study the diversity of microorganisms in the

environment, the richness and diversity of the microbiome was calculated using the diversity within the individual samples. This was achieved through the species Venn chart analysis and community composition analysis as described in Lam et al. (2016). The Circos sample and species diagrams with visual circles were used to provide description on the correspondence between samples and species, to reflect the proportion of the dominant species composition of each (or group) sample, and the different samples of each dominant species were used to determine the relationship between samples and species (Krzywinski et al., 2009). Species variance analysis was applied to detect differences in abundance between different groups (or samples) of the microbiome through determination of the difference significant test between groups and through LEfSe multi-level species differential analysis.

Sample comparison and functional predictive analysis

The alpha diversity of the microbial communities present in the soil samples under different treatments was determined through the use of Ace, Chao, Shannon and Simpson indices as described in Chernov et al. (2015). Beta Diversity Analysis and Sample grouping analyses were accomplished as described in Legendre and Condit (2019). The beta diversity analysis was undertaken through sample hierarchical clustering. The Hierarchical clustering of distance matrices was done to determine how close the sample branches were. This was undertaken through the Partial least square's discriminant analysis (PLS-DA). Using the COG libraries, the functional predictive analysis was undertaken through PICRUSt to show the effect of the 16S marker gene in the genome of the species using. The cluster of orthologous groups (COG) was determined as described in Tatusov et al. (2000) and were constructed by applying the criterion of consistency of genome-specific best hits to the results of an exhaustive comparison of all protein sequences from these genomes.

Soil enzymatic activity and agronomic traits

The level of soil acid phosphatase (S-ACP), soil urease (S-UE), soil sucrase (S-SC) and soil polyphenol oxidase (S-PPO) enzymatic activities were determined in this study as these are the major enzymes having been strongly associated with continuous cropping and soil quality (Liu et al., 2015). The analysis for the soil enzymatic activities were conducted in quadruplicate for all the soil sample. Determination of agronomic traits involved the measurement of the Tilling number, number of kernels per ear, grain weight per plant, thousand seed weight per plant, number of spikelets per plant and main ear length per plant. The soil microbial biomass carbon and soil microbial biomass nitrogen were determined under each treatment following the procedures outlined in Makarov et al. (2016) on substrate-induced respiration method. Briefly, Moist soil containing 10 ± 40 g oven-dry soil, were amended with a series of glucose concentrations (0.5, 1, 2, 4, 6 and 10 mg gÿ1 o.d. soil) in solution (to adjust soil moisture to 120% WHC). The soils were then incubated at 258 °C with shaking at 150 rev min per minute after adding liquid glucose, and CO_2 evolution was determined over after the addition of glucose.

Statistical data analysis

Data was analyzed using SPSS, QIIME and R software. Mean differences were calculated at a p value of 0.05. Significant differences among the means were

determined using the LSD test. T-tests and Metastats (http://metastats.cbcb.umd.edu/) in Mothur were used to compare the differences, and all p-values were adjusted with the false discovery rate (FDR) using the Benjamini-Hochberg (BH) method with the mt.rawp2adjp function in R.

