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Detection of Arbuscular Mycorrhizal Fungi in the Root of *Allium fistulosum* Grown in the Fumigated Field

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Abstract

Soil fumigation is an important pre-plantation practice to maximize land productivity. This practice not only effectively eliminate soilborne pests and pathogens, but also affects the beneficial soil microbial community, including arbuscular mycorrhizal (AM) fungi. In this study, the AM fungi associated with the *Allium fistulosum* roots were identified to understand the effect of fumigation treatment on the fungi. The roots have been collected from fumigated and non-fumigated fields. The DNA has been extracted, and then the fungal large subunit (LSU) ribosomal RNA gene has been amplified and sequenced. Two and three AM fungal operational taxonomic units (OTUs) have been detected from the fumigated and non-fumigated fields, respectively. Decomposer and pathogenic fungi were detected to coexist with the AM fungi, suggesting the resilience of these fungi upon fumigation treatment.

1. Introduction

Soil fumigation is a widely used pre-plantation practice in intensive agriculture aiming to manage soilborne pests, pathogens, and weeds. By temporarily sterilizing the soil, fumigation helps protect early-stage crops and promotes uniform establishment. With rising global food demand, the application of fumigants has become essential for optimizing productivity on limited agricultural land. Several chemical fumigants, including methyl bromide, chloropicrin, phosphine, dazomet, and metam sodium, have been introduced (Yan et al. 2025), and their effectiveness has been studied in various plant commodities (Gao et al. 2016; Yan et al. 2019, 2022).

While effective in pest and disease control, soil fumigation can negatively impact non-target soil organisms. Studies have reported declines in beneficial microbial communities, including bacteria, saprotrophs, and mycorrhizal fungi, following fumigation (Dangi et al. 2017; Fang et al. 2020; Li et al. 2022). These microbes play vital roles in nutrient cycling, organic matter decomposition, and overall soil health. Loss of microbial diversity can compromise long-term soil fertility and sustainability, raising concerns about the ecological costs of chemical soil treatments.

Soil fumigation is commonly applied in *Allium fistulosum*, an important horticultural species valued for both culinary and medicinal uses (Kim et al. 2023). This species is cultivated globally, with major production in East Asia, particularly China, Japan, and Korea (Padula et al. 2022). With the growing demand for *A. fistulosum*, farmers are facing emerging soilborne diseases such as leaf sheath rot (Misawa et al. 2017), leaf blight (Wang et al. 2021), and basal rot (Le et al. 2021). These challenges often necessitate the use of chemical inputs, including fumigants and fungicides, to maintain yield and crop quality.

On the other hand, *A. fistulosum* is known to associate with arbuscular mycorrhizal (AM) fungi, important soilborne symbionts that colonize plant roots and enhance nutrient uptake, particularly phosphorus, while improving plant resilience to environmental stressors. In return, AM fungi receive carbohydrates from the host plant (Smith and Read 2008). Field inoculation of *A. fistulosum* has been studied and reported to increase the yield and reduce dependence on chemical fertilizers (Tawaraya et al. 2012; Suzuki et al. 2021). Given the sensitivity of AM fungi to soil disturbances, including fumigation, it is essential to understand how chemical treatments affect their presence in the associated host plant roots. Disruptions to these symbiotic communities could compromise

their functional benefits and impact long-term crop productivity and soil health. Thus, a study investigating the effects of fumigation on AM fungi in *A. fistulosum* is necessary.

In this study, we observe AM fungi associated with *A. fistulosum* roots grown in fumigated and non-fumigated soils. We hypothesize that fumigation alters the AM fungal community structure, resulting in different taxa being detected between treated and untreated fields. Understanding these changes is crucial for evaluating the broader ecological implications of soil fumigation and for developing more sustainable agricultural practices that consider both crop performance and soil microbial conservation.

2. Materials and Methods

2.1. Root Sample Collection

Root samples were collected from post-harvest Dazomet-fumigated and non-fumigated *A. fistulosum* fields, with a history of *Rhizophagus clarus* (previously known as *Glomus clarum*) field inoculation. Tap water was used to wash the root samples and to remove soil particles. Clean root samples were cut into small pieces (around 1–2 mm long) and dried on a paper towel. Then, the roots were stored in a -20 °C freezer for one night for further analysis.

