EFFECT OF SHORT-TERM NITROGEN ADDITION ON THE SOIL NITROGEN-FIXING MICROBIAL COMMUNITY IN A KARST REGION

LU, Q. T. – LI, A. L. – LIU, Y. Z. – HU, G. – HU, C. – XU, C. H. – ZHONG, C. F.* – ZHANG, Z. H.*

Key Laboratory of Wildlife Evolution and Conservation in Mountain Ecosystem of Guangxi, College of Environmental and Life Sciences, Nanning Normal University, Nanning 530001, China

**Corresponding authors*
e-mail: zhong_cf@163.com; gxtczzh@126.com

(Received 10th Sep 2025; accepted 11th Dec 2025)

Abstract. While nitrogen (N) addition is known to alter microbial community structure and activity, it remains unknown how it affects the soil N-fixing community, particularly in karst ecosystems. This study implemented a field control experiment in the Nonggang karst region of Guangxi Province, with three distinct N addition treatments: control (CN, 0 kg N ha^{-1} yr^{-1}), low N (LN, 50 kg N ha^{-1} yr^{-1}), and high N (HN, 100 kg N ha^{-1} yr^{-1}). The response of N-fixing microbial composition and community structure was examined through *nifH* gene amplicon sequencing. Our results demonstrated that N addition significantly increased the Shannon diversity of the N-fixing community ($p < 0.05$) and markedly altered the community structure as evidenced by PCoA analysis results ($R^2 = 0.379$, $p = 0.001$). LN treatment led to a significant increase in the relative abundance of both the Firmicutes and Cyanobacteria phylum. In contrast, the *Bradyrhizobium* genus exhibited a different response pattern: its abundance was significantly reduced under both LN and HN treatments. LEfSe analysis further identified several differentially abundant biomarker taxa under different N addition treatments. This study confirmed that exogenous N addition can profoundly alter the composition of the soil N-fixing microbiome in karst areas, which may have a profound impact on N cycling processes and sustainability in this ecosystem.
Keywords: *karst soil, diversity, nitrogen fixation, nifH gene, microbial composition*

Introduction

Karst ecosystems are inherently fragile, with poor soil quality, and their nutrient cycling, especially the nitrogen (N) cycling, is highly susceptible to external N input interference (Chen et al., 2019). In light of continuous and often irrational applications of N fertilizers over time, elevated N inputs have profoundly impacted karst ecosystems. Preliminary studies have begun exploring how N addition affects karst plant communities, as well as soil carbon cycles, among other aspects (Wang et al., 2023a; Yuan et al., 2025). Nevertheless, the impact on key microbial functional groups within soils, especially nitrogen-fixing microorganisms, remains poorly understood.

The N fixation by microorganisms can convert molecular N into ammonia and other N-containing compounds (Kariman et al., 2022). Microbial N fixation is a crucial process for N input and plays a significant role in the N cycle (Dixon and Kahn, 2004). Soil N-fixing microorganisms exhibit various survival strategies, including autotrophs and symbiosis, and are widely distributed across terrestrial ecosystems (Fierer et al., 2007, 2011). Their metabolic activity is highly responsive to the soil's N status: in natural or low-N environments, their N fixation is often activated to mitigate N limitations; Conversely, the application of exogenous N fertilizers may induce feedback inhibition of their activity (Yang et al., 2024).

The application of N fertilizer is a critical strategy for achieving high crop yields in modern agricultural practices. However, extensive and frequent applications have

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_18531862

significantly impacted soil ecosystems (Wang et al., 2018; Zhong et al., 2020). Existing studies indicate that external N inputs can lead to alterations in the community structure of N-fixing microorganisms (Liu et al., 2020; Zhang et al., 2022). This typically manifests as a simplification of microbial communities involved in N fixation along with a succession trend towards dominance by eutrophic microorganisms. In forest and grassland ecosystems, the addition of N generally results in reduced diversity among N-fixing microbes while increasing the relative abundance of Proteobacteria (Zeng et al., 2016; Ling et al., 2017). Nevertheless, under certain extreme environmental conditions, these communities may display different trajectories of change (Sinsabaugh et al., 2015). Studies have demonstrated that N-fixing microorganisms present in karst soils predominantly exhibit heterotrophic characteristics and primarily belong to the phyla Proteobacteria, Firmicutes, and Cyanobacteria (Qi et al., 2025). Their genomes frequently contain the *nifH* gene responsible for encoding nitrogenase and have developed unique adaptive mechanisms over time (Reed et al., 2010; Kumar et al., 2017). However, driven by exogenous nitrogen input, how will the community structure, diversity, and key groups of N-fixing microorganisms change in karst soil? This gap represents a significant area for further research.

