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Impacts of anthropogenic disturbances on the community structures and functions of soil microorganisms

（人為的攪乱が土壤微生物群集の組成や機能に及ぼす影響）

In this thesis, I have written about three research themes: (1) Investigation of the community structure of soil ammonia-oxidizing bacteria and archaea, and their ammonia-oxidizing activity, targeting land-use changes in Zambia; (2) Investigation of the relationship between the heterogeneity of soil prokaryotic and fungal communities and their functions, targeting land-use changes in Kenya and Malawi; and (3) Evaluation of changes in microbial communities and functions by the non-native earthworm invasion in northern North America. All the themes focused on how anthropogenic activities have altered ecosystem functioning that are facilitated by soil microorganisms. Given that soil serves as a reservoir of nutrients crucial for living organisms, and soil microorganisms play vital roles in nutrient cycling (Schloter et al., 2018), it is necessary to study how human activities have impacted their functions. Such research is essential for evidence-based policymaking related to conservation.

【Research 1】 Community structure of ammonia-oxidizing bacteria was altered by land use change, while that of ammonia-oxidizing archaea was more site-specific in Zambia

Background

In sub-Saharan Africa, soil degradation such as loss of soil nitrogen (N) has been caused by excess cultivation due to the rapidly increasing population in the region (Ohigashi et al., 2021; Sanchez, 2002; Tully et al., 2015). It is said that nitrification by microbes is the largest part of the N loss from the system in the N cycle (Oita et al., 2016). The nitrification process is facilitated by microbes referred to as ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), nitrite-oxidizing bacteria (NOB), and complete ammonia oxidation (commamox) bacteria (Norton & Ouyang, 2019). Besides, tolerance to ammonia and preferable pH depend on the nitrifying species (Tourna et al., 2010). Thus, it is important to investigate how farming management, which includes ammonia-N fertilization, changes nitrifier's community structure and their resulting functions to examine whether farming accelerates further N loss in this region from the microbial perspective.

Objectives

To check whether nitrifier's community structural changes have been caused and whether these changes were related to the changes in nitrification potentials targeting land-use changes in Zambia.

Materials and Methods

Soils were sampled from pairs of neighboring land uses (natural land and farmland) in three sites (A, B, C) in Zambia in January 2019. Before the measurement of inorganic N (ammonia and nitrate), 5 g of soil was shaken with 50 mL of 10% KCl for one hour. The mixture was then centrifuged and filtered. The extract was subjected to the colorimetric method with a flow injection analyzer to measure $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. Nitrification potential was determined using the shaken-slurry method (Hart, 1994). For DNA analysis, the slurry was sampled 1 hour after starting the incubation. The extracted DNA was subjected to a quantitative polymerase chain reaction (qPCR) and an amplicon sequencing targeting the ammonia monooxygenase α subunit (*amoA*) gene with Ion PGM. The AOB

and AOA clusters were determined by creating phylogenetic trees with MEGA X and referring to the previous studies (Alves et al., 2018; Avrahami & Conrad, 2003). The community structures were analyzed by non-metric multidimensional scaling (NMDS), permutation analysis of variance (PERMANOVA), and *ancombc* function in R (Lin & Peddada, 2020).

Results and Discussion

The nitrification potential was 4.3 to 9.1 times higher in Site A compared to Site B and Site C, respectively. In each site, farmed soils exhibited 1.5 times higher nitrification potentials than natural lands on average, possibly leading to further N loss through land use. The qPCR revealed that AOB abundance tended to be larger in farmlands than in natural lands while AOA abundance was lower in all farmlands compared to the neighboring natural lands. This is consistent with the previous studies conducted in other regions (Carey et al., 2016), but it was not significantly correlated with the nitrification potential. Besides, the AOB community structure was differentiated between the land uses while the AOA community structure showed a site-dependency rather than being differentiated between the land uses. Taken together with the qPCR result, some specific AOB species relatively increased in farmlands, although AOA species mostly generally decreased in farmlands. Indeed, the relative abundance of species belonging to the AOB clusters 3a.2 and 3b tended to increase in farmlands in the *ancombc* test. The AOB species in cluster 3b is relatively tolerant against ammonia (Tourna et al., 2010; Webster et al., 2005). Therefore, N application in farming might benefit the species in this cluster. On the other hand, there was an uncertainty in either cluster 3a.1 or 3a.2 increases under farming management including N application. While the soils that enhanced cluster 3a.1 abundance decreased soil pH under farming (Kong et al., 2019; Wang et al., 2019), the soils that increased cluster 3a.2 abundance increased or maintained soil pH (Ohigashi et al., 2021; Zhong et al., 2016). Hence, the regulation of soil pH under farming management might be the key to fostering either of these clusters.