Results

Classification and taxonomic status identification

More than 400 000 valid reads were generated with an average mean length of 400 bp after MiSeq Illumina sequencing. The sequences were distributed among 24,417 OTU and 97% identity applied as the cutoff. Taxonomic composition analysis of the OTU's were generated and an abundance distribution compiled through community bar plot analysis for the top 10 taxonomies for each treatment. Based on that, the top 10 dominant phyla were Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, Bacteriodetes, Gemmatimonadetes, Firmicutes, Verrucomicrobia, Nitrospira, and Planctomycetes (*Fig. 1a*). The dominant genera representatives from the dominant phyla were; *Arthrobacter* (phylum actinobacteriota), *Sphingomonas* (phylum proteobacteria), *Gaiella* (phylum actinobacteriota), *Bacillus* (phylum firmicutes), *Massilia* (phylum Proteobacteria), *Knoellia* (phylum actinobacteriota), *Mycobacterium* (phylum actinobacteriota) and *Pedobacter* (phymum bacteroidota). The dominance of these genera had significant difference across the cropping years ($P < 0.050$), and their numbers increased with increase in the cropping years. Through Kruskal-Wallis H test for genus variation between cropping years, there was significant statistical difference ($p < 0.05$). Through Venn analysis, the highest number of species (1311) occurred at five years of continuous cropping, followed by 1304 at ten years of continuous cropping. The similarity was highest in species between five and ten years of continuous cropping and both had biggest difference with species under the control (CK) treatment (*Fig. 1b*), indicating the uniqueness and shift in the soil microbial characteristics across the cropping years.
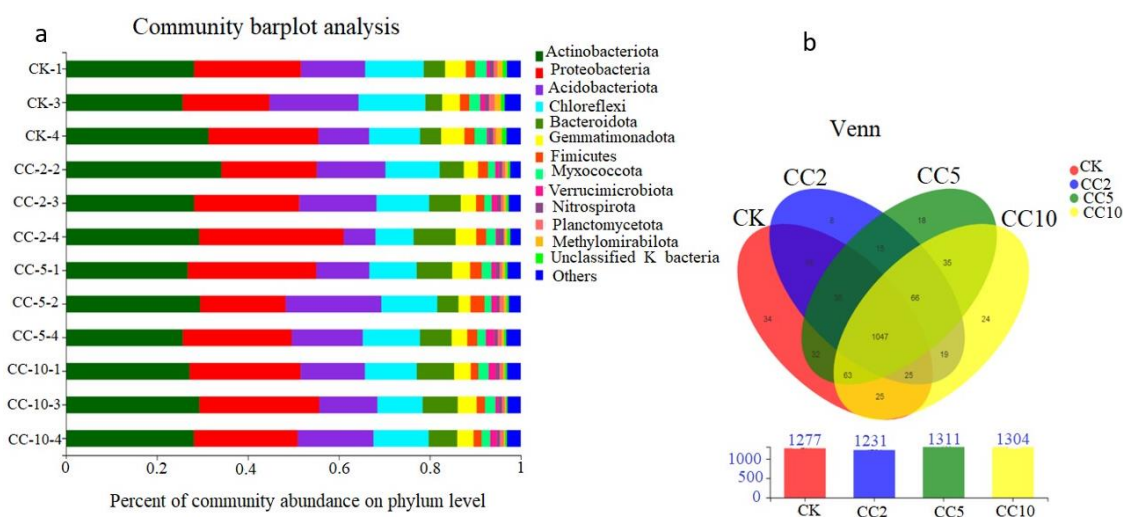
***Figure 1.** Species distribution and relationship through community bar plot (a) and Venn analyses (b)*

Microbial taxonomic composition

Based on the alpha diversity analysis through the use of Ace, Chao, Shannon and Simpson indices, there was statical differences in species abundance across the treatment years P < 0.05) (*Fig. 2*). This also indicated possible uniqueness and difference in microbial characteristics across the cropping years.
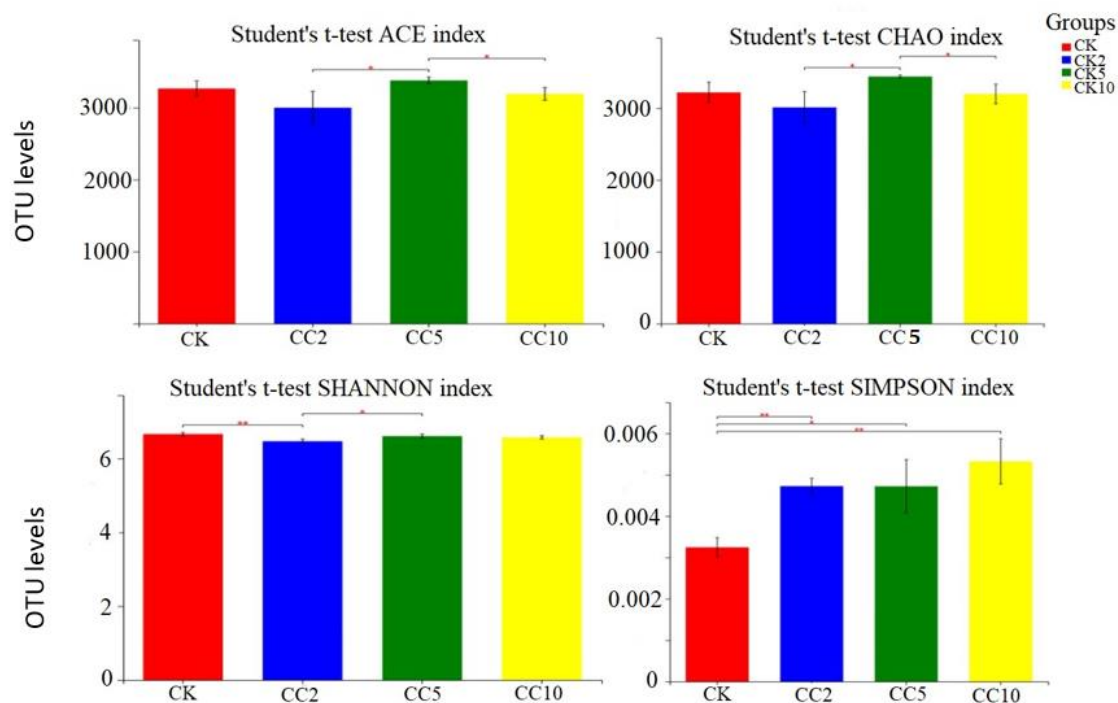
Figure 2. *Microbial community diversity between treatments based on Ace, Chao, Shannon and Simpson indices. Error bars indicate standard error of the means and asterisks indicates bars with no statistical differences*

Sample similarities and function classification

The species relationship between treatment groups through Partial Least Square Discriminant Analysis (PLS-DA) is provided in *Figure 3*. Through PLS-DA analysis at species level, it was noted that the control treatment had the least co-relation with the other treatments. This equally indicated possible uniqueness and differences in microbial characteristics between the non-cropped and continuously cropped soils. The Clusters of Orthologous Groups (COG) classification is provided in *Figure 4* and indicated that the functional characteristics of communities across the treatments were majorly on energy production and conversion, amino acid transport and metabolism, general function prediction and signal transduction mechanism.

