The effects of livestock grazing on physicochemical properties and bacterial communities of perlite-rich soil (#104113)

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The effects of livestock grazing on physicochemical properties and bacterial communities of perlite-rich soil

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This study investigated the impact of livestock grazing on soil physiochemical properties and microbial communities in post-mining perlite-rich soil surrounding a former perlite mine in Thailand. The aim of this study was to provide insights for post-mining reclamation, land-use planning and microbial resource conservation. The perlite-rich soil was found to be sandy, acidic and infertile. Interestingly, livestock grazing significantly improved its texture and nutrient content, suggesting potential as a cost-effective reclamation strategy. However, further research is needed to optimize grazing practices for long-term sustainability. The 16S metagenomic sequencing analysis revealed that microbial communities, which are crucial for soil quality, were also impacted by livestock grazing. The dominant bacterial phyla shifted with increases in Firmicutes and Chloroflexi and a decrease in Actinobacteria. Concerns about increased levels of pathogenic Enterobacteriaceae due to livestock grazing were not substantiated in perlite-rich soil. Analysis of all soil samples revealed consistently low levels of these bacteria, regardless of livestock grazing. This potentially due to the sandy texture and distinct microbial community composition in this soil type. This study further observed a rich and diverse population of Streptomycetaceae, a bacterial family known for producing antibiotics and other valuable secondary metabolites. Notably, 16S rRNA gene sequences revealed the presence of previously uncharacterized Streptomycetaceae within the perlite-rich soil, suggesting its potential as a source for novel antibiotic and secondary metabolite discovery. However, livestock grazing negatively impacted both the abundance and diversity of Streptomycetaceae in this specific soil type. This study, aligns with previous research, demonstrated that the response of soil microbial communities to livestock grazing varies significantly depending on soil type and perlite-rich soil appears to exhibit a unique response compared to other soil types. This finding emphasizes the importance of soil-specific research for understanding how livestock grazing affects microbial communities. Understanding the interplay between livestock grazing, soil type, and

microbial communities is critical for developing sustainable land management practices. Future research should focus on optimizing grazing for perlite-rich soil and characterizing the Streptomycetaceae community for potential antibiotic and secondary metabolite discovery. This knowledge will ultimately promote effective post-mining reclamation using livestock grazing and conservation of valuable microbial resources.

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Abstract

This study investigated the impact of livestock grazing on soil physiochemical properties and microbial communities in post-mining perlite-rich soil surrounding a former perlite mine in Thailand. The aim of this study was to provide insights for post-mining reclamation, land-use planning and microbial resource conservation. The perlite-rich soil was found to be sandy, acidic and infertile. Interestingly, livestock grazing significantly improved its texture and nutrient content, suggesting potential as a cost-effective reclamation strategy. However, further research is needed to optimize grazing practices for long-term sustainability. The 16S metagenomic sequencing analysis revealed that microbial communities, which are crucial for soil quality, were also impacted by livestock grazing. The dominant bacterial phyla shifted with increases in Firmicutes and Chloroflexi and a decrease in Actinobacteria. Concerns about increased levels of pathogenic Enterobacteriaceae due to livestock grazing were not substantiated in perlite-rich soil. Analysis of all soil samples revealed consistently low levels of these bacteria, regardless of livestock grazing. This potentially due to the sandy texture and distinct microbial community composition in this soil type. This study further observed a rich and diverse population of Streptomycetaceae, a bacterial family known for producing antibiotics and other valuable secondary metabolites. Notably, 16S rRNA gene sequences revealed the presence of previously uncharacterized Streptomycetaceae within the perlite-rich soil, suggesting its potential as a source for novel antibiotic and secondary metabolite discovery. However, livestock grazing negatively impacted both the abundance and diversity of Streptomycetaceae in this specific soil type. This study, aligns with previous research, demonstrated that the response of soil microbial communities to livestock grazing varies significantly depending on soil type and perlite-rich soil appears to exhibit a unique response compared to other soil types. This finding emphasizes the

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54 **Introduction**

55 Perlite is a type of amorphous volcanic rock that has a relatively high-water content, typically
56 formed by the rapid cooling of viscous lava or magma and mainly consisting of aluminosilicate
57 compounds (Reka et al., 2019; Stefanidou, Pachta & Konstantinidis, 2023). Perlite is widely used
58 for many applications such as construction industries, thermal insulators, filtration materials,
59 catalysts, removal of pollutants and agriculture due to its beneficial physicochemical properties
60 such as low density, high porosity, low thermal conductivity, high heat resistance, chemical
61 inertness and non-toxicity (Maxim, Niebo & Mcconnell, 2014; Reka et al., 2019; Khoshraftar,
62 Masoumi & Ghaemi, 2023; Yan et al., 2024). Perlite mining is operated in many countries
63 around the world including Greece, China, Iran, Turkey, USA, Japan, Hungary, Italy, Russia,
64 Ukraine, Macedonia and Thailand (Saisuthichai, 2006; Maxim, Niebo & Mcconnell, 2014; Reka
65 et al., 2019).

66 The Fa-La-Mee mountain located in the Lam Narai volcanic field, Lopbui province is the
67 only economic source of perlite in Thailand (Saisuthichai, 2006). A perlite mine was operated in
68 Fa-La-Mee mountain between 1992 to 2017 (Department of Primary Industries and Mines,
69 2024). Following the concession's end, the mine was left without any restoration plan as perlite

mining is considered to be safe for human health and has limited environmental impact (Maxim, Niebo & Mcconnell, 2014; Stefanidou, Pachta & Konstantinidis, 2023). After being abandoned for several years, the flatten parts of the mining area underwent a natural reclamation process and gradually turned to grasslands that created new pastures for livestock grazing. The livestock grazing in these areas involves the mixed breed cow and Siamese buffalo. The livestock were herded into the areas during wet season (June - November) and relocated to other areas during dry season (December - May). There are some remaining areas that are steep or covered in dry dipterocarp forest that are inaccessible for livestock due to the challenging terrain. Perlite often accumulates near the earth's surface; thus, the surface soil of both the grazing and non-grazing areas contain high amounts of crude perlite creating the unique perlite-rich soil (Lexa et al., 2021). Although perlite mining has occurred in several countries, the data on the physicochemical properties and microbial diversity in perlite-rich soil are limited.

Microbial communities play crucial roles in geochemical cycles, soil structure and soil ecosystem functioning (Banerjee & van der Heijden, 2023; Dai et al., 2023). Moreover, some soil microorganism such as Actinobacteria, especially *Streptomyces*, are important natural resources for antibiotics and a vast array of natural products (Donald et al., 2022; Alam et al., 2022). Mining activities, particularly the removal of surface topsoil and vegetation, disrupt soil microbial communities, leading to a decline in diversity and function (Rossum et al., 2016; Sansupa et al., 2021; Gabay et al., 2023). Following perlite mining, the flatten parts of the study area underwent natural rehabilitation and the land use was shifted to livestock grazing. Livestock grazing also influences soil physicochemical properties and shapes the composition of microbial communities in ways potentially different from the original state (Xun et al., 2018; Wu et al., 2022; Mhuireach, Dietz & Gillett, 2022). Previous studies have demonstrated the potential of

livestock grazing as a cost-effective strategy for post-mining reclamation due to its positive effects on soil aggregation, nutrient availability, and the creation of soil ecosystems that support plant growth (Steward, 2006; Teague & Kreuter, 2020). On the other hand, several reports revealed the association between livestock grazing and a rise in antibiotic resistant Enterobacteriaceae in soil, which potentially affect human and animal health (Amador et al., 2017; Sharma et al., 2018; Black et al., 2021).