In this study, we conducted a short-term field experiment involving N additions at multiple levels (0, 50, and 100 kg N ha^{-1} yr^{-1}) in a typical karst region of Nonggang in Guangxi Province. The objective was to elucidate the response patterns of N-fixing microorganisms in karst soil under conditions of N deposition while systematically exploring several scientific questions: (1) How does N addition affect the diversity of the N-fixing microbial community in karst soil? (2) Does and how does N addition change the community composition of N-fixing microorganisms and screen out the key biomarker groups that are sensitive and tolerant?

Materials and methods

Study site

The field experiment on N addition was carried out in an area located at coordinates 106°48′E and 22°31′N within Nonggang, Chongzuo City, Guangxi Province—an area characterized by typical karst landforms. The climate is subtropical monsoon with an annual average temperature ranging from approximately 20°C to 28°C; additionally, the soil is classified as calcareous. Dominant above-ground plant species found in this region include *Alocasia macrorrhiza*, *Bidens pilosa*, *Pueraria lobata*, *Cipadessa baccifera*, *Alchornea trewioides*, *Vitex kwangsiensis*, *Amoora yunnanensis*, *Cleistanthus sumatranus*, *Ficus hispida*, *Excentrodendron tonkinense, Deutzianthus tonkinensis*, *Litsea variabilis*, etc.

Soil treatment and sample collection

The soil N addition experiment included three treatments: control (no N addition, CN), low N (50 kg N ha^{-1} yr^{-1}, LN), and high N (100 kg N ha^{-1} yr^{-1}, HN). The N addition levels were selected based on their consistency with experimental gradients used in previous karst ecosystem studies (Duan et al., 2023; Li et al., 2025), thereby facilitating cross-study comparisons. Additionally, the design was informed by the regional nitrogen deposition rate (<100 kg N ha^{-1} yr^{-1}) as reported by Wen et al. (2020). The N was added in the form of CH_4N_2O and applied in two equal amounts in April and October 2023. To minimize cross-interference between sample plots, a distance of 10 m was maintained between each treatment plot.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_18531862

Soil samples were collected in May 2024. Each treatment included six replicates, resulting in a total of 18 samples. Soil samples from 0 to 10 cm above the surface were collected using the diagonal sampling method. After removing visible litter, root systems, and stones, each sample was placed into a sterile 50 mL centrifuge tube and immediately frozen on dry ice for storage. Upon returning to the laboratory, the samples were stored at -80°C until DNA extraction could be performed.

DNA extraction and PCR amplification

For each soil sample, an aliquot of 0.3 g of dry weight soil was used for total genomic DNA extraction utilizing a DNA extraction kit. The quality and concentration of the extracted DNA were assessed using NanoDrop One (Thermo Fisher Scientific, MA, USA). The *nifH* gene fragment was amplified via real-time polymerase chain reaction (PCR) employing forward primer nifHF (AAAGGYGGWATCGGYAARTCCA) and reverse primer nifHR (TGSGCYTTGTCYTCRCGGATBGGCAT), yielding an amplification product approximately 400 bp in length. The PCR mixture consisted of a total volume of 50 μL containing: 3 μL of DNA template (20 ng/μL), 1 μL of each forward and reverse primer (10 μM), along 25 μL of 2x Premix Taq; nuclease-free water was added to complete the volume. Amplification conditions involved: 5 min at 94°C for initialization; 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 52°C, and 30 s extension at 72°C; followed by 10 min final elongation at 72°C. Sequence generation was conducted on the Illumina Nova6000 platform.

The generation of the operational taxonomic unit (OTU) table was conducted following the UPARSE pipeline. OTU sequences were selected at a 97% sequence similarity threshold and subsequently aligned with reference sequences. Sequences exhibiting poor alignment performance were discarded. Following this, all OTU sequences underwent manual verification to eliminate chimeras and pseudogenes.