Microbial biomass content, enzyme activities and agronomic traits within and across the cropping systems

There was statistically significant difference in the microbial biomass carbon content between the control, two years continuous cropping and the five years continuous

cropping. However, there was no statistically significant difference in the microbial biomass carbon content between the control and the ten years continuous cropping. In contrary, there was statistically significant difference in the microbial biomass nitrogen content between the control and the two years continuous cropping, but no statistically significant difference in the microbial biomass nitrogen content between the control, five years and ten years continuous cropping (*Fig. 5*). Additionally, results on soil enzymatic activities indicated a steady increase in both the soil acid phosphatase enzymatic activity (S-ACP) and soil sucrase enzymatic activity (S-SC) with the increase in continuous cropping years (CK to CC10). Furthermore, the highest value of soil urease enzymatic activity (S-UE) was at the five years continuous cropping, while the soil polyphenol oxidase enzymatic activity (S-PPO) was highest at the control treatment. All the values for the reported enzymatic activities had statistically significant difference between the treatments (*Fig. 6*). Moreover, there was a steady decline in all the agronomic traits (Tillering number, number of kennels per ear, grain weight per plant, thousand seed weight, number of spikelets and the main ear length with increase in continuous cropping years. All the agronomic traits had general decline with increase in continuous cropping years of the Tibetan barley (*Fig. 7*).
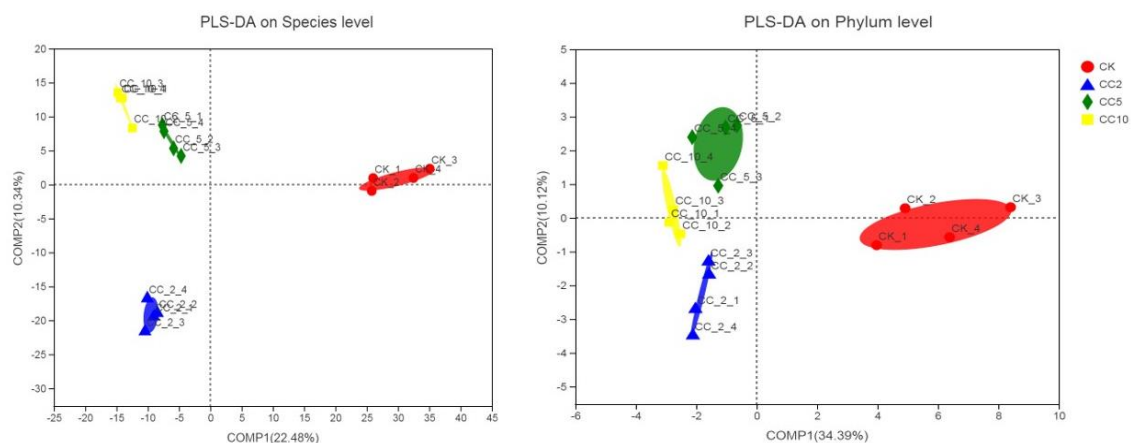
Figure 3. *Relationship in species between treatment groups through Partial Least Square Discriminant Analysis*

Discussion

Continuous cropping remains one of the most debatable farming practices in regards to its impact on soil and plant quality (Wienhold et al., 2006). From the top ten phyla, Actinobacteria, Proteobacteria, Acidobacteria and Chloroflexi were the most dominant. Indeed, previous studies have shown that actinobacteria majorly occurs in the soil and plays significant role in the decomposition of the organic matter of dead organisms so that the molecules can be taken up anew by plants. The proteobacteria include a wide variety of pathogens, while others are free-living, and include many of the bacteria responsible for nitrogen fixation (Yang, et al., 2020).