2.2. Molecular Identification of AM Fungi

One hundred mg of frozen root sample was ground in a 2 ml bead-containing tube. DNA extraction of the root sample was performed using the UltraClean™ Soil DNA Isolation Kit following the instructions from the manufacturer. The D1/D2 region of the large subunit ribosomal RNA gene (LSU rDNA) was amplified in a 20 µl reaction mixture of the Expand High-Fidelity kit containing 0.5 µM of LR1 (GCATATCAATAAGCGG) and FLR2 (GTCGTTTAAAGCCATT) primer pairs, and 2 µl of template DNA solution using PCR Thermal Cycler with the following program: 120 seconds of initial denaturation at 94 °C, 29 cycles of denaturation at 94 °C for 15 seconds, 60 seconds of annealing at 50 °C, and 80 seconds of extension at 72 °C. The success of amplification was confirmed with an electrophoresis system by running the PCR products on 1% agarose gel in TBE buffer. Six hundred to 800 bp bands were cut (size of PCR products was expected to be 680–780 bp, Kawahara and Ezawa 2013) and purified by the MonoFas® DNA Purification Kit.

The purified DNA was inserted into pT7Blue T-Vector with DNA Ligation Kit, cloned into competent cells of *Escherichia coli*, and grown in Luria Bertani (LB) medium supplemented with IPTG, X-gal, and ampicillin. Plates with transformed *E. coli* were incubated for 16 hours at 37 °C and stored in a dark room. Ten randomly chosen single colonies of transformed *E. coli* per plate were amplified using the direct colony PCR technique with a mixture of GoTaq® DNA Polymerase, U19 (GTTTTCCCAGTCACGACT) and T7 (TAATACGACTCACTATAG) primer pairs, under the above-mentioned PCR conditions. The products of the colony PCR were used for sequencing and analyzed in a sequencer. The sequence data were assigned to fungal operational taxonomic unit (OTU) by conducting a BLASTN search against the NCBI database with ≥97% similarity and a BLAST e-value of < 1e-50.

3. Results and Discussion

3.1. Isolation of Targeted DNA

The first detection of the AM fungi in the root of *A. fistulosum* was conducted by checking the presence of the amplified DNA in an electrophoresis gel and comparing it to the 1,000/100 bp DNA ladder. The amplified DNA of *A. fistulosum* root from the fields with and without fumigation showed the targeted band after examination in the electrophoresis gel (Table 1). Four bands in the range of 600–800 bp were selected, purified, and cloned into the *E. coli* competent cell and incubated for 16 hours. A total of 39 colonies were chosen for colony PCR and sequenced.

Table 1. Detection of 600–800 bp band from the PCR product by electrophoresis

Root sample	Detected band range (bp)	
	600–700	700–800
Fumigated	+	+
Non-fumigated	+	+

*Note: the (+) mark indicates the presence of the band

3.2. AM fungal sequence detected from *A. fistulosum* root

AM fungal species colonizing the root of *A. fistulosum* in both fields had been identified. The PCR products of the 39 colonies were successfully sequenced and assigned to fungal sequences. Twenty sequences (51%) among the

total sequences were assigned to the OTU of AM fungal species, while 19 sequences (49%) were assigned to other fungi (Figure 1a). The number of sequences that were assigned to AM fungal OTU and non-AM fungal OTU was almost similar in this study, where AM fungal sequences were mostly detected from the 700–800 bp band, while the lower band (600–700 bp) shows non-AM fungi (Figure 1b).