Statistical analyses

Statistical and graphical analyses were performed within the R environment (Team, 2023). The α-diversity of nitrogen-fixing communities across different treatments was assessed using the Shannon-Wiener diversity index and Chao1 index, with calculations performed via the vegan package (Oksanen et al., 2022). To evaluate β-diversity, principal coordinate analysis (PCoA) based on Bray-Curtis distance was employed through the vegan package. Additionally, permutational multivariate analysis of variance (Adonis) was utilized to assess differences among treatments. The linear discriminant analysis effect size (LEfSe) was applied to identify biomarkers of each treatment (Segata et al., 2011). Furthermore, linear discriminant analysis (LDA) was implemented to evaluate the impact of significant species (Zhao et al., 2024), employing a logarithmic LDA score cutoff of >2.0 and a Kruskal-Wallis test p-value <0.05. All graphs were generated using the ggplot2 software package (Hadley et al., 2016).

Results

Soil N-fixing microbial community structure in response to N addition

The relative abundance of N-fixing microorganisms exhibited sensitivity to N addition. At the phylum level (*Fig. 1a*), regardless of the treatment, the Proteobacteria,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_18531862

Cyanobacteria, and Firmicutes were consistently dominant across CN, LN, and HN treatments; however, the relative abundance of Proteobacteria exhibited a slight decrease in the LN treatment that was not statistically significant. In comparison to CN, both Cyanobacteria and Firmicutes showed a significantly increased relative abundance in LN ($p < 0.05$), while a decline in Cyanobacteria was noted in HN that did not reach statistical significance. At the genus level (*Fig. 1b*), taxonomic patterns among different treatments were more complex: Compared to the CN treatment, the relative abundance of *Bradyrhizobium* significantly decreased in both LN and HN ($p < 0.05$). Conversely, *Hyphomiocrobium* and *Sulfurivermis* experienced significant increases ($p < 0.05$). In addition, *Paenibacillus* also showed a notable increase in LN ($p < 0.05$), while *Azoarcus* significantly increased in HN ($p < 0.05$). A specific comparison between LN and HN treatments revealed a decline in *Calothrix* within HN; meanwhile, *Hyphomiocrobium* and *Azoarcus* experienced increases that were not statistically significant.

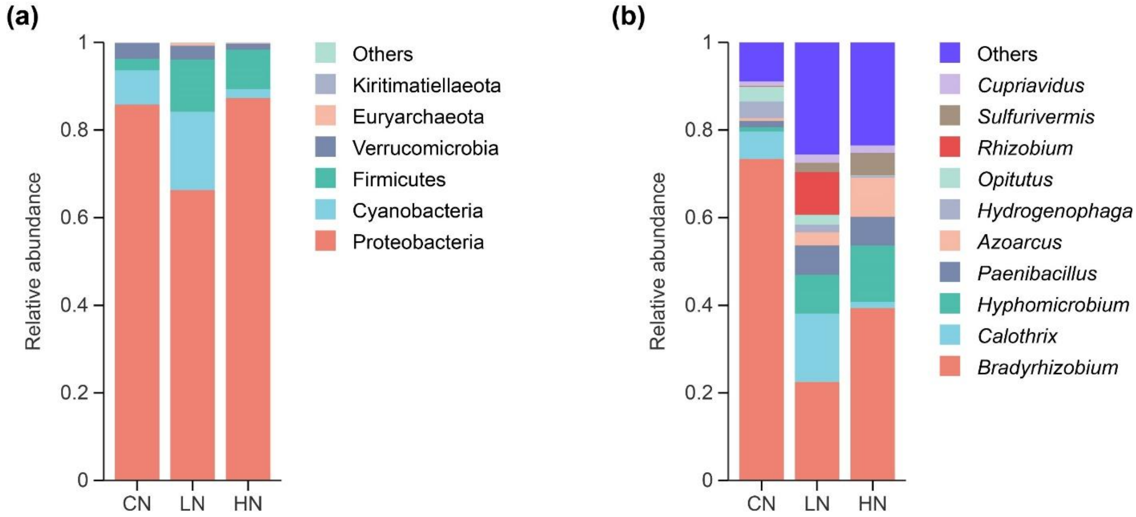

Figure 1. *Relative abundance and composition of the N-fixing microbial community containing the nifH gene at the phylum level (a) and genus level (b) in the three N addition treatments*

Effects of N addition on the diversity of N-fixing microbial communities

N addition had differential effects on diversity indices of the soil N-fixing microbial community. Although no significant differences were detected for the Chao1 index across treatments ($p > 0.05$, *Fig. 2a*), both LN and HN treatments significantly increased the Shannon index when compared to CN ($p < 0.05$, *Fig. 2b*). However, there was no significant statistical difference between the Shannon index for LN and HN treatments.