From previous studies, most of the dominant genera; *Sphingomonas, Gaiella, Bacillus, Massilia, Knoellia, Mycobacterium* and *Pedobacter* from the top 10 dominant phylum have been found to enhance the process of nitrogen fixation while others like the *Sphingomonas* are specifically common in degraded soils (Walsh et al., 2019). *Bacillus* spp. Have been recorded to serve multiple ecological functions in soil

ecosystem from nutrient cycling to conferring stress tolerance to plants. Members of the genus *Bacillus* are known to have multiple beneficial traits which help the plants directly or indirectly through acquisition of nutrients, overall improvement in growth by production of phytohormones, protection from pathogens and other abiotic stressors. Studies have also shown that *Massilia* colonized and proliferated on the seed coat, radicle, roots, and also on hyphae of phytopathogenic *Pythium aphanidermatum* infecting crops (Ofek et al., 2012). Moreover, *Mycobacterium* has been associated with acidic soils (Walsh et al., 2019). Members of the *Sphingomonas* and *Pedobacter* genera are often isolated from contaminated soils due to their unique abilities to support in situ bioremediation (Zhou et al., 2016).
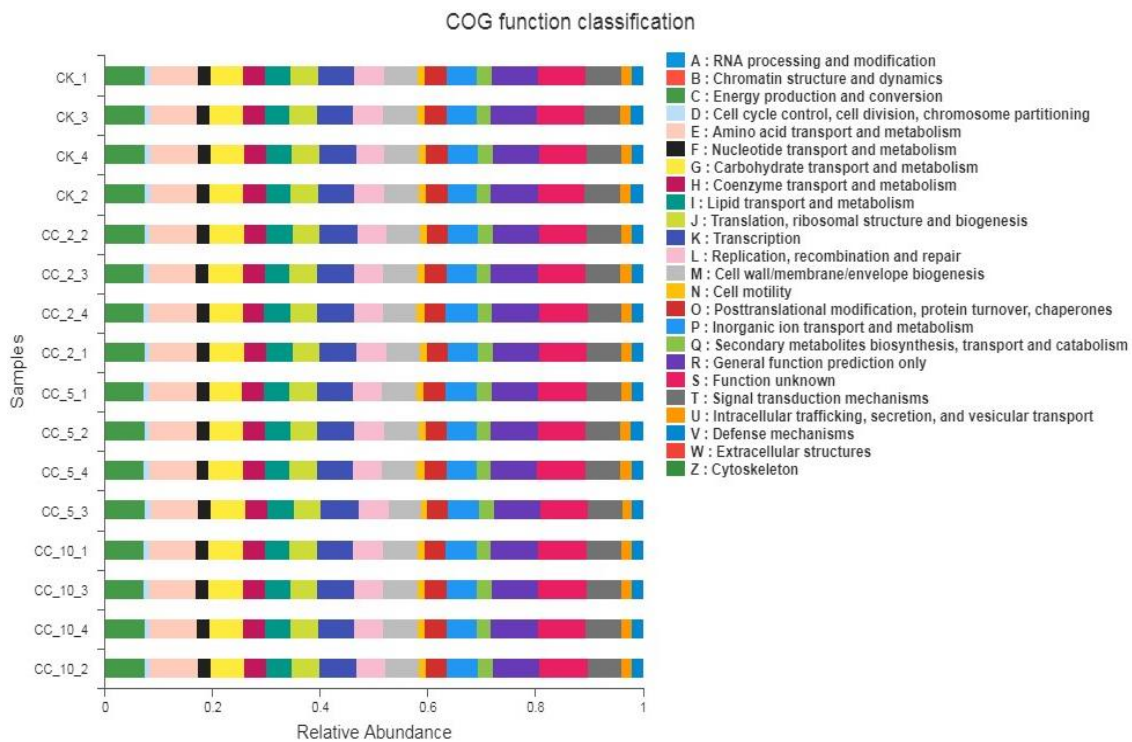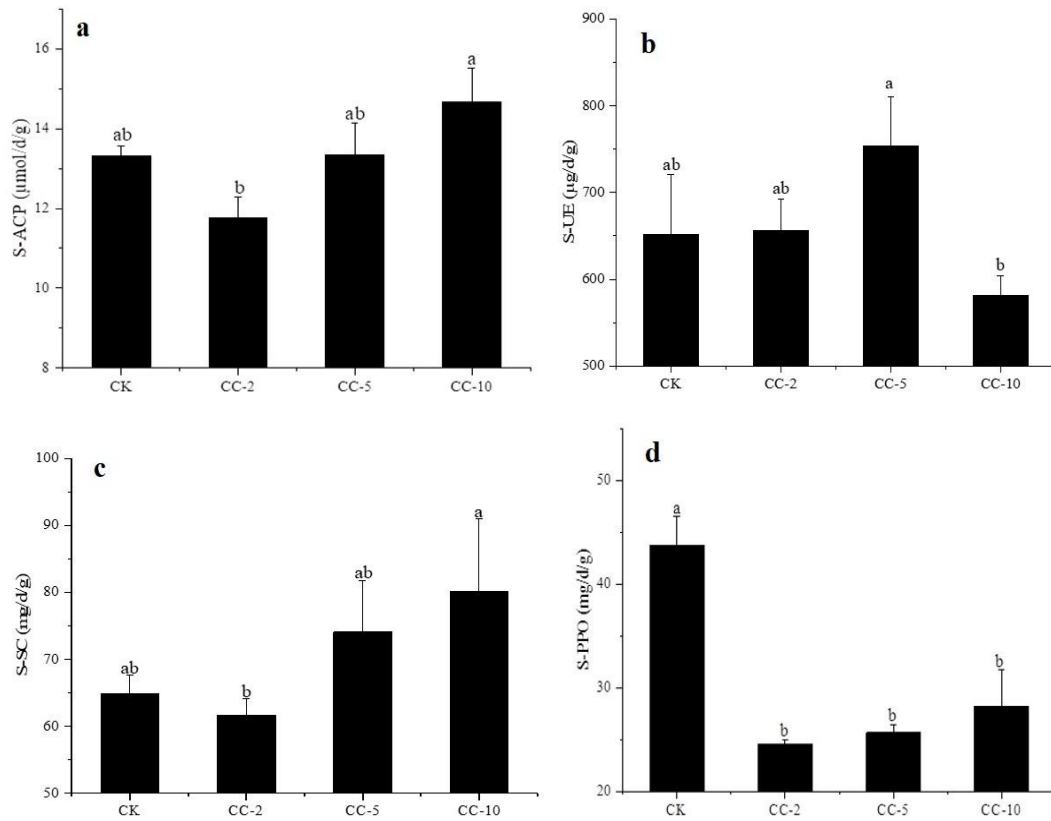

