https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.

Biogeosciences Discussions Open Access EGU

1 **TITLE PAGE:**

2

3 **Dark septate endophytic fungi associated with pioneer grass inhabiting volcanic deposits**

4 **and their functions in promoting plant growth**

5

6 HAN SUN[1,2*], TOMOYASU NISHIZAWA[1,2], HIROYUKI OHTA[1,2], KAZUHIKO NARISAWA[1,2]

7

8 [1]*United Graduate School of Agricultural Science, Tokyo University of Agriculture and*

9 *Technology, 3-5-8 Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan*

10 [2] *Ibaraki University College of Agriculture, 3-21-1 Chuo, Ami-machi, Ibaraki 300-0393, Japan*

11

12 **Running Headline:** Endophytic fungi in volcanic deposits

13

14 **Type of the Study:** Original Research Article

15

16 ***Corresponding Author*:**

17 Han Sun

18 E-mail: sunh1211@163.com

19 Tel: +81-029-888-8667

20 Fax: +81-029-888-8667

21

22

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.

Biogeosciences Discussions Open Access EGU

Dark septate endophytic fungi associated with pioneer grass inhabiting volcanic deposits and their functions in promoting plant growth

HAN SUN[1,2*], TOMOYASU NISHIZAWA[1,2], HIROYUKI OHTA[1,2], KAZUHIKO NARISAWA[1,2]

[1]*United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan*

[2] *Ibaraki University College of Agriculture, 3-21-1 Chuo, Ami-machi, Ibaraki 300-0393, Japan*

Abstract

Growth of the pioneer grass *Miscanthus condensatus,* one of the first vegetation to be established on volcanic deposits, is promoted by root-associated fungi, particularly dark septate endophytes (DSE). Fungal taxa within DSE colonize the root of *Miscanthus condensatus* in oligotrophic *Andosol*, and their function in plant growth promotion remains largely unknown. We, therefore, comprehensively assessed the composition of the DSE community associated with *Miscanthus condensatus* root in volcanic ecosystems using the approaches of both metabarcoding (next-generation sequencing) and isolation (culturing). Also, their promotion effects of DSE on plant growth (rice as a proxy) were evaluated by inoculation of core isolates to rice roots. Here, we found: i) 70% of culturable fungi that colonized *Miscanthus condensatus* phylogenetically belonged to DSE, ii) 7 orders were identified by both sequencing and culturing methods, and iii) inoculation of DSE isolates (*Phialocephala fortinii, P. helvetica, and Phialocephala sp.*) validated their effects on rice growth, particularly under an extremely low pH condition (compared to control without inoculation, rice biomass enhanced by 7.6 times after inoculation of *P. fortinii*). This study helps improve our understanding of the community of *Miscanthus condensatus*-associated DSE fungi and their functions in promoting plant growth.

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


Key words: *volcanic deposits*, *pioneer grass*, *dark septate endophytic fungi*, *culture-non-culture approaches*

Introduction

Numerous studies demonstrated that symbiotic fungi play a significant role in the establishment of pioneer vegetation in harsh environments or agricultural soils with extremely low pH. The association of these fungal micro-organisms that promote plant colonization in extreme conditions is mainly to: improve host nutrient uptake (Usuki and Narisawa, 2007; Yadav *et al*., 2009), defend against pathogens (Busby *et al*., 2016), promote tolerance to abiotic stress (Rodriguez *et al*., 2008; Gill *et al*., 2016), and modify trophic interactions (Clay, 1996; Omacini *et al*., 2001; Bultman *et al*., 2003).

One of the most common groups of monocotyledonous root endophytes is dark septate endophytes (DSE), which usually colonize in tissues intracellularly and intercellularly of more than 600 living herbaceous and woody plant species (Jumpponen and Trappe, 1998). DSE, which are characterized by their morphology of melanized, septate hyphae and structure like microsclerotia, also confer the ability to improve plant performance through enhanced nutrient uptake, and increased ability to withstand adverse environmental conditions (Khastini and Jannah, 2021). Increasing evidence shows that DSE gradually become the most prevalent root colonizers under extreme environmental conditions of different ecosystem (Deram *et al*., 2008; Regvar *et al*., 2010). For example, Huusko *et al*. (2017) reported DSE-dominated colonization in *Deschampsia flexuosa* roots along a postglacial land uplift gradient. Gonzalez Mateu *et al*. (2020) reported that DSE inoculation *Phragmites australis* had higher aboveground biomass under mesohaline conditions. DSE, e.g., *Phialocephala fortinii*, promote host plant growth and

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


71 adaptation to the hostile environment by: i) increasing resistance to heavy metal contamination
72 and heat/drought stress via producing melanized cell walls and, ii) facilitating uptake of
73 nutrients such as nitrogen and phosphorous (Jumpponen *et al*., 1998; Surono and Narisawa,
74 2017).