Beta diversity analysis further demonstrated a significant effect of N application on the community structure of N-fixing microorganisms. Principal coordinate analysis (PCoA) based on Bray-Curtis distances showed clear separation among samples under different N treatments (Adonis, $R^2 = 0.379$, $p = 0.001$), indicating that N addition explained 37.9% of the variation in community structure (*Fig. 3*). The first principal coordinate (PCoA1) explained 36.5% of this variance, while the second (PCoA2) accounted for an additional 11.33%. These results suggest that although N application did not alter the richness of N-fixing microbial communities, it significantly enhanced community diversity and markedly reshaped their community structure.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_18531862

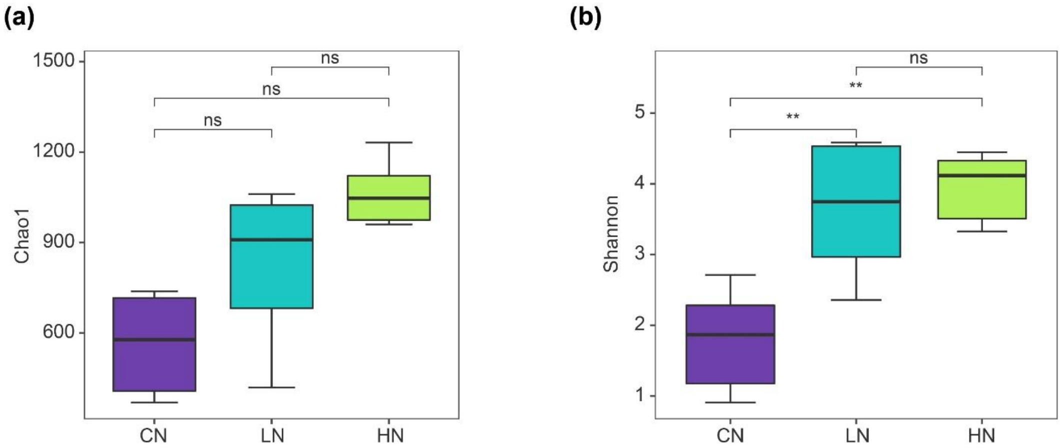

Figure 2. *Comparison of nitrogen-fixing microbial alpha-diversity under different nitrogen addition treatments: (a) Chao1 index and (b) Shannon index*

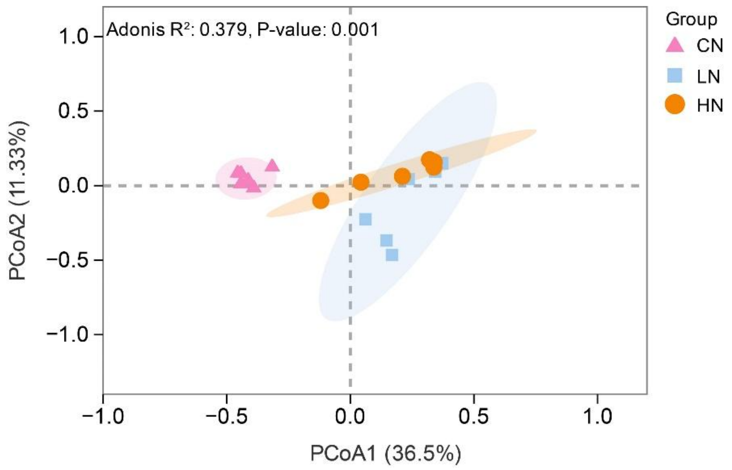

Figure 3. *Principal coordinate analysis (PCoA) of N-fixing community compositions (OTU level) under different N addition treatments*

Biomarker under different N addition treatments

LEfSe analysis revealed significant differences in the composition of soil N-fixing microbial taxa among the three N addition treatments (LDA score (log 10) >2.0, Kruskal-Wallis test $p < 0.05$; *Fig. 4*). Specifically, three biomarkers (Bradyrhizobiaceae class, Hyphomicrobiales order, and *Bradyrhizobium* genus) were found to be enriched in the CN treatment. Under the LN treatment, a total of 28 taxa were identified as significantly enriched biomarkers across multiple taxonomic levels, including the Cyanobacteria phylum, Bacilli class, Nostocales order, Paenibacillaceae family, and *Geomonas* genus. Most of these microorganisms were oligotrophic or facultative trophic bacteria, indicating that the low N addition may confer a selective advantage for these taxa. In contrast, the HN treatment resulted in the detection of 30 specific biomarkers, among them were copiotrophic bacteria such as the Enterobacterales order, Enterobacteriaceae family, Erwiniaceae family, and *Pantoea* genus. This indicates that high N input significantly promoted the enrichment of microbial taxa capable of rapidly utilizing abundant nutrient resources.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_18531862