*Figure 4. Clusters of Orthologous Groups (COG) classification*
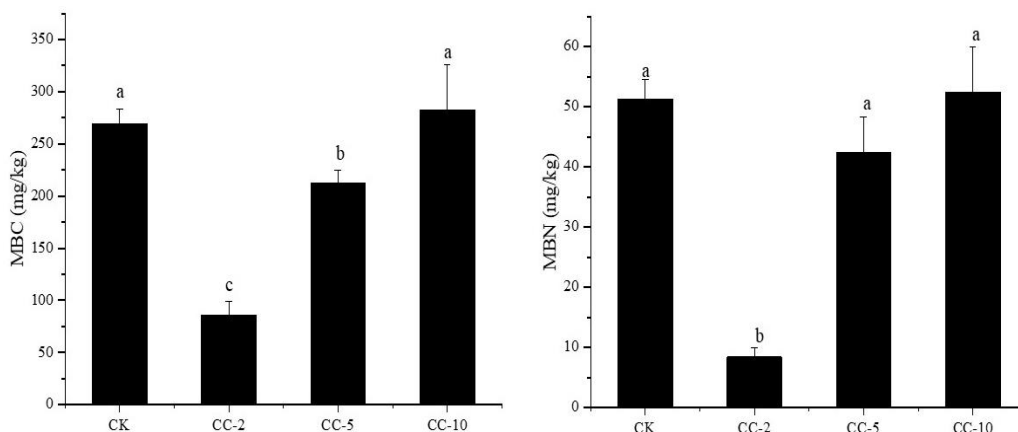
Figure 5. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN).. (Bars with the different letters at the top under the same treatment are statistically different)

Figure 6. *(a) Soil Acid phosphatase enzymatic activity (S-ACP), (b) Soil urease enzymatic activity (S-UE), (c) Soil sucrase enzymatic activity (S-SC), (d) Soil polyphenol oxidase enzymatic activity (S-PPO). (Bars with the different letters at the top under the same treatment are statistically different)*
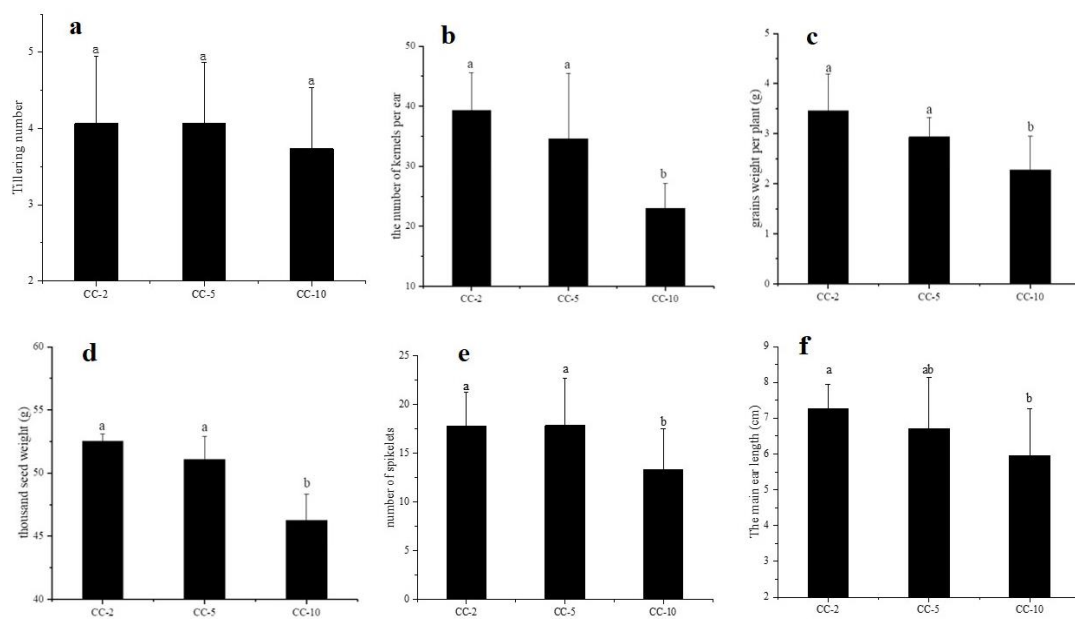
Figure 7. *Tilling number (a), number of kennels per ear (b), grain weight per plant (c), weight of a thousand seed per plant (d), number of spikelet (e), main ear length (f). (Bars with the different letters at the top under the same treatment are statistically different)*

