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Influence of seasonality and agricultural practices on soil microbes in Kamishihoro, Japan

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Key words: Land use; season; fertilizer; microorganisms; sequencing

Abstract

Soil microbes are important for maintenance of soil fertility and ecosystem functions. Agricultural practices such as land use, seasonality and fertilizer application effect the soil microbial community structure. However, the effect of these management practices on soil microbes and related functions, especially in one specific region, is still not clear.

Therefore, the study was conducted in Kamishihoro, Japan, to determine effect of land use (cropland, grassland), season (summer, spring) and fertilizer (anaerobic digestate, control) on soil microbial community and functions. Experimental procedure included DNA extraction, quantification, purification, amplification of 16S rRNA (bacterial gene) and Next Generation Sequencing (Ion PGM). Data analysis included Qiime-bacterial community structure, PICRUST-bacterial functional diversity predicted based on 16S rRNA data and STAMP- statistics.

Results showed that land use had the strongest influence on microbial communities, with higher relative abundance recorded in grassland in phyla *Nitrospirae*, *Crenarchaeota* etc. At family level, grassland had microbes that were found in cropland and 244 more unique microbes. Effect of season was higher in cropland in phyla *Acidobacteria*, *Nitrospirae* etc. At family level, summer had more unique microbes in cropland and spring had more unique microbes in grassland. Land use had a significant ($p < 0.05$) effect on more soil functions related to metabolism, cell communication among others, compared to season and fertilizer. Effect of season was more pronounced in cropland on functions related to metabolism, biosynthesis etc. Although on few functions (4), fertilizer had significant ($p < 0.05$) effect in cropland and grassland related to transcription and metabolism.

The study showed that land use was the most important factor in determining soil microbial community structure and functions, compared to season and fertilizer. Specifically, grassland had higher relative abundance and functionality compared to cropland. Future research should consider long term effects of imposed treatments on the same.

Introduction

Soil microbial communities are significant to the maintenance of soil health by providing ecosystem services (Singh et al., 2014), such as decomposition of organic matter and nutrient cycling (Marque et al., 2014). Microbial diversity is an essential part of the soil microbial community (Bender et al., 2016). High microbial diversity enhances soil ecosystem services and tolerance to disturbances such as land use change, seasonality and fertilizer application (Griffiths and Philippot, 2013). A decrease in diversity would lead to potential loss of soil functions, reducing the systems stability (Trivedi et al., 2016). Thus, soil microbes should be diverse to ensure sustainable provision of ecosystem services. In agriculture, soil disturbances are common and may be caused by management practices such as land use and fertilizer application (Li et al., 2002). Soil disturbances can also occur naturally through seasonal changes (Luo et al., 2019). Nonetheless, the influences of these factors, i.e., land use, seasonal changes and fertilizer application on soil microbial diversity and related soil functions are still not fully explored.

The study therefore aimed to address the following key question 1) In a regional scale, which is the biggest factor (land use, season or fertilizer) controlling the soil microbiome and 2) Is it possible identify the key microbes and functions impacted by each factor? We therefore conducted research on soils from cropland and grassland applied with biogas slurry, mainly made of dairy wastes, during summer and spring.

[Methods and Study Site]

[Study Site] The study site was in Kamishihoro (43°14'N, 143°18'E), Hokkaido, Japan.

[Treatments] Treatments were two land use regimes; cropland (beet-*Beta vulgaris*-farm) and grassland (four plots with two grass species i.e timothy-*Phleum pretense* and alfalfa-*Medicago sativa*); two seasons; summer (August to October-2018) and spring (April to June-2018) and two fertilizer treatments; Anaerobic

digestate and no treatment control. During the experimental period spring (April to June) had average temperature of 10.6°C and cumulative rainfall of 252 mm, while summer (August to October) had average temperature of 14°C and cumulative rainfall of 474 mm.

[DNA extraction and sequencing] Soil DNA was extracted by phenol-chloroform extraction method (Sagova-Mareckova et al., 2008). First and second Polymerase Chain Reaction (PCR) was conducted targeting V4 region of 16S bacterial gene. DNA sequencing was done with the Ion PGM next generation sequencer.

[Analysis of 16S rRNA based microbial community structure] Raw sequenced data was processed using QIIME software package version 2018.11 (Boyle et al., 2009). Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) software was used to predict microbial functional attributes (Langille et al., 2013).

[Analysis of community composition] Venn diagrams were constructed using VennDiagram package in R software version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria)

[Statistical Analysis] Statistical analysis of taxonomic and functional profiles (STAMP) software was used to analyze statistical differences between treatments using Welch's *t*-test (Parks et al., 2014). Taxa and functions that had greater than 1 percent mean proportions were selected for this study.