75 Wild plant species may live in symbiosis with mycoflora that may have been lost during
76 breeding of the cultivars used in agriculture (Yuan *et al*., 2010). Whilst, some of symbiotic
77 fungi, that can assist plants to adapt to a given stress in a natural habitat, might increase
78 tolerance of crop species to that stress in an agriculture system. Thus, from an agricultural point
79 of view, the plant symbiotic fungi could be seen as an extended source for crop adaptation and
80 growth in agronomy. In attempts to domesticate "wild" symbiotic fungi, some of these DSE
81 species in natural system have been successfully transferred to agricultural species from their
82 original host, providing benefits to the inoculated crops.

83 Rice (*Oryza sativa*) is the principal food grain crop (one of the four major food crops) for
84 more than 3 billion people, and its consumption exceeds 100 kg per capita annually in many
85 Asian countries (Yuan *et al*., 2010). During the last several decades, there have been major
86 climatic events, including global warming, soil acidification, etc, that decreased agricultural
87 productivity of rice around the world. Soil pH is a highly sensitive factor to determine plant
88 survival, distribution, and interactions with microorganisms, which are vital for the availability
89 of essential nutrients and plant growth (Luo *et al*., 2013). Acidic soils occupy around 40-50%
90 of the world's potentially arable land. Plants commonly encounter deficient and toxic levels of
91 mineral elements (soluble ionic Al, mainly Al^{3+})when grown in acidic (pH<5) soil. About 13%
92 of the world's rice is produced in acid soil. Compared with other crops, rice has relatively
93 stronger Al toxic resistance (Famoso *et al*., 2010), and is also the most complex cereal crop
94 with Al resistance genes (Ma *et al*., 2002). Nevertheless, as for other crops, heavy metal toxicity

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


in acid soil limits rice growth and nutrients uptake, and subsequently reduces grain yield (Chen *et al*., 2020). The optimal pH range for rice growth is 5.0-8.5, which shows the likely reduction of yield in the soil with the extended pH range. To improve these soil acidity, liming is often used but is practically difficult and unsustainable.

Microorganisms inoculation is a sustainable approach to potentially promote plant resistance to acidic stress. For instance, plant-associated fungi, such as arbuscular mycorrhizal fungi (AM fungi), reportedly play a key role in the protection of plants in acidic soils. Yet, high concentrations of H^+ and Al^{3+} can inhibit hyphal growth and spore germination in AM fungi, thereby decreasing the possibility of colonizing plant roots (Clark, 1997; Van Aarle *et al*., 2002; Postma *et al*., 2007). Comparably, DSE show marked potential to help host plants resist acidity because of their higher H^+ tolerance than other colonizing fungi (Postma *et al*., 2007). Still, there is a lack of reports of DSE improving host crop (e.g., rice) growth under acidic conditions, especially an extremely acidic condition (pH 3.0).

Re-vegetation in volcanic soil, characterized by a dominance of biological processes, is difficult due to: i) strong acidity of volcanic deposits, ii) high concentration of toxic elements, and iii) deficiencies in essential nutrients. *Miscanthus sinensis,* an unique pioneer grass plant during recovery after volcanic eruption, is the first to be established on volcanic deposits, and frequently found as primary vegetation in lahar deposited by volcanic eruptions (Watanabe *et al*., 2006; Hirata *et al*., 2007; An *et al*., 2008; Ezaki *et al*., 2008). This is because *M. sinensis* can tolerate a wide range of environmental stresses due to the trait of C4 photosynthesis, leading to high productivity and low-nutrient requirement (Stewart *et al*., 2009). Apart from *Miscanthus* traits that adapt to the volcanic soil, the root-associated fungal communities are widely reported to benefit the growth and promote the adaptation of host plants to stress, such as aridity (Wu and Xia, 2006), salinity (Porcel *et al*., 2012), and oligotrophic conditions (Jeewani *et al*., 2021).

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


A better understanding of plant-microbe interactions, therefore, can help improve our understanding of vegetation recovery and plant growth promotion including agricultural application scene. The isolation and culture of fungal species, therefore, are indispensable as they complement taxonomic databases and validate taxa revealed by sequencing. Bai *et al*. (2015) established *Arabidopsis* root-derived bacterial culture collections representing the majority of species that were reproducibly detectable by culture-independent community sequencing. Laval *et al*. (2021) investigated fungal and bacterial communities in soils receiving wheat and oilseed rape residues, and confirmed the feasibility of combined culture-unculture approaches that revealed consistent community profiles. The role of keystone taxa revealed by the sequencing data-based co-occurrence network can be further validated by culturing and followed inoculation. For example, isolation was used to test whether the interaction between micro-organisms predicted by metagenomic sequencing actually occurs (Laval *et al*., 2021). By isolation and inoculation, (Zheng *et al*., 2021) identified the strong decomposition ability of keystone taxa such as the genera *Chryseobacterium* (bacteria), *Fusarium*, *Aspergillus*, and *Penicillium* (fungi), which are consistent with the keystone taxa revealed by the co-occurrence network. The combination of sequencing and culturing methods, therefore, is powerful for the identification of putative taxa (either individually or creation of synthetic communities). Yet, studies on DSE in volcanic ecosystems by culture-unculture approaches are lacking, and inoculation to validate the function in rice growth still awaits further investigation.