In this study, we compared the physicochemical properties and bacterial diversity of perlite-rich soil between areas impacted by livestock grazing and those that remained unaffected by livestock. Our findings provide new insights into the impact of livestock grazing on perlite-rich soil which can be applied for future reclamation, land-use planning and microbial resource conservation.

Materials & Methods

Study site, experimental design and sample collection

The study site was the former perlite mine located on Fa-La-Mee mountain, Sa Bot district, Lopburi province, Thailand. Soil samples were collected in December 2022 (dry season) from five different locations surrounding the mine (Fig. 1). At each location, two areas were designated: one with livestock grazing and one without. This resulted in a total of 10 sampling sites (5 grazing and 5 non-grazing). At each study site, five replicate plots (1 m x 1 m) were established. Prior to sample collection, any loose debris on the topsoil was removed. Within each plot, five soil cores were collected from a depth of 10 cm. These cores were then combined to form a composite sample per plot. The plot-level composite samples were then combined into a single, larger composite sample representing the entire site. The site-level samples were passed through a 2-mm sieve to remove stones, roots and organic litter. Approximately 1 kg of the

combined sample was collected in a sterile container, stored at 4 °C and transported to the laboratory (method adapted from Sansupa et al., 2021). The details for the study sites are given in Table S1 and Fig. S1.

Soil physicochemical property analysis

Approximately 900 g of soil from each sample site was submitted to Department of Soil Science, Kasetsart University for soil physicochemical property analysis (Kasetsart University, Bangkok, Thailand). The following properties were measured: pH, texture (sand, silt, cray), electrical conductivity (EC), organic matter (OM), organic carbon (OC), total nitrogen (TN), ammonia (NH_4), nitrate (NO_3), available phosphorus (P), available potassium (K), available calcium (C), available magnesium (Mg), available zinc (Zn), available manganese (Mn), available iron (Fe), available sulphate (SO_4) and soluble silicon (Si). The references for soil physicochemical property analysis are listed in Table S2.

DNA extraction and 16S ribosomal RNA (16S rRNA) gene sequencing

For analysis of bacterial composition, 10-gram of soil samples were submitted to Zymo Research (Irvine, California, United States) for sequencing of the V3–V4 region of the 16S rRNA gene. Soil DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Irvine, United States). Bacterial 16S rRNA gene targeted sequencing was performed using the Quick-16S™ NGS Library Prep Kit with Quick-16S™ Primer Set V3-V4 (forward primer: 5'-CCTACGGGDGGCWGCAG-3', 5'-CCTAYGGGGYGCWGCAG-3' and reverse primers 5'-GACTACNVGGGTMTCTAATCC-3'. The forward primer is a mixture of the two sequences listed) (Zymo Research, Irvine, United States). The final library was sequenced on Illumina® MiSeq™with a v3 reagent kit (600 cycles). The sequencing was performed with 10% PhiX spike-in control library (Illumina, San Diego, United States).

Bioinformatics analysis

Raw DNA reads were processed using QIIME2 (Bolyen et al., 2019). Forward and reverse primers were trimmed, followed by quality filtering, merging of sequences, and chimera removal using DADA2 (Callahan et al., 2016). Subsequently, similar sequences were clustered into amplicon sequence variants (ASVs). ASVs with less than ten sequencing reads were removed. Finally, bacterial taxonomy assignment was performed using the Silva v.138 database (Quast et al., 2012). All bioinformatics analyses were performed in Galaxy Sever (Abueg et al., 2024). Maximum likelihood phylogenetic trees were constructed using MEGA11 (Tamura, Stecher & Kumar, 2021) with the 16S rRNA V3-V4 sequences. The resulting phylogenetic trees were visualized using iTOL (https://itol.embl.de/).

Statistical analysis

Statistical analyses were performed by PAST software (Hammer et al., 2001), R program (R Core Team, 2021) and GraphPad Prism version 9 (GraphPad Software, Boston, MA, USA). The soil physicochemical properties were analyzed by Non-metric MultiDimensional Scaling (NMDS) using Bray-Curtis distance. The alpha diversity was calculated based on the presenting bacterial richness (observed Operational Taxonomic Unit, OTU) and diversity indexes (Chao1, Simpson and Shannon). Beta diversity, presenting bacterial community composition, was visualized by Principal Co-ordinates Analysis (PCoA) based on Bray-Curtis distance of ASV composition. Spearman's rank correlation was used to determine the effect of soil physicochemical properties on the abundance of bacterial phyla. All these analyses were performed by PAST software. OTUs that have significant abundance among different groups (logarithmic score > 2 and p-value < 0.05) were identified by Linear Discriminant Analysis Effect Size (LEfSe) (Segata et al., 2011). This analysis was performed using the LEfSe

implementation available on the Huttenhower Lab Galaxy Server (https://huttenhower.sph.harvard.edu/lefse/). LEfSe leverages the relative abundance of amplicon ASVs in each sample, identifies taxa with statistically significant differences between groups using a Kruskal-Wallis test, and estimates their effect sizes through Linear Discriminant Analysis. Paired t-test was performed by GraphPad Prism and Sign test was performed by R.

Results

Soil physicochemistry

All soil samples appeared sandy with white, light gray or gray in color, indicated the presence of perlite (Fig. S2). Physicochemical analyses revealed that perlite-rich soil across both livestock grazing and non-grazing areas had sandy texture, acidic pH, and low nutrient content. High variability in most measured parameters across the study sites necessitated the use of both paired t-test and Sign test to assess differences between livestock grazing and non-grazing areas. Livestock- grazing soils exhibited significantly higher levels of clay content, EC, OM, OC, TN, NH_4, NO_3, available P, available K, available Ca, available Mn, extractable Mn, extractable Fe, extractable SO_4 and soluble Si compared to the non-grazing soils (Table 1). Livestock grazing and non-grazing samples were clustered separately when analyzed by NMDS (Fig. 2A). The soil physiochemistry raw data were summarized in Table S3.

Bacterial composition differences between the livestock-grazing and non-grazing areas

Rarefaction curves of observed OTUs from each sample plateaued at the analyzed sequencing depth (20,000 reads per sample), indicating that a sufficient number of OTUs were detected to represent the bacterial community captured by our method (Fig. S3). Alpha-diversity analysis of the 18,870 observed ASVs revealed no significant differences in biodiversity between grazing and non-grazing areas (Fig. S4). Despite the similarities in biodiversity summary statistics, beta-

diversity analysis revealed distinct microbial communities in the two areas (Fig. 2B). According to the OTU annotation results, a total of 6,314 OTUs, belonging to 30 phyla, 70 classes, 139 orders, 262 families and 790 genera were identified across all samples. The most abundant phyla in perlite-rich soil included Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes and Chloroflexi. The relative abundance of dominant bacterial phyla, especially Actinobacteria, Firmicutes, and Chloroflexia, were distinctly different between livestock grazing and non-grazing area (Fig. 3). Further analysis also showed that livestock exposure samples showed a significant increase in Firmicutes (11.6±0.06% grazing vs 7.6±0.03% non-grazing) and Chloroflexi (7.7±0.02% grazing vs 3.6±0.01% non-grazing), while Actinobacteria decreased significantly (21.7±0.06% grazing vs 33.0±0.16% non-grazing) (Fig 4). LeFSe analysis was used to identify taxa that differ significantly between sample groups (p-value < 0.05 and LDA score > 2). Samples from livestock grazing areas exhibited significantly higher abundance of Chloroflexi, Methonomicrobia, Coriobacteriaceae, Holophagaceae, Syntrophaceae, Geobacteraceae and Rhodocyclaceae while non-grazing areas exhibited significantly higher abundance of Rhizobiales, Streptomycetaceae, Burkholderiaceae, Iamiaceae and Haliangiaceae (Fig. 5).