[Results]

[Effect of imposed treatments on microbial abundance and distribution]

At phylum level, land use had the most effect on changes in relative abundance of soil microbes (Fig. 1A). compared to season and fertilizer. Regarding the distribution of soil microbes at family level, land use had the largest effect on soil microbes with grassland having more unique microbial taxa compared to cropland (Fig. 1B). The effect of season varied based on land use. In cropland summer had more unique microbes while in grassland spring had more unique microbes.

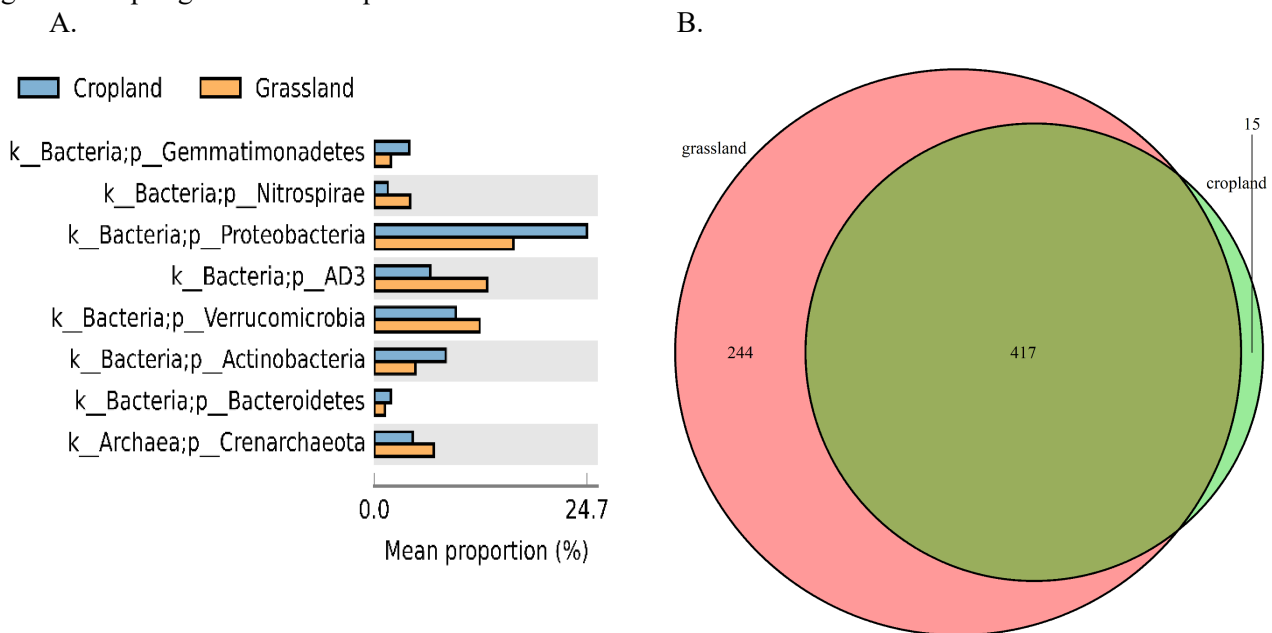


Figure 1; A) Mean proportions of selected phyla that had significant ($p < 0.05$) differences based on land use B) Venn diagram showing shared and unique microbes at family level based on land use.

[Effect of imposed treatments on soil functions]

Land use had the most effect on soil functions with higher functionality recorded in grassland (Fig.2). Although on few functions, season (summer) and fertilizer (organic liquid) had significant effects on function related to metabolism and transcription.