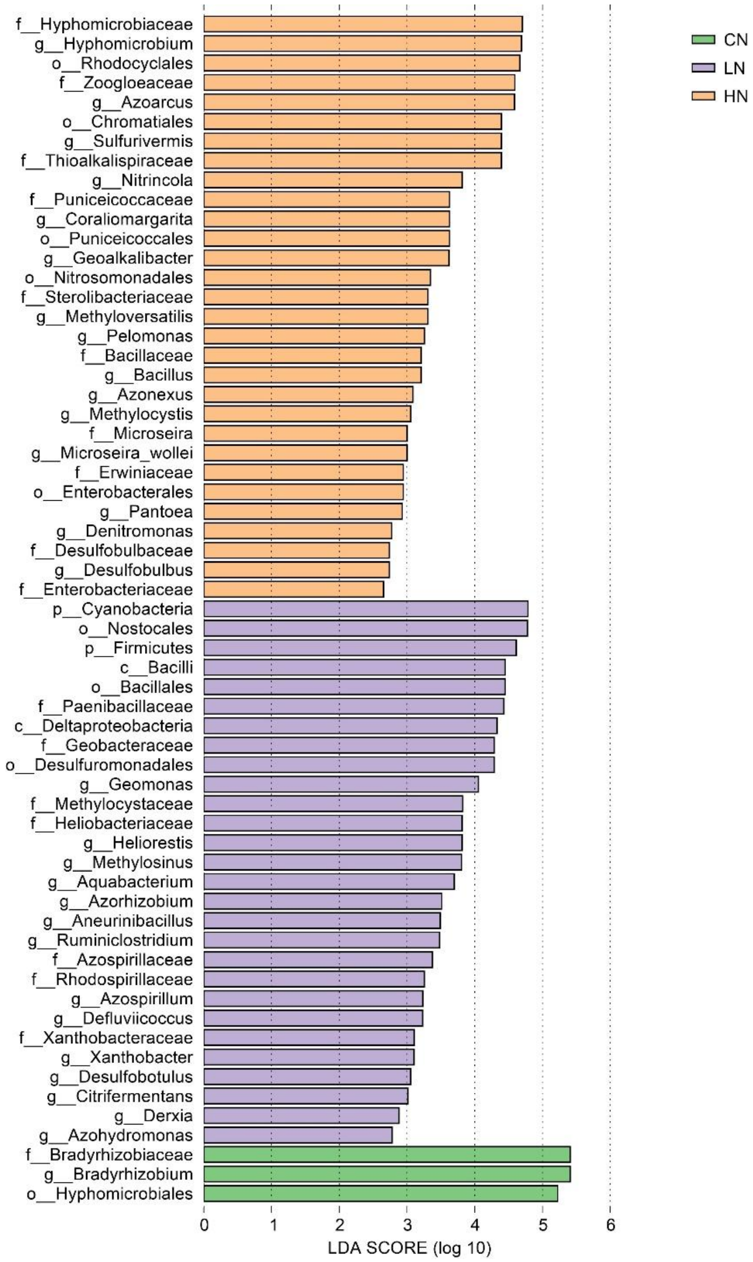

Figure 4. *Differentially abundant N-fixing bacterial taxa as assessed using LEfSe analysis in the three N addition treatments of karst soils*

Discussion

This study investigated the changes in the community structure of N-fixing microorganisms in karst soil under different levels of N addition. The findings revealed significant differences in the response patterns of different taxonomic units to N input. Specifically, the Proteobacteria, Cyanobacteria, and Firmicutes emerged as the dominant phyla in the N-fixing microbial communities of karst soil. N addition was found to alter the composition of soil N-fixing microbial community, leading to alterations in the relative abundance of specific taxa. For example, our results indicated that the relative abundance of the Firmicutes increased under LN treatment, while the Verrucomicrobia exhibited a slight decline. This suggests that N addition can increase

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_18531862

the relative abundance of eutrophic bacteria (such as the Firmicutes) at the expense of oligotrophic bacteria (such as the Verrucomicrobia), by alleviating their N limitation (Meng et al., 2019; Hu et al., 2021), which is consistent with our observations. In addition, notable differences were observed in N-fixing community structures resulting from various levels of N addition. For example, while the Cyanobacteria showed an increase in relative abundance under LN treatment, their abundance declined when exposed to HN conditions. These findings corroborate previous studies indicating that added N exerts a gradient effect on different N-fixing microorganisms (Zhu et al., 2018; Wang et al., 2022; Liu et al., 2020).