Abundance and biodiversity of Enterobacteriaceae and Streptomycetaceae between grazing and non-grazing areas

In this study, 16S rRNA metagenomic sequencing revealed a relatively low abundance (0 – 0.1%) of Enterobacteriaceae in the perlite-rich soil, with no significant difference between grazing and non-grazing samples (Fig. 6). Interestingly, LeFSe analysis revealed significant differences in Streptomycetaceae abundance between livestock grazing and non-grazing areas. This prompted a more detailed analysis of the abundance and biodiversity of this bacterial

family. The non-grazing areas had a significantly higher abundance of Streptomycetaceae compared to the grazing areas (1.11±0.49 % and 1.76±0.23 % for grazing and non-grazing areas, respectively) (Fig. 6). For biodiversity, 16S rRNA metagenomic sequencing identified 122 ASVs belonging to the Streptomycetaceae family. Of these, 115 ASVs were assigned to the *Streptomyces* genus, while 4 and 2 ASVs were mapped to *Kitasatospora* and *Streptacidiphilus*, respectively. One ASV remained unclassified at the genus level. The non-grazing areas harbored a higher diversity of Streptomycetaceae with 90 ASVs compared to 60 ASVs found in grazing areas. Notably, 28 ASVs were found in both locations. The abundance bar plot visualizes the overall abundance and diversity of Streptomycetaceae between grazing and non-grazing samples (Fig. 7). Phylogenetic analysis of 16S rRNA gene sequences (V3-V4 region) revealed a high degree of diversity among Streptomycetaceae populations within the perlite-rich soil, as evidenced by their segregation into distinct clades within the phylogenetic tree (Fig. 8).

Relativity between bacterial composition and soil physicochemical properties

Spearman's rank correlation analysis between bacterial composition in phyla level and soil physicochemical properties revealed significant positive correlations between the relative abundance of Firmicutes, Chloroflexi and soil OM, along with several nutrients (e.g., Fe, Mn). On the contrary, available K, Mn and SO_4 showed negative correlations on the abundance of Acidobacteria, Planctomycetes and Verrucomicrobia. Additionally, NO_3 and soluble silicon displayed negative correlations with Actinobacteria. (Fig. 9).

Discussion

Perlite is a volcanic aluminosilicate mineral that is used in various applications. Perlite mines are operated in many countries around the world which leave behind post-miming lands in need of reclamation. In this study, we comparatively analyze the physicochemical properties and

bacterial community structure of perlite-rich soil from livestock-grazing and non-grazing areas surrounding a former perlite mine in Thailand. This analysis aims to provide a deeper understanding of how livestock grazing influences perlite-rich soil characteristics and its potential role in reclamation efforts.

Physicochemical analysis of perlite-rich soil indicated a sandy texture, acidic pH and limited nutrient availability. Interestingly, livestock grazing led to a significant improvement in soil texture and nutrient availability. This finding aligns with previous studies on other soil types which demonstrated positive effects of rational grazing on soil aggregation, nutrient content, and overall plant growth-supporting ecosystems (de Faccio Carvalho et al., 2010; Teague & Kreuter, 2020). Livestock grazing can be a cost-effective strategy for reclamation of some post-mining lands (Steward, 2006). However, uncontrolled or heavy grazing can negatively impact soil quality and ecosystem (Lai & Kumar, 2020). Therefore, further research is crucial for identifying suitable grazing practices for long-term sustainability and ecological resilience of the reclaimed land.

Microbial communities are recognized as keystone components of soil ecosystems, influencing crucial biogeochemical cycles, soil structure development, and overall ecosystem function (Xun et al., 2018; Wu et al., 2022; Mhuireach, Dietz & Gillett, 2022). In this study, 16S rRNA gene sequencing indicated that Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, and Chloroflexi were the most abundant bacterial phyla in the perlite-rich soil, which aligns with findings from previous microbiome studies on various soil types (Xun et al., 2018; Wang et al., 2021; Cui et al., 2021). The results of this study revealed the potential effects of livestock grazing on bacterial community in perlite-rich soil. Soil samples from grazing areas displayed a significant increase in the relative abundance of Firmicutes and Chloroflexi, accompanied by a

decrease in Actinobacteria. Previous research demonstrated that livestock grazing impacted soil microbial communities differently depending on soil type. A study on meadow steppe soils observed an increase in Firmicutes accompanied by a decrease in Chloroflexi after grazing (Xun et al., 2018). In contrast, a study on chestnut soil found a significant increase in Chloroflexi abundance with grazing (Zhang et al., 2023). Typical steppe soils exhibited a rise in both Firmicutes and Actinobacteria following grazing (Usman et al., 2024), while a separate study on chernozem (black soil) reported that livestock grazing decreased both Firmicutes and Actinobacteria (Wang et al., 2021). Additionally, a study on desert soil showed an increase in all three bacterial phyla (Firmicutes, Chloroflexi, and Actinobacteria) following grazing (Cui et al., 2021). These diverse findings strongly suggest that the effect of livestock grazing on soil microbial communities is highly dependent on soil type and likely influenced by other factors such as grazing practices and environmental factors. Many studies suggested that manure deposition by grazing animals alters the physicochemical characteristics of soil, which can subsequently impact the composition of microbial communities (Xun et al., 2018; Zhang et al., 2023; Usman et al., 2024). Our findings align with this concept, as a positive correlation was observed between the increased abundance of Firmicutes/Chloroflexi and elevated levels of soil OC and other nutrients (e.g., Mg, Fe) in grazing areas. Several studies have shown a positive correlation between the abundance of Firmicutes and increasing levels of soil OC and nutrients. This reflects the copiotrophic nature (rapid growth at high abundant resource) of Firmicutes and their capacity for decomposing recalcitrant carbon sources (Xun et al., 2018; Cui et al., 2021; Lourenço, Cantarella & Kuramae, 2023). However, the responses of Chloroflexi to OC and soil nutrients appeared to depend on soil type (Trivedi et al., 2016; Xun et al., 2018; Cui et al., 2021; Lourenço, Cantarella & Kuramae, 2023). Firmicutes species are widely studied for their

multifaceted contributions to bioremediation and sustainable agriculture. These beneficial bacteria promote plant growth by enhancing nutrient acquisition, influencing plant hormone production, and acting as biological control agents against plant pathogens (Aguilar-Paredes et al., 2023). The phylum Chloroflexi consists of a diverse group of organisms, including both photosynthetic and decomposer species. Despite being recognized as one of the dominant taxa in soils, the ecological significance of Chloroflexi remains poorly understood due to limited data (Thiel et al., 2019).