Through Venn analysis, it was shown that the highest number of bacterial species occurred at five years continuous cropping and there was highest relationship in species between five years continuous cropping and ten years continuous cropping treatments, supporting the contribution of continuous cropping on the emergence of the noted bacterial communities. Furthermore, based on the Ace, Chao, Shannon and Simpson diversity indices, it was shown that the microbial diversity had significant statistical variation between the treatment group, with Simpson clearly pointing out the increase in diversity with increase in cropping years. Indeed, through PLS-DA, it was noted that the control treatment had the least co-relation with the other treatment years, and the functional characteristics of communities across the treatment years were majorly on energy production and conversion, amino acid transport and metabolism, general function prediction and signal transduction mechanism. These findings showed the temporal variation in microbial community structure and associated functional characteristics under long term continuous cultivation of highland barley as had earlier been report by Nemadodzi et al. (2020). Moreover, the COG classification indicated that the functional characteristics of communities across the treatments were majorly on energy production and conversion, amino acid transport and metabolism, general function prediction and signal transduction mechanism, indicating the most needed microbial functions with increase in cropping years which result to the degradation in the quality of the soil.

Soil microbial biomass is the main driving force in the decomposition of organic materials and is frequently used as an early indicator of changes in soil properties that result from soil management and environment stresses in agricultural ecosystems. Furthermore, microbial interaction within the soil elevates the spatial heterogeneity of both the soil microbial biomass Carbon and Nitrogen (Richter et al., 2018). The microbial carbon and nitrogen biomass were both lower within the two years of continuous cropping treatment than the other treatments. This could be based on the clearance of the available carbon and nitrogen at the early stages of the continuous cropping before the replenishment take over in the following years of continuous cropping, due to the emergence in the identified microbial genera (Mooshammer et al., 2017).

Acid phosphatase is an enzyme widely found in soil that frees the attached phosphate group from other molecules and is used by microorganisms to access organically-bound phosphate (Bacmaga et al., 2015). Sucrose plays an important role in biological systems by influencing the osmoregulation, tolerance to temperature and desiccation, cell signaling and carbon transport and storage (Gu et al., 2019). The enzymatic hydrolysis of sucrose by the enzyme sucrase results in the formation of its two monosaccharide components. According to the present study, continuous cropping promoted the occurrence of these two classes of soil enzymatic activities. However, there was reduction in the occurrence of Soil urase enzymatic activity (S-UE) and the Soil polyphenol oxidase enzymatic activity (S-PPO) with increase in continuous cropping. Soil urease is majorly considered to be of microbial in origin, and its activity in soils is due to accumulated urease. This enzyme is remarkably stable in organo-mineral complexes found in soil (Dharmakeerthi et al., 1996). The enzyme urease plays numerous roles in the survival of most plants. It is mainly involved in the nitrogen metabolism, where urea is primarily used as a source of nitrogen necessary for growth. It also catalyzes the hydrolysis of urea to ammonium and carbamate ions, which decompose to carbon dioxide and ammonia. Urease activity is widely distributed in the

soil where it also plays essential role in nitrogen metabolism (Kumar, 2015). Studies have shown that phenolic compounds exuded by roots are significant in the allelopathic interactions among plants and root enzymes that destroy phenolics and may protect plants against allelopathic inhibition and thus aid in invasiveness. Phenolic-degrading enzymes are mostly found in aboveground plant parts, but have also been previously reported in root tissues (Sinsabaugh, 2010). Furthermore, the decline in all the agronomic traits (Tilling number, number of kennels per ear, grain weight per plant, thousand seed weight, number of spikelets and the main ear length) with increase in continuous cropping years was an indication of the effect of continuous cropping on the Tibetan barley phenotypic traits. Indeed, studies have shown that that continuous cropping greatly affect the yield of most highland crops which may be for this case include the Tibetan barley (Li et al., 2020).