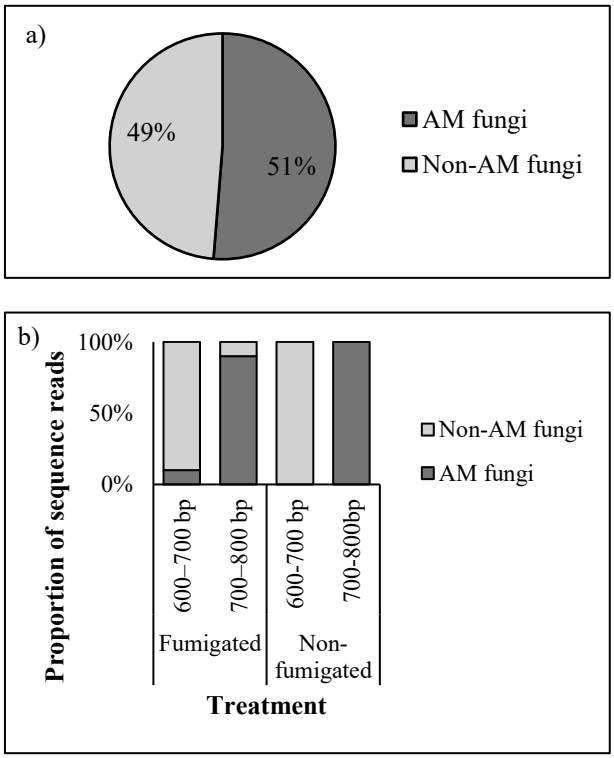

Figure 1. a) Composition and b) proportion of AM fungal and non-AM fungal sequences detected from *A. fistulosum* root.

Four AM fungal OTUs were detected in the root of *A. fistulosum*, in which two and three OTUs were detected from the sample collected in the fumigated field and non-fumigated field, respectively. In the fumigation treatment, *Rhizophagus clarus* (previously known as *Glomus clarum*) and *Gigaspora margarita* were detected. Whereas, in the non-fumigated treatment, *R. clarus*, *Claroideoglomus claroideum* (previously known as *G. claroideum*), and uncultured Glomeromycota were detected (Table 2). *R. clarus* was found in both fumigated and non-fumigated fields (Table 2), which can be due to the field inoculation history of this species. This species is known as a ruderal fungus that can rapidly occupy the niche.

The presence of *Gi. A margarita* under fumigation treatment may indicate its ability to survive and thrive in a chemically disturbed area. AM fungi were reported to respond differentially to disturbance types, with the Glomeraceae being most tolerant to various types of disturbance, the Gigasporaceae being tolerant to chemical disturbance, and the Acaulosporaceae being the least tolerant to all disturbance (van der Heyde et al. 2017). *Gi. margarita* is characterized by a huge white spore ranging from 260–480 µm (Bonfante 2022), invests more biomass in the extraradical mycelium, and regenerates mainly from spore propagules (Hart and Reader 2002). The large size of the spore may facilitate the survival of *Gi. margarita* during contact with the chemical released during the decomposition of the fumigant. In the non-fumigated field, however, *Gi. margarita* was absent. This may be due to the competition with *R. clarus* and *C. claroideum*.

3.3. Non-AM fungal species detected from *A. fistulosum* root

In this study, nearly half of the fungal reads were identical to non-AM fungal OTU, indicating the coexistence of those fungi with the AM fungal species in the ecosystem. A total of six non-AM fungal OTUs were detected, of which four OTUs were detected in fumigated and three were detected in non-fumigated fields (Table 3). The composition of non-AM fungal OTUs was different in both fields. Three decomposer species and one pathogen were detected in the fumigated field. In contrast, all the species detected in the non-fumigated field were pathogenic fungi. This result indicates that the fumigation reduces the pathogenic fungi while maintaining the beneficial fungi, consistent with the previous studies in other agricultural commodities (Chen et al. 2022; Lin et al. 2024; Mao et al. 2024).

Rhizoctonia spp. was reported to cause leaf sheath rot in *A. fistulosum (Misawa et al. 2017)*, while *Ceratobasidium sp.* was reported to cause root rot in *A. sativum* (Yin et al. 2020) and peanut (Li et al. 2025). *Rhizoctonia butinii* was absent in the fumigated field, but *Ceratobasidium sp.* was still present, indicating that fumigation is only effective against certain species. Limited information was found on the effect of *Ceratobasidium sp.* on *A. fistulosum* growth and productivity; thus, further study is necessary. The decomposer species, such as *Mrakia aquatica*, *Tetradium furcatum*, and *Tetracladium sp*., were detected in the fumigated field. This can be a good sign that not all beneficial fungi are affected by the fumigation treatment and therefore can maintain their ecological role in the ecosystem. However, further study is needed to understand the extent to which fumigation treatments do not affect the activity of those beneficial fungi.