The experiments involving N addition conducted during this study took place within karst ecosystems characterized by relatively poor nutrient availability where microbial populations are typically constrained by limited sources of N (Yang et al., 2023b). Notably, there was no significant impact on the Chao1 index for the N-fixing community due to N addition, suggesting that such additions did not substantially alter species richness within this soil community. This outcome aligns with findings reported by Wang et al. (2023b), indicating that the number of species may not be sensitive to N input in the short term. Compared to CN treatment, both LN and HN treatments significantly increased the Shannon diversity index. This enhancement can be attributed to the additional N input alleviating the N source limitation for microorganisms, thereby promoting the diversity of soil N-fixing microbial communities (Averill and Waring, 2018). This finding aligns with previous research indicating that N input fosters the growth of originally N-restricted microbial groups (Yang et al., 2023a; Duan et al., 2023). In contrast, Cao et al. (2024) reported a decrease in bacterial diversity within subtropical forest following N additions, suggesting that the effects of N addition on bacterial diversity are variable and likely dependent on site-specific conditions.

Furthermore, this study demonstrated that N addition significantly changed microbial community structure, which may be associated with varying levels of N application. Different treatments also resulted in distinct microbial biomarkers within soil N-fixing communities. These variations could be explained by changes in certain taxa sensitive to N addition. The CN treatment was characterized by oligotrophic taxa such as the *Bradyrhizobium*, consistent with prior studies indicating that nutrient-poor soils favor *k*-strategists adapted to nutrient scarcity (Gao et al., 2025). These organisms potentially rely on symbiotic N fixation as a compensatory mechanism for limited exogenous N inputs, an adaptive strategy commonly observed in unfertilized ecosystems. In contrast, the LN treatment exhibited a distinct profile dominated by N-fixing bacteria (*Azospirillum*, *Derxia*) and the Cyanobacteria (Nostocales). This indicates that moderate N application can stimulate autotrophic organisms to fix atmospheric N_2 effectively while enhancing overall microbial-driven N supply. Yin et al. (2025) also noted that short-term N addition might effectively alleviate soil nutrient limitations; thus, responses among oligotrophic and copiotrophic microbial taxa differ based on their ecological strategies regarding N availability. Conversely, HN treatment was associated with an enrichment of copiotrophic *r*-strategists such as the Enterobacteriaceae and *Bacillus*, alongside numerous taxa involved in anaerobic processes like the Desulfobulbus and *Denitromonas*. This pattern aligns with existing concepts positing that excessive N input promotes the growth of fast-growing microorganisms while simultaneously increasing oxygen consumption through enhanced microbial activity, leading to microsite anaerobiosis.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_18531862

Conclusions

This study demonstrated that short-term N addition significantly altered the community structure of N-fixing microbes in a karst region of southern China. The input of N has enhanced the dominance of N-sensitive microbial taxa, thereby facilitating the emergence of a more diverse microbial community. Furthermore, while short-term N addition did not affect Chao1 richness, it did increase the Shannon index of N-fixing communities. Different levels of N addition markedly influenced the biomarkers of soil N-fixing microbial communities. Our study was conducted through short-term field experiments. Moving forward, it will be essential to monitor the impact of continuous N addition on soil N-fixing microbes to provide a foundation for predicting changes in environmental conditions in karst areas resulting from N enrichment.

Acknowledgements. This work was supported by the Guangxi Natural Science Foundation (2025GXNSFBA069350), the National Natural Science Foundation of China (42301073, 32260062, and 42467008), and the Guangxi Science and Technology Base and Talent Special Project (AD22080023).