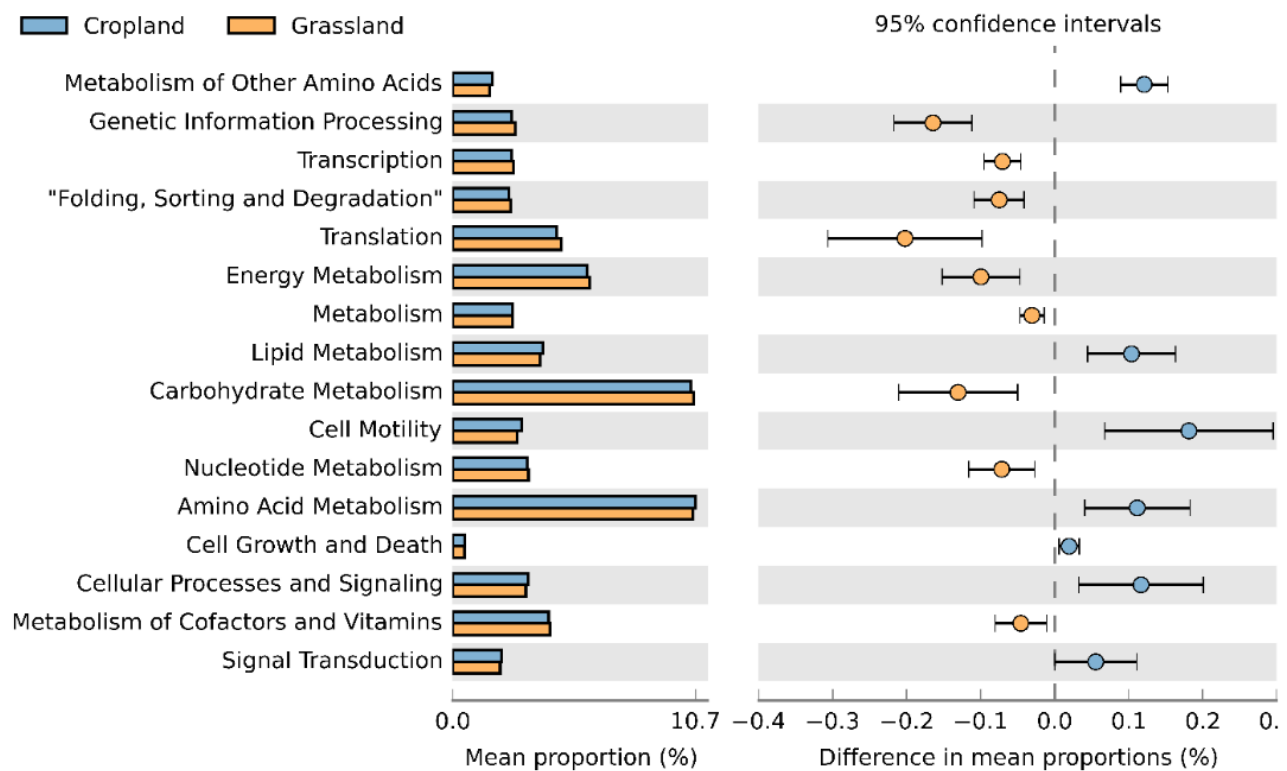


Figure 2; Mean proportions of selected soil related functions that had significant ($p < 0.05$) differences based on land use treatments.

Discussion [Conclusions/Implications]

Land use had the most effect on microbial abundance, distribution, and soil functions. This may have been caused by differences in management practices between the two land use regimes. Specific to our study, grassland management involved anaerobic digestate application and minimal soil disturbance, while cropland was characterized by relatively larger frequency of tillage, monoculture and the addition of relatively larger amounts of inorganic fertilizers. Our results agree with Bissett et al. (2014) who reported that microbial community differences in cropland and grassland could be related to management practices that influenced soil physical and chemical properties, initiating changes in soil microorganisms' structure. Our results also indicate that agricultural management practices related to agricultural land use are critical in shaping microbial community structures in agricultural soils, by their influence on microbial abundance, distribution and soil functions. Additionally, higher microbial abundance, distribution and soil functions were recorded in grassland compared to cropland. This may be due to cropland having lower soil pH (5.23) compared to grassland (5.89). Low soil pH in cropland may have been caused by continuous use of ammonium sulphate, an inorganic nitrogen fertilizer (Garvin and Carver, 2003). Msimbira and Smith (2020) found that the ideal pH for most soil microorganisms in agricultural soils was from 5.5 to 6.5, therefore their growth and related functions would be enhanced in grassland (pH 5.89) compared to cropland (pH 5.23) in our study. Our results suggest that management practices that directly or indirectly influence soil pH such as inorganic nitrogen fertilizer additions are important in shaping microbial community structures in agricultural soils.

In cropland, summer had more unique microbes than spring at family level. Our findings can be attributed to higher rainfall experienced during summer (474mm) compared to spring (252mm), that increased soil moisture content and subsequently microbial diversity. Luo et al. (2019) conducted research on the effect of seasonal changes (summer, spring and autumn) on soil microbial diversity in agricultural soils. Based on their results, summer recorded higher diversity compared to spring, due to more rainfall during summer in agreement with our findings. However, in grassland, spring had more unique microbes at family level than summer. This may have been due to higher pH in spring (5.98) compared to summer (5.8) that may have encouraged microbial growth (Msimbira and Smith, 2020).

These results show the importance of land use and seasonal changes on soil microorganisms and the resultant provision of related soil functions within the agricultural environment. The study should provide more insight on the effect of combined effects of land use and seasonal treatments on specific soil microorganisms and

related functions within the agricultural system at a regional level. Additionally, the study should contribute towards development of sustainable best management practices within the agricultural environment, that will ensure the continued provision of microbial ecosystem services.

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