Conclusion and recommendation

The highest number of species occurred at five years of continuous cropping. The species similarity was highest between five and ten years of continuous cropping and both had biggest difference with species under the control, indicating the uniqueness and shift in the soil microbial characteristics across the cropping years. Indeed, through the different indices, there was statical differences in species abundance. The Clusters of Orthologous Groups (COG) classification indicated that the functional characteristics of communities across the treatments were majorly on energy production and conversion, amino acid transport and metabolism, general function prediction and signal transduction mechanism. All the values for the reported enzymatic activities had statistically significant difference between the treatments and the agronomic traits had general decline with increase in continuous cropping years of the Tibetan barley. Therefore, a long-term study on the correlation between the changes in specific soil properties and the soil microbial community structure, enzymatic characteristics and the plant agronomic traits is recommended.

REFERENCES

[1]   Baćmaga, M., Kucharski, J., Wyszkowska, J. (2015): Microbial and enzymatic activity of soil contaminated with azoxystrobin. – Environmental Monitoring and Assessment 187(10): 615. https://doi.org/10.1007/s10661-015-4827-5.

[2]   Chernov, T. I., Tkhakakhova, A. K., Kutovaya, O. V. (2015): Assessment of diversity indices for the characterization of the soil prokaryotic community by metagenomic analysis. – Eurasian Soil Sc. 48: 410-415. https://doi.org/10.1134/S1064229315040031.

[3]   Costa, S. S., Guimarães, L. C., Silva, A., Soares, S. C., Baraúna, R. A. (2020): First steps in the analysis of prokaryotic pan-genomes. – Bioinformatics and Biology Insights 14: 1-9. https://doi.org/10.1177/1177932220938064.

[4]   Dharmakeerthi, R., Thenabadu, M. (1996): Urease activity in soils: a review. – Journal of the National Science Foundation of Sri Lanka 24(3): 159-195. DOI: http://doi.org/10.4038/jnsfsr.v24i3.5548.

[5]   Gao, Q., Meng, X. Z., Yu, H. F. (2006): Reason analysis and control methods ofsuccession cropping obstacle. – Shandong Agricultural Sciences 3: 60-63 (in Chinese with English abstract).

[6]   Gu, C., Zhang, S., Han, P., Hu, X., Xie, L., Li, Y., Brooks, M., Liao, X., Qin, L. (2019): Soil enzyme activity in soils subjected to flooding and the effect on nitrogen and phosphorus uptake by oilseed rape. – Frontiers in Plant Science 10: 368. https://doi.org/10.3389/fpls.2019.00368.

[7]   Tang, H-M., Xiao, X-P., Tang, W-G., Lin, Y-C., Wang, K., Yang, G-L. (2014): Effects of winter cover crops residue returning on soil enzyme activities and soil microbial community in double-cropping rice fields. – PloS ONE 9(6): e100443. https://doi.org/10.1371/journal.pone.0100443.

[8]   Han, G., Lan, J., Chen, Q., Yu, C., Bie, S. (2017): Response of soil microbial community to application of biochar in cotton soils with different continuous cropping years. – Scientific Reports 7(1): 10184. https://doi.org/10.1038/s41598-017-10427-6.

[9]   Krupenikov, I. A., Boincean, B. P., Dent, D. (2011): Farming and Soil Health. – In: The Black Earth. International Year of Planet Earth. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-0159-5_17.

[10]   Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., Jones, S. J., Marra, M. A. (2009): Circos: an information aesthetic for comparative genomics. – Genome research 19(9): 1639-1645. https://doi.org/10.1101/gr.092759.109.

[11]   Kumar, S. (2015): Plant Ureases: physiological significance, role in agriculture and industrial applications. A review South Asian J. – Food Technol. Environ. 1(2): 105-115.

[12]   Lam, F., Lalansingh, C. M., Babaran, H. E., Wang, Z., Prokopec, S. D., Fox, N. S., Boutros, P. C. (2016): VennDiagramWeb: a web application for the generation of highly customizable Venn and Euler diagrams. – BMC Bioinformatics 17: 401. https://doi.org/10.1186/s12859-016-1281-5.

[13]   Legendre, P., Condit, R. (2019): Spatial and temporal analysis of beta diversity in the Barro Colorado Island forest dynamics plot, Panama. – For. Ecosyst. 6: 7. https://doi.org/10.1186/s40663-019-0164-4.

[14]   Li, M., Yang, F., Wu, X., Yan, H., Liu, Y. (2020): Effects of continuous cropping of sugar beet (Beta vulgaris L.) on its endophytic and soil bacterial community by high-throughput sequencing. – Ann Microbiol 70(39). https://doi.org/10.1186/s13213-020-01583-8.