REFERENCES

[1] Averill, C., Waring, B. (2018): Nitrogen limitation of decomposition and decay: How can it occur? – Global Change Biology 24: 1417-1427.
[2] Cao, J., Li, L., Han, Y., Liu, Z., Lai, F., Yang, Y. (2024): Warming mitigates the effects of nitrogen addition on the soil diazotrophic community in a subtropical forest. – Applied Soil Ecology 203: 105686.
[3] Chen, H., Li, D., Mao, Q., Xiao, K., Wang, K. (2019): Resource limitation of soil microbes in karst ecosystems. – Science of the Total Environment 650: 241-248.
[4] Dixon, R., Kahn, D. (2004): Genetic regulation of biological nitrogen fixation. – Nature Reviews Microbiology 2: 621-631.
[5] Duan, P., Xiao, K., Wang, K., Li, D. (2023): Responses of soil respiration to nitrogen addition are mediated by topography in a subtropical karst forest. – Catena 221: 106759.
[6] Fierer, N., Bradford, M. A., Jackson, R. B. (2007): Toward an ecological classification of soil bacteria. – Ecology 88: 1354-1364.
[7] Fierer, N., Lauber, C. L., Ramirez, K. S., Zaneveld, J., Bradford, M. A., Knight, R. (2011): Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. – The ISME Journal 6: 1007-1017.
[8] Gao, Y., Zhou, J., Lin, T.-C., Li, Y., Zeng, Q., Chen, S., Xiong, D., Zhang, Q., Yang, Z., Yang, Y. (2025): The dominance of K-strategy microbes enhances the potential of soil carbon decomposition under long-term warming. – Applied Soil Ecology 206: 105854.
[9] Hadley, W., Winston, C., Lionel, H., Thomas, L. P., Kohske, T., Claus, W., Kara, W., Hiroaki, Y., Dewey, D., Teun, V. D. B. (2016): ggplot2: Elegant Graphics for Data Analysis. – Springer-Verlag, New York.
[10] Hu, J., Richwine, J. D., Keyser, P. D., Li, L., Yao, F., Jagadamma, S., Debruyn, J. M. (2021): Nitrogen fertilization and native C4 grass species alter abundance, activity, and diversity of soil diazotrophic communities. – Frontiers in Microbiology 12(2021): 675693.
[11] Kariman, K., Moreira-Grez, B., Scanlan, C., Rahimlou, S., Boitt, G., Rengel, Z. (2022): Synergism between feremycorrhizal symbiosis and free-living diazotrophs leads to improved growth and nutrition of wheat under nitrogen deficiency conditions. – Biology and Fertility of Soils 58: 121-133.

[12] Kumar, U., Panneerselvam, P., Govindasamy, V., Vithalkumar, L., Senthilkumar, M., Banik, A., Annapurna, K. (2017): Long-term aromatic rice cultivation effect on frequency and diversity of diazotrophs in its rhizosphere. – Ecological Engineering 101: 227-236.
[13] Li, J., Zhao, J., Liao, X., Wang, W., Long, X., Liu, Y., Xiao, J., Zhang, W., Wang, K. (2025): Nitrogen deposition exhibits limited influence on soil nematode energy fluxes and soil carbon and nitrogen mineralization in a typical karst ecosystem. – Soil Ecology Letters 7: 250298.
[14] Ling, N., Chen, D., Guo, H., Wei, J., Bai, Y., Shen, Q., Hu, S. (2017): Differential responses of soil bacterial communities to long-term N and P inputs in a semi-arid steppe. – Geoderma 292: 25-33.
[15] Liu, W., Jiang, L., Yang, S., Wang, Z., Tian, R., Peng, Z., Chen, Y., Zhang, X., Kuang, J., Ling, N., Wang, S., Liu, L. (2020): Critical transition of soil bacterial diversity and composition triggered by nitrogen enrichment. – Ecology 101: e03053.
[16] Meng, H., Zhou, Z., Wu, R., Wang, Y., Gu, J.-D. (2019): Diazotrophic microbial community and abundance in acidic subtropical natural and re-vegetated forest soils revealed by high-throughput sequencing of nifH gene. – Applied Microbiology and Biotechnology 103: 995-1005.
[17] Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. D., Durand, S., Evangelista, H. B. A., Fitzjohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M. O., Lahti, L., Mcglinn, D., Ouellette, M.-H., Cunha, E. R., Tyler Smith, A. S., Braak, C. J. F. T., Weedon, J. (2022): vegan: Community Ecology Package. R package version 2.6-4. – https://CRAN.R-project.org/package=vegan.
[18] Qi, Y., He, Y., Yao, L., Yan, Q., Wu, C., Wu, Y., Wang, J. (2025): Relationships between nitrogen-fixing bacteria community structure in Vicia villosa nodules, soil properties and rocky desertification degree in karst area southwest China. – PLOs ONE 20: e0329408.
[19] Reed, S. C., Townsend, A. R., Cleveland, C. C., Nemergut, D. R. (2010): Microbial community shifts influence patterns in tropical forest nitrogen fixation. – Oecologia 164: 521-531.
[20] Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., Huttenhower, C. (2011): Metagenomic biomarker discovery and explanation. – Genome Biology 12: R60.
[21] Sinsabaugh, R. L., Belnap, J., Rudgers, J., Kuske, C. R., Martinez, N., Sandquist, D. (2015): Soil microbial responses to nitrogen addition in arid ecosystems. – Frontiers in Microbiology 6(2015): 819.
[22] Team, R. C. (2023): R: A Language and Environment for Statistical Computing. – R Foundation for Statistical Computing, Vienna.
[23] Wang, C., Liu, D., Bai, E. (2018): Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. – Soil Biology and Biochemistry 120: 126-133.
[24] Wang, C., Zhou, Z., Li, Y., Kong, J., Dong, H. (2023a): Effects of changes in land use structure on nitrogen input in the Pingzhai Reservoir watershed, a karst mountain region. – Heliyon 9: e16262.
[25] Wang, L., Zhang, H., Wang, J., Wang, J., Zhang, Y. (2022): Long-term fertilization with high nitrogen rates decreased diversity and stability of diazotroph communities in soils of sweet potato. – Applied Soil Ecology 170: 104266.
[26] Wang, X., Feng, J., Ao, G., Qin, W., Han, M., Shen, Y., Liu, M., Chen, Y., Zhu, B. (2023b): Globally nitrogen addition alters soil microbial community structure, but has minor effects on soil microbial diversity and richness. – Soil Biology and Biochemistry 179: 108982.
[27] Wen, Z., Xu, W., Li, Q., Han, M., Tang, A., Zhang, Y., Luo, X., Shen, J., Wang, W., Li, K., Pan, Y., Zhang, L., Li, W., Collett, J. L., Zhong, B., Wang, X., Goulding, K., Zhang,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_18531862