Table 2. AM fungal OTU detected from *A. fistulosum* root

Treatment	Band range (bp)	AM fungi detected	Similar sequence	Accession number	Similarity
Fumigated	600–700 bp	*Rhizophagus clarus*	*Glomus clarum* Att894-7 clone pHS029-28	FM865542.1	99
	700–800 bp	*Rhizophagus clarus*	*Glomus clarum* Att894-7 clone pHS029-28	FM865542.1	100
		Gigaspora margarita	*Gigaspora margarita* clone BI2_4_3	HF968907.1	98
Non-fumigated	700–800 bp	*Rhizophagus clarus*	*Glomus clarum* Att894-7 clone pHS029-28	FM865542.1	100
		Uncultured Glomeromycota	Uncultured Glomeromycota clone SHN2_117	KM208501.1	99
		Claroideoglomus claroideum	*Glomus claroideum* isolate SW201-1	AM040316.1	99

Table 3. Non-AM fungal OTU detected from *A. fistulosum* root

Treatment	Band range (bp)	Fungal species detected	Ecological role
Fumigated	600–700 bp	*Mrakia aquatica*	Decomposer (Tsuji et al. 2019)
		Tetracladium furcatum	Decomposer (Lazar et al. 2022, 2024)
		Ceratobasidium sp.	Pathogen (Leiva et al. 2023; Li et al. 2025)
	700–800 bp	*Tetracladium sp.*	Decomposer (Lazar et al. 2022, 2024)
Non-fumigated	600–700 bp	*Ceratobasidium sp.*	Pathogen (Leiva et al. 2023; Li et al. 2025)
		Rhizoctonia butinii	Pathogen (Misawa et al. 2017)
		Uncultured fungus	-

4. Conclusion

Molecular methods were employed to understand the effect of fumigation on the AM fungal community in the *A. fistulosum* roots. We found that some AM fungal species and non-AM fungal species survive and coexist in the fumigated field. Further study is required to characterize the fungal adaptation strategy to continuous and long-term chemical inputs. The result of this study provides insight into the resilience of the AM fungal community in chemically disturbed ecosystems and may contribute to the improvement of sustainable agricultural management practices.

References

Bonfante, P. 2022. Microbe Profile: *Gigaspora margarita*, a multifaceted arbuscular mycorrhizal fungus. Microbiology (United Kingdom) 168. https://doi.org/10.1099/mic.0.001202

Chen, R., Jiang, W., Xu, S. 2022. An emerging chemical fumigant: two-sided effects of dazomet on soil microbial environment and plant response. Environmental Science and Pollution Research 29:3022–3036. https://doi.org/10.1007/s11356-021-15401-4

Dangi, S.R., Tirado-Corbalá, R., Gerik, J., & Hanson, B. D. 2017. Effect of long-term continuous fumigation on soil microbial communities. Agronomy 7. https://doi.org/10.3390/agronomy7020037

Fang, W., Wang, X., Huang, B. 2020. Comparative analysis of the effects of five soil fumigants on the abundance of denitrifying microbes and changes in bacterial community composition. Ecotoxicol Environ Saf 187. https://doi.org/10.1016/j.ecoenv.2019.109850

Gao, S., Sosnoskie, L. M., Cabrera, J. A. 2016. Fumigation efficacy and emission reduction using low-permeability film in orchard soil fumigation. Pest Manag Sci 72:306–314. https://doi.org/10.1002/ps.3993

Hart, M. M., & Reader, R. J. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytologist 153:335–344. https://doi.org/10.1046/j.0028-646X.2001.00312.x

Kawahara, A., & Ezawa, T. 2013. Characterization of arbuscular mycorrhizal fungal communities with respect to zonal vegetation in a coastal dune ecosystem. Oecologia 173:533–543. https://doi.org/10.1007/s00442-013-2622-y

Kim, S. H., Yoon, J. B., Han, J. 2023. Green onion (*Allium fistulosum*): An aromatic vegetable crop esteemed for food, nutritional and therapeutic significance. Foods 12. https://doi.org/10.3390/foods12244503

Lazar, A., Mushinski, R. M., & Bending, G. D. 2022 Landscape scale ecology of *Tetracladium spp*. fungal root endophytes. Environmental Microbiomes 17. https://doi.org/10.1186/s40793-022-00431-3

Lazar, A., Phillips, R.P., Kivlin, S. 2024. Understanding the ecological versatility of *Tetracladium* species in temperate forest soils. Environ Microbiol 26. https://doi.org/10.1111/1462-2920.70001

Le, D., Audenaert, K., & Haesaert, G. 2021. Fusarium basal rot: profile of an increasingly important disease in *Allium spp*. Trop Plant Pathol 46:241–253. https://doi.org/10.1007/s40858-021-00421-9/Published