Several studies have linked livestock grazing to an increase in antibiotic-resistant Enterobacteriaceae in soil which poses a potential threat to human and animal health (Amador et al., 2017; Sharma et al., 2018; Black et al., 2021). However, in this study, 16S rRNA metagenomic sequencing revealed a relatively low abundance (0 – 0.1%) of Enterobacteriaceae in the perlite-rich soil. Furthermore, no significant difference was observed between grazing and non-grazing samples. A previous study demonstrated that the survival of *Escherichia coli*, a member of the Enterobacteriaceae family, varied with the soil type and the survivability of *E. coli* was minimal in sandy soil (Alegbeleye & Sant'Ana, 2023). Additionally, research has revealed a strong correlation between soil microbial communities, which differ across soil types, and the survival of Enterobacteriaceae in soil (Moynihan et al., 2015). Therefore, the low abundance of Enterobacteriaceae observed in the perlite-rich soil, regardless of grazing, is likely a consequence of both the physicochemical properties and the distinct microbial communities present in this soil type.

This present study also revealed a high abundance and diversity of Streptomycetaceae within the perlite-rich soil. This observation is likely attributable to the established biofilm formation capabilities of Streptomycetaceae on perlite granules (Domínguez-González et al., 2022).

Streptomycetaceae are well-recognized for their production of vast numbers and varieties of antibiotic compounds. Furthermore, members of Streptomycetaceae are able to produce numerous secondary metabolites employed in antifungal, antiparasitic, chemotherapeutic, and immunosuppressant applications. Thus, Streptomycetaceae bacteria are considered as a valuable reservoir for novel antibiotic discovery and other beneficial applications (Donald et al., 2022; Alam et al., 2022). Analysis of the Streptomycetaceae family in perlite-rich soil revealed the presence of three genera: *Streptomyces*, *Kitasatospora*, and *Streptacidiphilus*. Notably, *Streptomyces* is the most abundant genus within this family. *Streptomyces* is the largest antibiotic-producing genus of Actinomycetota which can produce a vast array of medically important antibiotics, including streptomycin, tetracycline, and chloramphenicol (Donald et al., 2022). Previous studies on phylogenetic analysis confirmed *Kitasatospora* as a distinct genus, separate from *Streptomyces* (Takahashi, 2017). *Kitasatospora* emerge as valuable sources of bioactive compounds due to their ability to produce a variety of biologically active compounds with extremely diverse structures and most of these compounds are unique to this genus (Zimmermann et al., 2023). The genus *Streptacidiphilus* represents a group of acidophilic members of actinobacteria within the family *Streptomycetaceae* (Song et al., 2018). Genome mining studies have identified *Streptacidiphilus* strains as intriguing candidates for further investigation due to their potential for producing novel secondary metabolites and exhibiting unique enzymatic activities (Malik, Kim & Kim, 2020). Intriguingly, analysis of the Streptomycetaceae community in the perlite-rich soil revealed the presence of several ASVs that did not match any identified members of Streptomycetaceae within the database. This suggests the possibility of novel, uncharacterized Streptomycetaceae species residing in the study area. Consequently, this finding highlights the potential of this area as a valuable source for

actinomyces capable of producing novel antibiotics and secondary metabolites. While livestock grazing can enhance soil quality, it has a negative effect on Streptomycetaceae populations within perlite-rich soils. LEfSe analysis highlighted a significantly higher abundance of Streptomycetaceae in non-grazing areas compared to grazing areas. This finding was further supported by a significant difference detected using a paired-t test. Notably, livestock grazing also affected the overall diversity of Streptomycetaceae in perlite-rich soil. These results differ from observations in paddock and chernozem soil types, which studies reported a positive correlation between Streptomycetaceae populations and livestock grazing (Wang et al., 2021; Mhuireach, Dietz & Gillett, 2022). This contrast highlights the potential influence of soil type on the relationship between Streptomycetaceae and grazing practices.

Taken together, this study and the existing literature emphasize the need for soil-specific research to understand how livestock grazing affects soil microbial communities. This knowledge is crucial for optimizing grazing practices to promote healthy soil ecosystems as well as microbial resource conservation in different environments.

Conclusions

This study investigated the impact of livestock grazing on the physicochemical properties and microbial communities of perlite-rich soil surrounding a former perlite mine in Thailand. Our findings revealed that livestock grazing improved soil texture and nutrient availability without increasing the abundance of pathogenic Enterobacteriaceae. This study also revealed a highly abundant and diverse Streptomycetaceae community within the perlite-rich soil. This finding suggests the potential of this environment as a valuable source for novel antibiotic and secondary metabolite-producing strains. However, livestock grazing led to a significant decrease in Streptomycetaceae abundance and diversity in the study areas. Therefore, further studies on

optimizing grazing practices and characterizing Streptomycetaceae in perlite-rich soil are required to achieve soil improvement for post-mining reclamation together with microbial resource conservation. Our findings, along with the results from previous studies, suggested that the effects of livestock grazing on soil microbial communities are varying depending on soil types. This highlights the critical need for soil-specific investigations to develop optimized grazing practices across diverse environments.

Acknowledgements

We would like to express our gratitude to Mr. Pho Laosena for his invaluable assistance in guiding us to the sampling sites and facilitating soil sample collection.

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Table 1(on next page)

Physicochemical properties of perlite-rich soil

1

Parameter	Area		t-test		Sign test	
	Grazing	Non-grazing	t	*p*-value	Z	*p*-value
pH	6.1 ± 0.5	5.5 ± 0.7	2.648	0.057	1.342	0.180
Sand (%)	60.2 ± 17.8	68 ± 15.9	-3.814	0.019	2.236	0.025
Silt (%)	30.4 ± 16.3	23.5 ± 9.1	1.458	0.219	1.342	0.180
Clay (%)	9.6 ± 5.4	6.8 ± 3.9	2.997	0.040	2.236	0.025
EC (dS/m)	0.06 ± 0.03	0.03 ± 0.01	1.835	0.140	2.236	0.025
OM (g/kg)	29.4 ± 23.8	12.7 ± 9.5	1.700	0.164	2.236	0.025
OC (g/kg)	17 ± 13.8	7.4 ± 5.5	1.703	0.164	2.236	0.025
TN (g/kg)	1.3 ± 1.1	0.6 ± 0.4	1.680	0.168	2.236	0.025
NH_4 (mg/kg)	6.7 ± 4.1	3.5 ± 2.7	1.906	0.129	2.236	0.025
NO_3 (mg/kg)	8.4 ± 4.1	3.0 ± 2.3	3.023	0.039	2.236	0.025
C/N ratio	11.3 ± 2	12.6 ± 3.4	-0.856	0.417	0.447	0.655
P (mg/kg)	306.2 ± 111.4	135.5 ± 58.4	3.336	0.029	2.236	0.025
K (mg/kg)	35.5 ± 51.5	5.3 ± 2.8	1.325	0.256	2.236	0.025
Ca (mg/kg)	959 ± 606	370.8 ± 226.2	2.397	0.075	2.236	0.025
Mg (mg/kg)	215.6 ± 164	63.6 ± 26.8	2.387	0.075	2.236	0.025
Zn (mg/kg)	1.1 ± 0.6	0.5 ± 0.4	1.247	0.280	1.342	0.180
Mn (mg/kg)	43 ± 21.7	23.6 ± 16.8	3.038	0.038	2.236	0.025
Fe (mg/kg)	54.1 ± 36.9	27.3 ± 25.7	1.771	0.151	2.236	0.025
SO_4 (mg/kg)	49.1 ± 20.8	38.6 ± 8.5	1.243	0.282	1.342	0.180
Si (mg/kg)	20.5 ± 14.8	8.6 ± 1.7	1.835	0.140	2.236	0.025

2

Figure 1

Study areas

Sampling sites are numbered 1–5. Yellow and blue arrows indicate grazing and non-grazing areas, respectively. The aerial image was captured from Google Earth Pro.