Conclusion

Our study has revealed that both site-specific characteristics and soil environmental changes through land-use changes influenced the AOB and AOA communities in Zambia where soil degradation is severe. Thus, when we set policies or measures regarding soil conservation, we need to consider both site-specific and relatively common phenomena through land-use changes. To establish more comprehensive and generalizable insights, further research involving a broader range of sites will be essential. Our study holds significant importance as it represents the first report to describe ammonia-oxidizing microbial community structures in sub-Saharan Africa. These findings provide fundamental evidence that can contribute to mitigating the issue of nitrogen runoff resulting from farming activities in this region.

[Research 2] Intensive land use induced both taxonomic and functional homogenizations of soil microbial communities and a rise of fungal pathogens in sub-Saharan Africa

Background

In sub-Saharan Africa, people face the issue of declining soil nutrients and productivity due to excessive farming management (Ohigashi et al., 2021; Sanchez, 2002; Tully et al., 2015). Land-use changes are followed by cultivation practices, such as removing weeds and growing a few crop species. Yet these practices act as moderate disturbances in terms of fostering diverse microbes, which has resulted in an increase in microbial sample-scale diversity (α diversity) in theoretical and empirical studies in various regions such as Europe, South America, and Africa (Labouyrie et al., 2023; Ohigashi et al., 2021; Tardy et al., 2015). On the contrary, some reports suggested that a reduction in the heterogeneity of microbial community (β diversity) in the locations due to environmental homogenization (Tatsumi et al., 2023). Nevertheless, it remains unexplored whether such taxonomic homogenization indeed causes the homogenization of actual microbial functions such as nutrient cycling (e.g., C and N cycling) or protection from undesired threats (preventing pathogens).

Objectives

To clarify whether soil microbial taxonomic homogenization has been caused by farming and whether such taxonomic homogenization indeed homogenize microbial functions or increase the possibility of invasion of pathogens within the location (one site) and/or across the region.

Materials and Methods

Soils were sampled from pairs of neighboring land uses (natural land and farmland) in five sites (D-H) in Kenya and Malawi in November 2019. After measurements of soil chemical properties (i.e., pH, moisture, total C, total N), DNA was extracted from the soils. Quantification of microbial abundance was conducted using qPCR, targeting 16S rRNA and ITS genes for prokaryotes and fungi, respectively. Using the same gene regions, amplicon sequencings were conducted. Then, the obtained DNA sequence data were then subjected to the analysis of microbial communities' dissimilarity. Additionally, to examine the effects of spatial distance, environmental factors (Farming, Moisture, total C, total N, and pH), their overlapping effect, and an unexplained effect, a variation partitioning was performed using *varpart* function in R. Using the same environmental factors, multiple regression analysis was conducted to identify factors driving microbial homogenization. The prokaryotic C cycle-related and N cycle-related functions were estimated using PICRUST2 while the fungal lifestyles were estimated using FungalTraits.

Results and Discussion

The NMDS demonstrated that both soil prokaryotic and fungal community structures were shifted similarly by the land-use change, even across the different sites. Previously in sub-Saharan Africa, a consistent effect of land-use change on soil prokaryotic and fungal community structures was observed using sites approximately 130-240 km apart at maximum, within Zambia and South Africa, respectively (Kangan Nkuekam et al., 2018; Ohigashi et al., 2021). The current study further expanded the scale (approximately 1500 km apart at maximum) at which a consistent effect of land-use change on soil microbial communities could be observed in sub-Saharan Africa. In addition, the taxonomic homogenization of prokaryotic and fungal communities has been caused by land-use changes within each site and across multiple sites. The heterogeneity of the prokaryotic community tended to be correlated with the heterogeneities of the C cycle- and N cycle-related functions at the location scale, but the trend was not observed at the metacommunity scale. Previous studies based on incubation have revealed that, under the same incubating conditions, bacterial metacommunities from different soil sources showed functional similarity rather than taxonomic compositional similarity (Langenheder et al., 2005, 2006). This might be due to the limit to the dispersion ability of bacteria (Choudoir & DeAngelis, 2022), although further study would be needed to clarify the actual mechanism. On the contrary to prokaryotes, the heterogeneity of the fungal community tended to correlate with the heterogeneity of fungal lifestyle both at the location scale and at the metacommunity scale. This might be caused by the physical disturbances resulting from farming, such as disrupting fungal hyphae during cultivation (Helgason et al., 1998; van der Wal et al., 2006). Furthermore, the metacommunity-scale fungal heterogeneity was negatively correlated with the relative abundance of pathotrophs. This indicates that pathogenic fungi become relatively prominent even among communities that are not directly interacting but are virtually homogenized. Recently, a framework was proposed for the "meta-ecosystems" to consider the metacommunity scale functions (Gounand et al., 2018; Loreau et al., 2003). This trend in fungal metacommunity can be the key to comprehending the influence of agriculture on soil fungal functional changes potentially affecting areas as wide as a continent. The local-scale taxonomic heterogeneity was driven not by farming management but by soil properties such as soil pH. However, the metacommunity- scale taxonomic heterogeneity was significantly negatively influenced by farming.