F., Liu, X. (2020): Changes of nitrogen deposition in China from 1980 to 2018. – Environment International 144: 106022.
[28] Yang, R., Yang, Z., Yang, S., Chen, L.-L., Xin, J., Xu, L., Zhang, X., Zhai, B., Wang, Z., Zheng, W., Li, Z. (2023a): Nitrogen inhibitors improve soil ecosystem multifunctionality by enhancing soil quality and alleviating microbial nitrogen limitation. – Science of The Total Environment 880: 163238.
[29] Yang, T., Zhang, H., Zheng, C., Wu, X., Zhao, Y., Li, X., Liu, H., Dong, L., Lu, Z., Zhou, J., Peng, X. (2023b): Bacteria life-history strategies and the linkage of soil C-N-P stoichiometry to microbial resource limitation differed in karst and non-karst plantation forests in southwest China. – Catena 231: 107341.
[30] Yang, Z., Dai, H., Huang, Y., Dong, B., Fu, S., Zhang, C., Li, X., Tan, Y., Zhang, X., Zhang, X. (2024): Driving mechanisms of soil bacterial α and β diversity under long-term nitrogen addition: subtractive heterogenization based on the environment selection. – Geoderma 445: 116886.
[31] Yin, H., Xu, M., Huang, Q., Xie, L., Yang, F., Zhang, C., Sha, G., Cao, H. (2025): Response of soil bacteria to short-term nitrogen addition in nutrient-poor areas. – Microorganisms 13: 56.
[32] Yuan, Y., Du, X., Yin, Y., Xia, G. (2025): Short-term nitrogen addition improves soil phosphorus availability by regulating phosphorus-cycling microbial communities in a karst forest. – Geoderma 461: 117478.
[33] Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., Chu, H. (2016): Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. – Soil Biology and Biochemistry 92: 41-49.
[34] Zhang, R.-T., Liu, Y.-N., Zhong, H.-X., Chen, X.-W., Sui, X. (2022): Effects of simulated nitrogen deposition on the soil microbial community diversity of a Deyeuxia angustifolia wetland in the Sanjiang Plain, Northeastern China. – Annals of Microbiology 72: 11.
[35] Zhao, S., Zhang, B., Yang, J., Zhou, J., Xu, Y. (2024): Linear discriminant analysis. – Nature Reviews Methods Primers 4: 70.
[36] Zhong, Y., Liu, J., Jia, X., Shangguan, Z., Wang, R., Yan, W. (2020): Microbial community assembly and metabolic function during wheat straw decomposition under different nitrogen fertilization treatments. – Biology and Fertility of Soils 56: 697-710.
[37] Zhu, C., Tian, G., Luo, G., Kong, Y., Guo, J., Wang, M., Guo, S., Ling, N., Shen, Q. (2018): N-fertilizer-driven association between the arbuscular mycorrhizal fungal community and diazotrophic community impacts wheat yield. – Agriculture, Ecosystems & Environment 254: 191-201.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_18531862