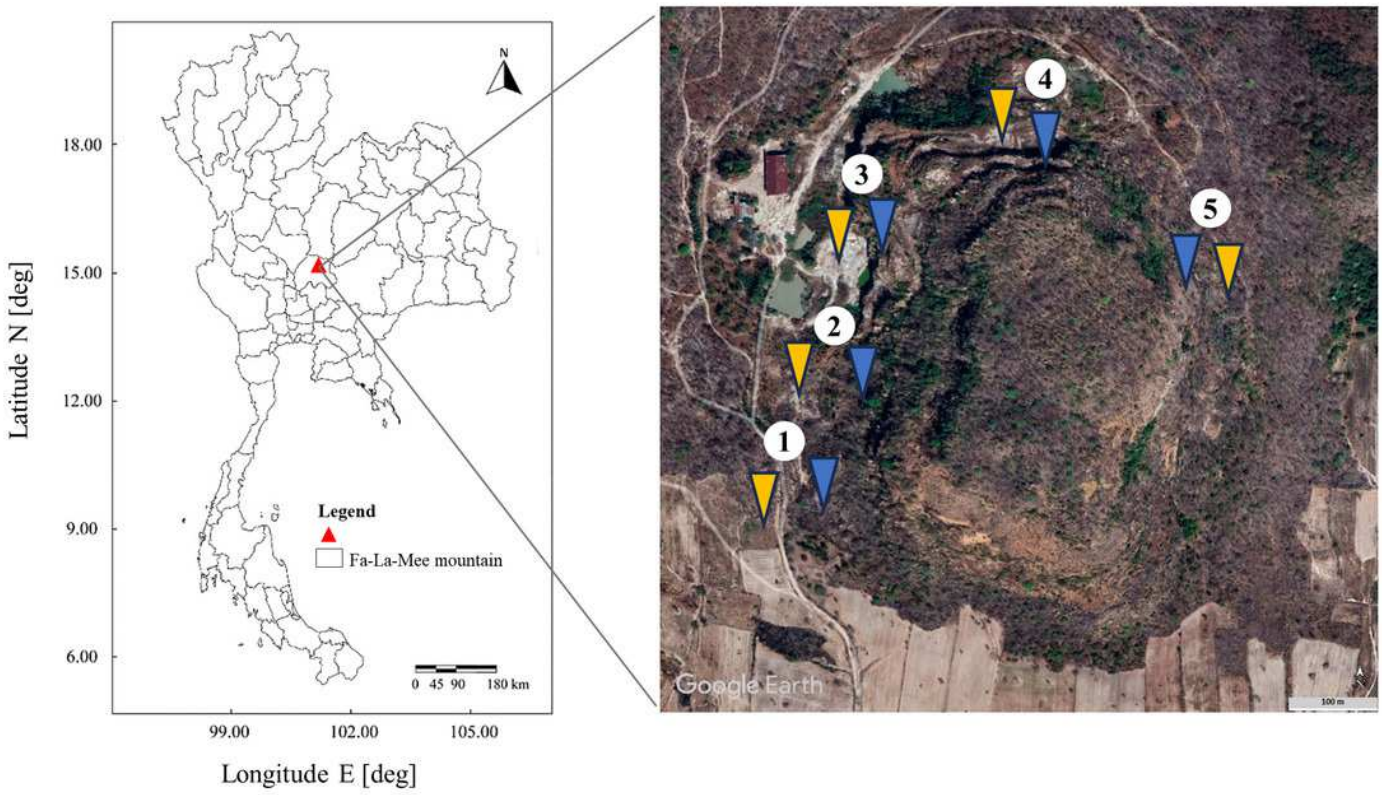

Figure 2

Comparison of the grazing and non-grazing areas through soil physicochemical properties (A) and bacterial communities (B)

(A) NMDS of the soil physicochemistry analyzed using Bray-Curtis distance. (B) PCoA analyzed using Bray-Curtis distance based on ASV composition.