Leiva, A. M., Pardo, J. M., Arinaitwe, W. 2023. *Ceratobasidium sp.* is associated with cassava witches' broom disease, a re-emerging threat to cassava cultivation in Southeast Asia. Sci Rep 13. https://doi.org/10.1038/s41598-023-49735-5

Li, X., Skillman, V., Dung, J., & Frost, K. 2022. Legacy effects of fumigation on soil bacterial and fungal communities and their response to metam sodium application. Environmental Microbiomes 17. https://doi.org/10.1186/s40793-022-00454-w

Li, Y., Zhang, X., Song, X. 2025. Identification of a novel pathogen of peanut root rot, *Ceratobasidium sp.* AG-A, and the potential of selected bacterial biocontrol agents. Journal of Fungi 11:472. https://doi.org/10.3390/jof11070472

Lin, Y. M., Li, M. H., Dai, C. Y. 2024. Dazomet fumigation modification of the soil microorganism community and promotion of Panax notoginseng growth. Front Microbiol 15. https://doi.org/10.3389/fmicb.2024.1443526

Mao, L., Liu, X., Sial, M. U. 2024. Soil application of dazomet combined with 1,3-dichloropropene against soilborne pests for tomato production. Sci Rep 14. https://doi.org/10.1038/s41598-024-83182-0

Misawa, T., Kurose, D., & Kuninaga, S. 2017. First report of leaf sheath rot of Welsh onion caused by nine taxa of *Rhizoctonia spp*. and characteristics of the pathogens. Journal of General Plant Pathology 83:121–130. https://doi.org/10.1007/s10327-017-0706-y

Padula, G., Xia, X., & Hołubowicz, R. 2022. Welsh Onion (*Allium fistulosum* L.) Seed Physiology, Breeding, Production and Trade. Plants 11. https://doi.org/10.3390/plants11030343

Smith, S. E., & Read, D. 2008. Mycorrhizal Symbiosis, 3rd edn. Academic Press. https://doi.org/10.2136/sssaj2008.0015br

Suzuki, T., Uno, T., Tajima, R. 2021. Optimum range of soil phosphorus fertility needed for effective arbuscular mycorrhizal inoculation of Welsh onions in a non-allophanic Andosol. Soil Sci Plant Nutr 67:540–544. https://doi.org/10.1080/00380768.2021.1977587

Tawaraya, K., Hirose, R., & Wagatsuma, T. 2012. Inoculation of arbuscular mycorrhizal fungi can substantially reduce phosphate fertilizer application to *Allium fistulosum* L. and achieve marketable yield under field condition. Biol Fertil Soils 48:839–843. https://doi.org/10.1007/s00374-012-0669-2

Tsuji, M., Kudoh, S., Tanabe, Y., & Hoshino, T. 2019. Basidiomycetous yeast of the genus *Mrakia*. In: Fungi in Extreme Environments: ecological role and biotechnological significance. Springer International Publishing, pp 145–156. https://doi.org/10.1007/978-3-030-19030-9_8

van der Heyde, M., Ohsowski, B., Abbott, L. K., & Hart, M. 2017. Arbuscular mycorrhizal fungus responses to disturbance are context-dependent. Mycorrhiza 27:431–440. https://doi.org/10.1007/s00572-016-0759-3

Wang, C. H., Tsai, Y. C., Tsai, I. 2021. Stemphylium leaf blight of Welsh Onion (Allium fistulosum): An emerging disease in Sanxing, Taiwan. Plant Dis 105. https://doi.org/10.1094/PDIS-11-20-2329-RE

Yan, D., Cao, A., Wang, Q. 2019. Dimethyl disulfide (DMDS) as an effective soil fumigant against nematodes in China. PLoS One 14. https://doi.org/10.1371/journal.pone.0224456

Yan, D., Liu, J., Wang, X. 2025. A review on the mechanisms of fumigant action. New Plant Protection 2. https://doi.org/10.1002/npp2.27

Yan, D., Wang, Q., Li, Y. 2022. Efficacy and economics evaluation of seed rhizome treatment combined with preplant soil fumigation on ginger soilborne disease, plant growth, and yield promotion. J Sci Food Agric 102:1894–1902. https://doi.org/10.1002/jsfa.11526

Yin, Y. S., Li, J. J., Zhang, F. B. 2020. First report of *Ceratobasidium sp*. causing root rot of garlic in China. Plant Dis. https://doi.org/https://doi.org/10.1094/PDIS-08-19-1679-PDN