Conclusion

The farming management in sub-Saharan Africa led to the homogenization of soil prokaryotic and fungal community structures within the location (local scale) and across different farmlands (metacommunity scale). The local-scale prokaryotic taxonomic homogenization tended to correlate

with the homogenizations of predicted functions of carbon and nitrogen cycles. Additionally, at both local-scale and metacommunity-scale fungal taxonomic homogenization had a correlation with the homogenization of fungal lifestyles at these scales. Besides, the more homogenized fungal community tended to contain a relatively larger abundance of pathogenic fungi. Local-scale taxonomic diversity was primarily shaped by soil properties, particularly soil pH, rather than farming practices. However, at the metacommunity level, farming had a significant negative impact on taxonomic diversity. Our findings highlighted the importance of assessment of microbial communities' heterogeneity using soils under intensive land use from separated sites, which can elucidate unrecognized microbial homogenization even across countries 1500 km apart at maximum.

【Research 3】 Impacts of earthworm invasion on soil nitrification in a former glacier northern hardwood forest in Minnesota, USA

Background

In the American northern hardwood forest, native earthworms were thought to be wiped out during the last Ice Age (Bohlen et al., 2004). The recent rapid invasion of earthworms has impacted ecosystem services such as soil nutrient availability. A previous study conducted in a forest near Leech Lake, Minnesota, in 2017 (Jang et al., 2022) revealed that earthworm invasion had resulted in a decrease in soil bacterial and archaeal genes linked to nitrification—producing nitrate, a vital nutrient for other organisms—particularly in the surface (0-10 cm). However, it remains unclear if nitrification activity indeed decreases after earthworm invasion as well as the mechanism behind it.

Objectives

To examine whether the decrease in microbial DNA related to nitrification indeed causes lower nitrification activity in the surface in the site highly invaded by non-native earthworms and whether the nitrifiers whose abundance was changed by the invasion contribute to nitrification activity.

Materials and Methods

Soils were collected from the A (0-5 cm) and E (10-15 cm in Site M; 15-20 cm in Site H) horizons at a minimally invaded site (Site M) and a highly invaded site (Site H), where were separated by 200 m from each, at a forest near Leech Lake in Minnesota, USA in 2022. The abundance of nitrifiers (AOA, AOB, and NOB) was quantified using qPCR. Then, to identify the nitrifiers actively contributing to the nitrification, the soils were incubated with 0.05% of ammonium sulfate (w/w) in chambers filled with 5% of $^{13}\text{CO}_2$ or $^{12}\text{CO}_2$ (v/v) at 20°C for 9 weeks. Nitrification potential was measured at the same condition. DNA was extracted from the incubated soils for ultra-centrifugation to separate ^{13}C -labeled and unlabeled DNA. Microbes enriched in the ^{13}C -labeled “heavy” fractions were identified by sequencing the 16S rRNA gene amplicons.

Results and Discussion

Similarly to the previous study by Jang et al. (2022), in the field soil, the AOA abundance was significantly lower in Site H compared to Site M. However, in the ^{13}C DNA-SIP, any AOA species were not detected. Instead, some AOB and NOB species were detected in soil layers other than the shallow layer in Site H. Thus, regardless of the varying abundance of nitrifying bacterial species in the field, it was suggested that the nitrification activity was low in the shallow layers of soils with high earthworm invasion levels. In previous studies at other earthworm invasion sites, it has been reported that the amounts of available copper and molybdenum decrease in the shallow soil layers as earthworm invasion levels increase (Richardson et al., 2018). Copper and molybdenum are involved in enzymes related to nitrification reactions (Chicano et al., 2021; Hodgskiss et al., 2023; Musiani et al., 2020). Hence, it is possible that the leaching of these metals into the lower layers may potentially hinder nitrification reactions.

Conclusion

While earthworms are often valued for their role in enhancing soil fertility as ecosystem engineers, their introduction into non-native regions can disrupt local ecosystem functioning. Our research, conducted in a former glacier forest near Leech Lake in Minnesota, USA, revealed that nitrification activity was slower at the A horizon in highly invaded sites compared to minimally invaded ones. This decline in nitrification activity is likely attributed to a potential decrease in copper (Cu) and/or molybdenum (Mo) levels in the shallow soil, as suggested by previous studies. Overall, our findings provide new insights into the mechanism that decrease in N availability in the soil invaded by non-native earthworms.

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