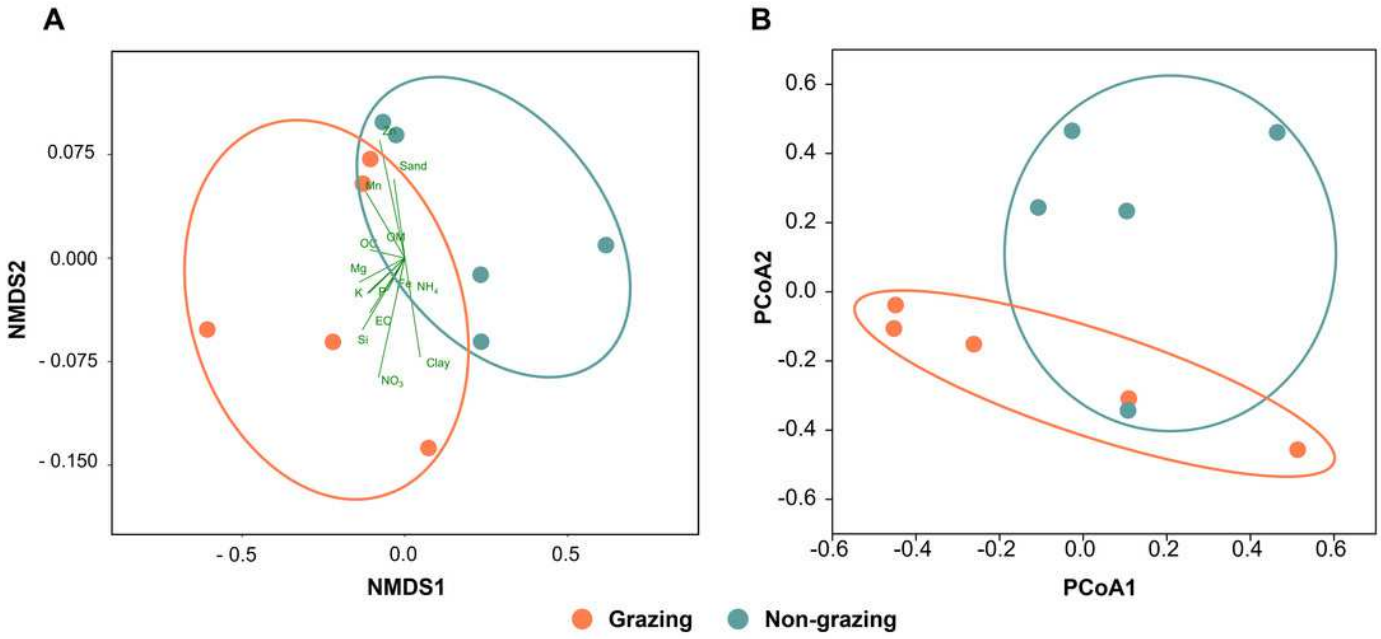

Figure 3

Relative abundance of top 10 most abundant phyla

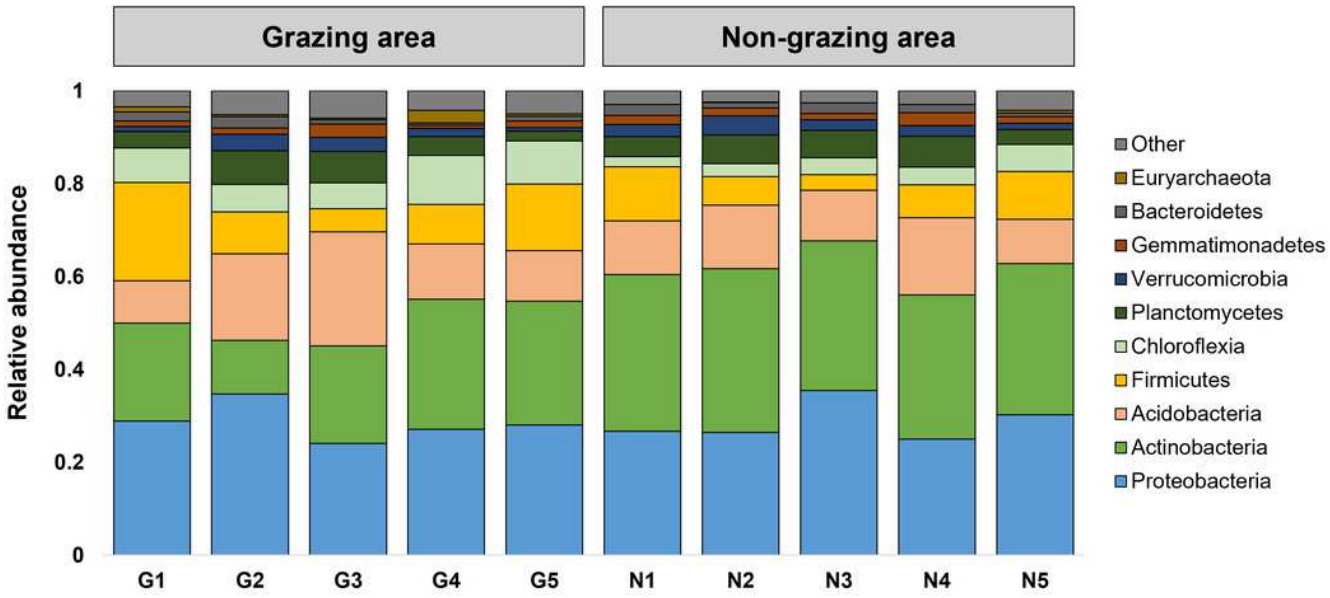

Figure 4

Relative abundance of Firmicutes, Chloroflexi and Actinobacteriain in the different areas

Significance was determined using pair t-test ($p < 0.05$).

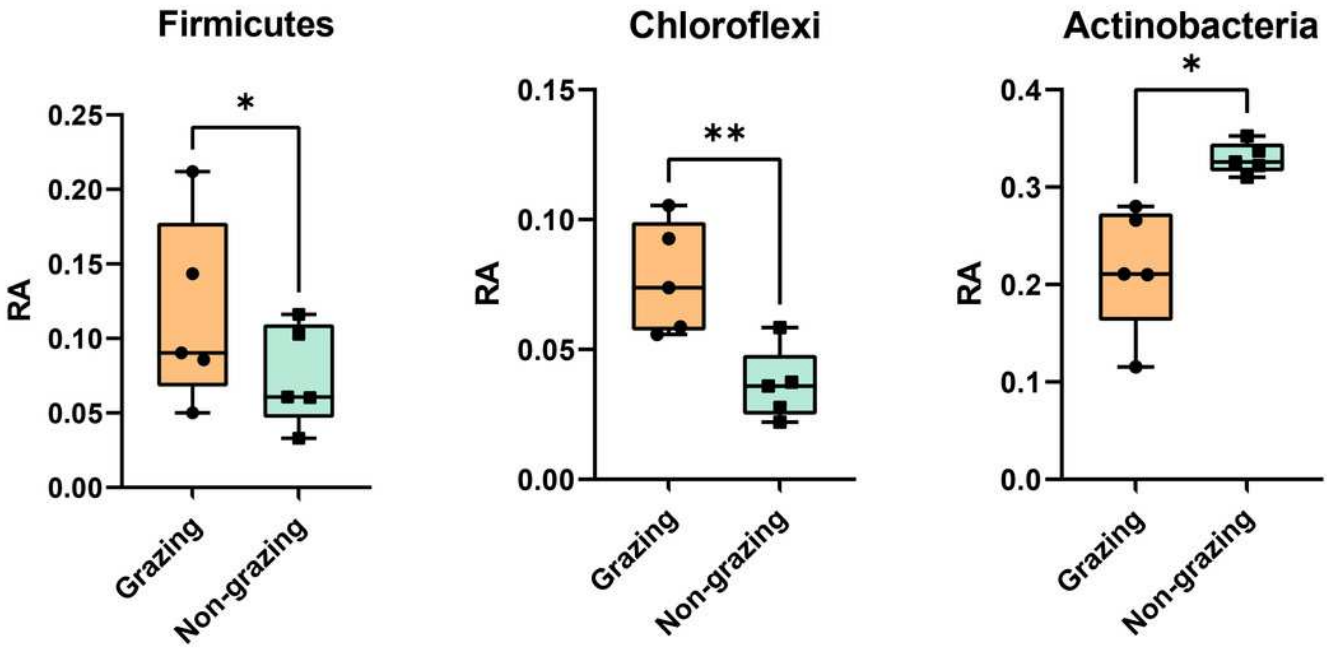

Figure 5

LEfSe analysis on OTUs identified in perlite-rich soil samples from grazing and non-grazing areas