[15]   Liu, R., Dai, Y., Sun, L. (2015): Effect of rhizosphere enzymes on phytoremediation in PAH-contaminated soil using five plant species. – PloS one 10(3): e0120369. https://doi.org/10.1371/journal.pone.0120369.

[16]   Lyu, J., Jin, L., Jin, N., Xie, J., Xiao, X., Hu, L., Tang, Z., Wu, Y., Niu, L., Yu, J. (2020): Effects of different vegetable rotations on fungal community structure in continuous tomato cropping matrix in greenhouse. – Front Microbiol. 11: 829. https://doi.org/10.3389/fmicb.2020.00829.

[17]   Makarov, M. I., Malysheva, T. I., Maslov, M. N., Kuznetsova, E. Y., Menyailo, O. V. (2016): Determination of carbon and nitrogen in microbial biomass of southern-Taiga soils by different methods. – Eurasian Soil Sc. 49: 685-695. https://doi.org/10.1134/S1064229316060053.

[18]   Monaci, E., Polverigiani, S., Neri, D., Bianchelli, M., Santilocchi, R., Toderi, M., D'Ottavio, P., Vischetti, C. (2017): Effect of contrasting crop rotation systems on soil chemical and biochemical properties and plant root growth in organic farming: first results. – Italian Journal of Agronomy 12(4). https://doi.org/10.4081/ija.2017.831.

[19]   Mooshammer, M., Hofhansl, F., Frank, A. H., Wanek, W., Hämmerle, L., Leitner, S., Schnecker, J., Wild, B., Watzka, M., Keiblinger, K. M., Zechmeister-Boltenstern, S., Richter, A. (2017): Decoupling of microbial carbon, nitrogen, and phosphorus cycling in response to extreme temperature events. – Science Advance 3: e1602781. http://advances.sciencemag.org.

[20] Nemadodzi, L. E., Vervoort, J., Prinsloo, G. (2020): NMR-based metabolomic analysis and microbial composition of soil supporting Burkea africana growth. – Metabolites 10(10): 402. https://doi.org/10.3390/metabo10100402.

[21] Ofek, M., Hadar, Y., Minz, D. (2012) Ecology of root colonizing Massilia (Oxalobacteraceae). – PLoS ONE 7(7): e40117. https://doi.org/10.1371/journal.pone.0040117.

[22] Richter, A., Huallacháin, D. O., Doyle, E., Clipson, N., Van Leeuwen, J. P., Heuvelink, J. B., Creamer, R. E. (2018): Linking diagnostic features to soil microbial biomass and respiration in agricultural grassland soil: a large-scale study in Ireland. – European Journal of Soil Science 68(3): 414-428. https://doi.org/10.1111/ejss.12551.

[23] Rigon, J. P. G., Calonego, J. C. (2020): Soil carbon fluxes and balances of crop rotations under long-term no-till. – Carbon Balance Manage 15: 19. https://doi.org/10.1186/s13021-020-00154-3.

[24] Sinsabaugh, R. L. (2010): Phenol oxidase, peroxidase and organic matter dynamics of soil. – Soil Biology and Biochemistry 42(3). 391-404. https://doi.org/10.1016/j.soilbio.2009.10.014.

[25] Tatusov, R. L., Galperin, M. Y., Natale, D. A., Koonin, E. V. (2000): The COG database: a tool for genome-scale analysis of protein functions and evolution. – Nucleic Acids Research 28(1): 33-36. https://doi.org/10.1093/nar/28.1.33.

[26] Walsh, C. M., Gebert, M. J., Delgado-Baquerizo, M., Maestre, F. T., Fierer, N. 2019. A global survey of mycobacterial diversity in soil. – Appl Environ Microbiol 85: e01180-19. doi: 10.1128/AEM.01180-19.

[27] Wienhold, B., Pikul, J., Liebig, M., Mikha, M., Varvel, G., Doran, J., Andrews, S. (2006): Cropping system effects on soil quality in the Great Plains: synthesis from a regional project. – Renewable Agriculture and Food Systems 21(1): 49-59. https://doi.org/10.1079/RAF2005125.

[28] Willis, A. D. (2019): Rarefaction, alpha diversity, and statistics. – Front. Microbiol. 10: 2407. https://doi.org/10.3389/fmicb.2019.02407.

[29] Yang, T., Siddique, K. H. M., Liu, K. (2020): Cropping systems in agriculture and their impact on soil health. A review. – Global Ecology and Conservation 23: e01118. https://doi.org/10.1016/j.gecco.2020.e01118.

[30] Zhou, L., Li, H., Zhang, Y., Han, S., Xu, H. (2016): Sphingomonas from petroleum-contaminated soils in Shenfu, China and their PAHs degradation abilities. – Brazilian Journal of Microbiology 47(2): 271-278. https://doi.org/10.1016/j.bjm.2016.01.001.