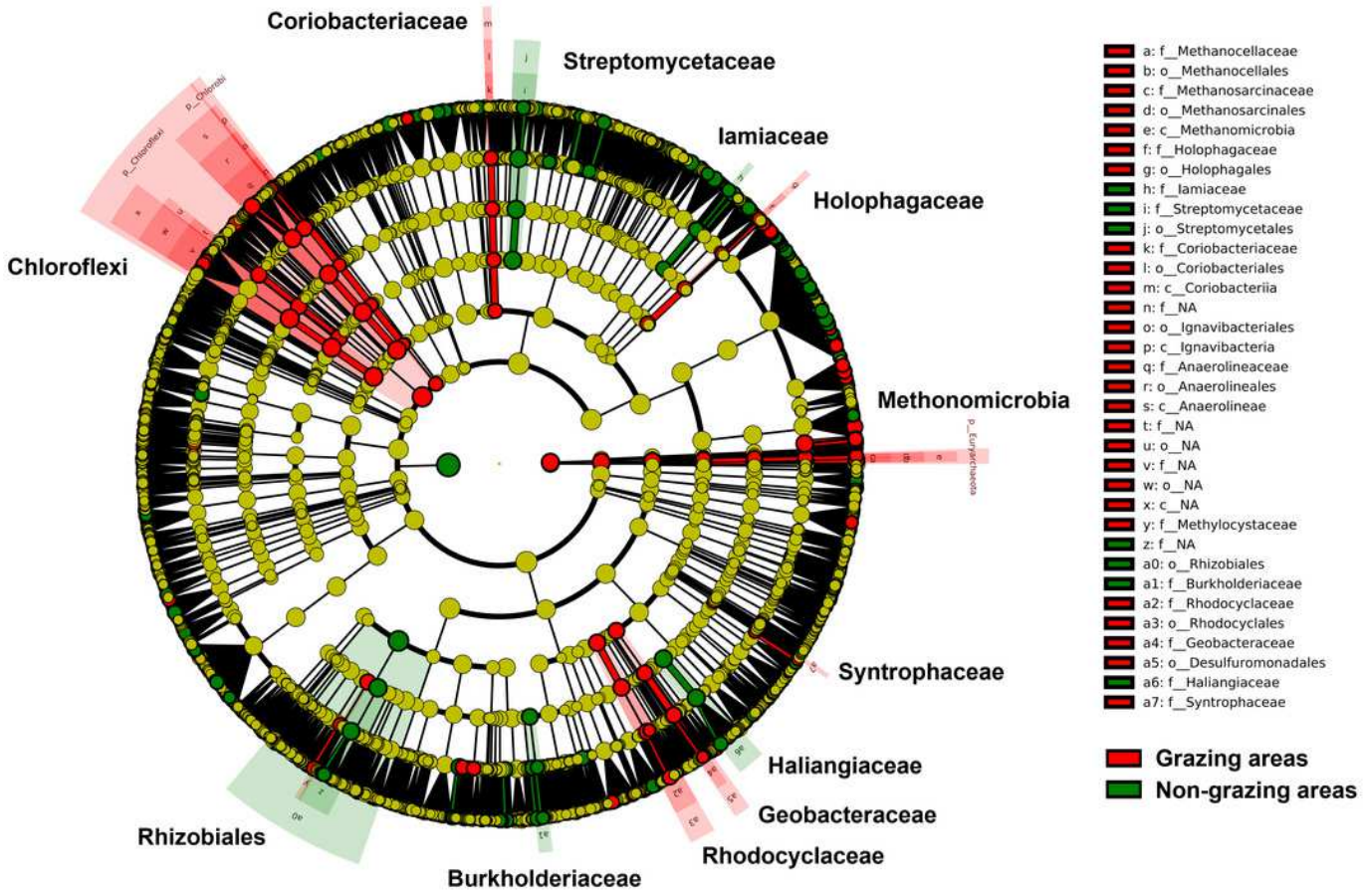

Figure 6

Relative abundance of Enterobacteriaceae and Streptomycetaceae in the different areas

Significance was determined using pair t-test ($p < 0.05$).

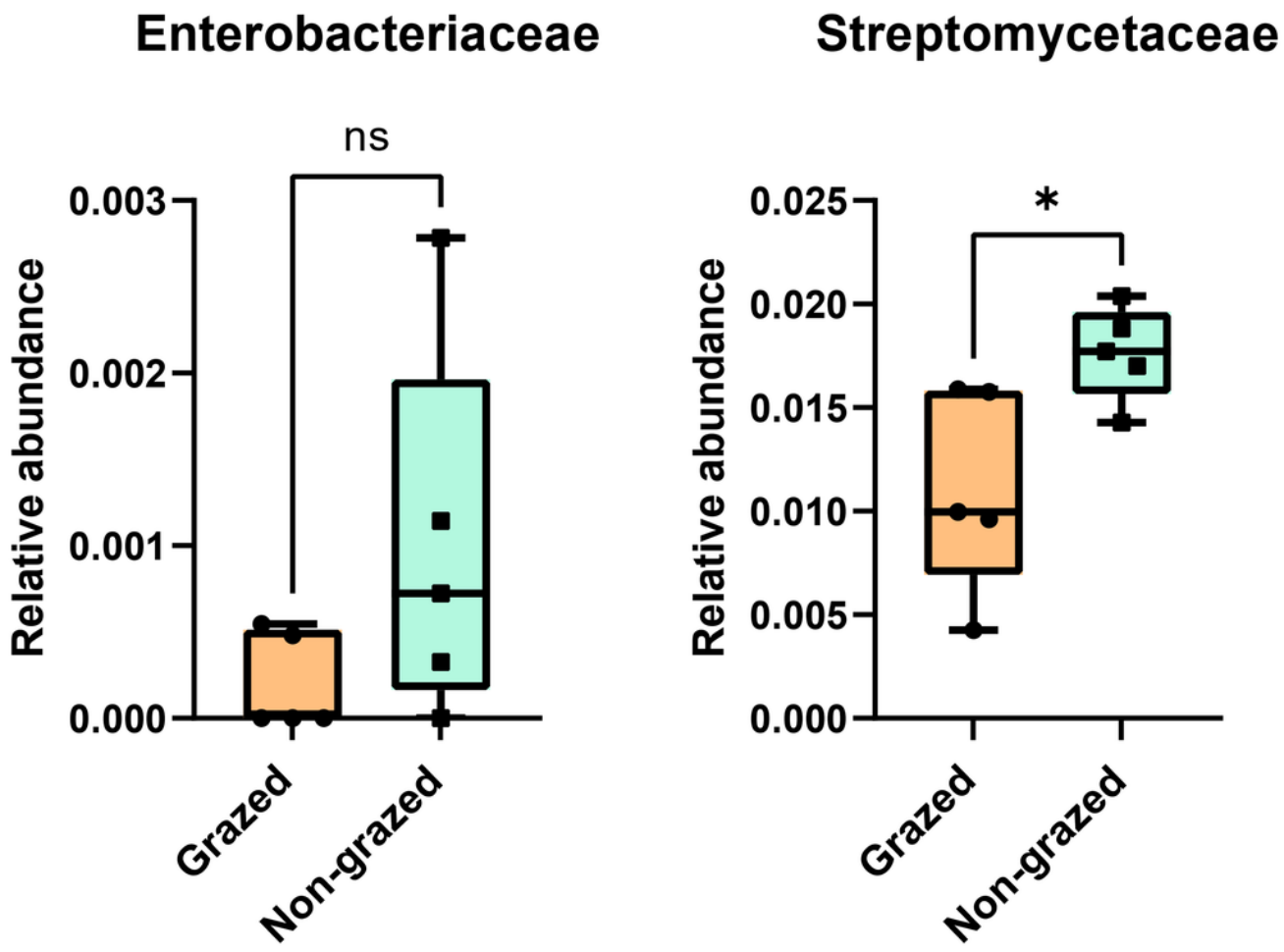

Figure 7

Figure 7 Abundance bar plot of Streptomycetaceae found in livestock-grazing and non-grazing perlite-rich soil.

The top 30 most abundant taxa are shown individually, all remaining taxa are grouped together as other.

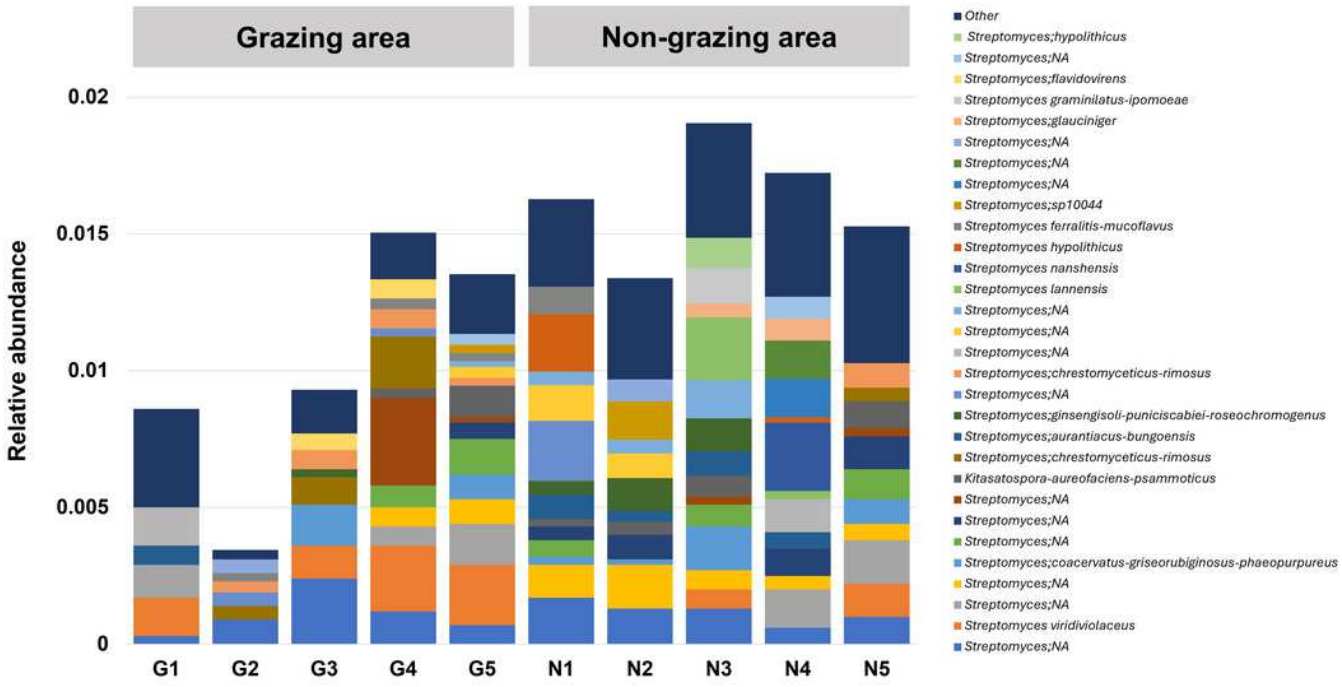

Figure 8

Phylogenetic analysis of Streptomycetaceae found in livestock grazing (A) and non-grazing (B) perlite-rich soil.

The phylogenetic tree was constructed based on 16S rRNA (V3-V4) sequences with the maximum-likelihood method using MEGA11.

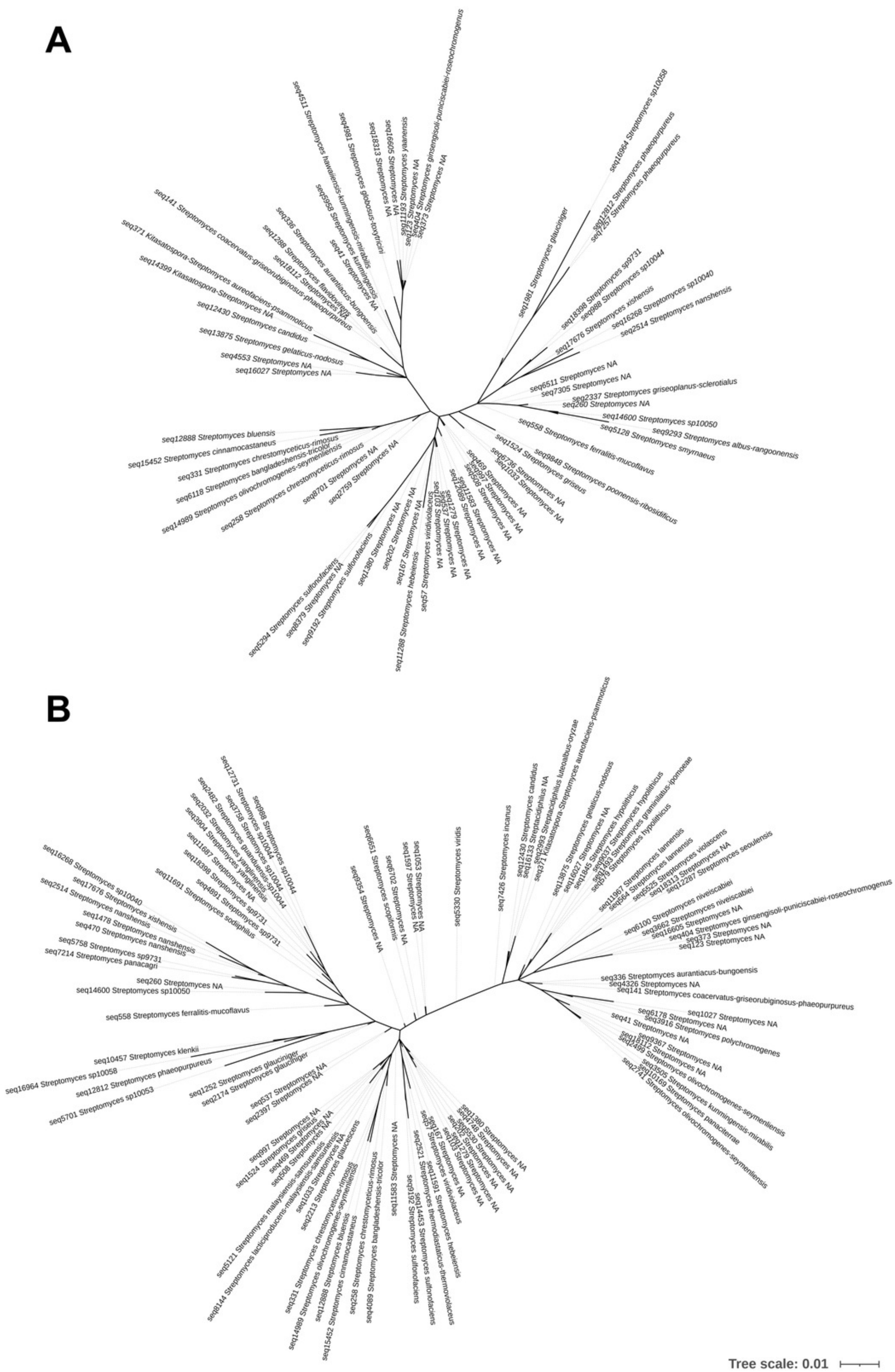
A
B
Tree scale: 0.01

Figure 9

Spearman’s rank correlation between the abundant phyla and soil physicochemical properties.

Circle with box indicates the significant correlation ($p < 0.05$).