For this purpose, both culture-dependent and culture-independent approaches were adopted, to comprehensively reveal the fungal communities of *Miscanthus*-associated, particularly DSE, from volcanic ecosystems. Their functions in promoting plant growth (via isolation-inoculation) in different pH soils were further evaluated. Here, we sampled soil and plants from Miyake-jima, as a model active basaltic volcanic island with an eruption in 2000.

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


143 It is located in the Pacific Ocean, and it ejected large amounts of volcanic ash and gases such
144 as sulfur dioxide and hydrogen sulfide (60% of vegetation on the island was affected). As a
145 result of SO_2 gas exposure, volcanic ash deposits were acidified due to SO_4^{2-} absorption. They
146 were characterized by strong acidity, with high levels of exchangeable Ca^{2+} and Al^{3+} (Fujimura
147 *et al*., 2016). This study, therefore, aimed to: i) reveal the fungal taxa associated with the roots
148 of *M. condensatus* during vegetation recovery by a combination of sequencing and culturing
149 approaches, and ii) inoculate the major food crop i.e., rice with these indigenous isolates
150 (overlapped with sequencing-revealed taxa) to evaluate their effects on rice growth, in
151 particularly under low pH condition. We hypothesized that abundant colonization by DSE fungi
152 occurs in the pioneer grass *M. condensatus* inhabiting volcanic deposits near the crater of
153 Miyake-jima, due to DSE's traits of preferential colonization under oligotrophic and acidic
154 conditions.
155
156 **Materials and Methods**
157 **Study site description and root sampling**
158 Miyake-jima (55.5 km^2 in area; highest point, 775 m), a basaltic volcanic island (34º05′ N,
159 139º31′E; Fig. 1), belongs to the Fuji volcanic southern zone in the East Japan volcanic belt.
160 Mount Oyama, located in the center of the island, is an active volcano. A large amount of
161 volcanic SO_2 gas (~54 kt d^{-1}) was ejected immediately from a newly created summit caldera
162 after the latest eruption in 2000 (Fujimura *et al*., 2016). The SO_2 gas exposure declined slowly
163 after the eruption, and as a result of this exposure, the volcanic ash deposits were acidified due
164 to SO_4^{2-} absorption. They were characterized by strong acidity [pH (H_2O), 3.1-4.0], with high
165 levels of exchangeable Ca^{2+} (33.5-115 cmolc kg^{-1}) and Al^{3+} (0.8-10.2 cmolc kg^{-1}) (Fujimura *et*
166 *al*., 2016). At 18 years after the eruption, the patchy vegetation of a pioneer grass, *Miscanthus*

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


167 *condensatus*, was established at site OY near the Miyake-jima summit crater (34º04.69′ N,
168 139º31.04′ E; 553m a.s.l; Fig. 1). The rhizosphere soils of *Miscanthus condensatus* were
169 collected at site OY in November 2017, and March and September 2018. From each period,
170 three healthy specimens of *M. condensatus* were collected, kept in sterile plastic bags, and
171 immediately stored on ice. Samples were divided into two portions, and: 1) kept at 4ºC and
172 processed within 48 h after collection for isolation, and 2) kept at -20 ºC until DNA extraction
173 and molecular analysis.
174
175 **Root surface sterilization and culturable endophytic fungal isolation**
176 In order to remove adhering soil and free-living microbes, which are unlikely to interact
177 with the roots of plants, root samples were gently rinsed with tap water. Individual roots were
178 severed aseptically in 1-cm-long sections with a sterile scalpel and put into 50-mL conical
179 centrifuge tubes. Then, they were superficially sterilized with 0.005% Tween 20 and then rinsed
180 with sterilized distilled water before the aseptic stepwise sterilization process was carried out.
181 Root sections were treated with 70% ethanol for 1 min, with a further step in the above process,
182 1% sodium hypochlorite was added and sterilized for 5 min. Finally, sections were rinsed with
183 sterilized distilled water three times. After surface sterilization, the final wash was spread plated
184 onto 1/2 Corn Meal agar medium (cornmeal, Difco 25 g L^{-1}, Bacto agar, Difco 15 g L^{-1}) to
185 confirm the disinfection and incubated for 2 weeks at 23ºC to examine for the presence of a
186 growth colony. Root sections were dried with sterile filter paper overnight and then placed onto
187 cornmeal agar medium containing 0.1 mg kg^{-1} streptomycin and incubated at 23ºC for 2 weeks.
188 After incubation, pure cultures were obtained by transferring single hyphae to cornmeal malt
189 yeast agar medium (CMMY; Malt extract 10 g L^{-1}, Yeast extract 2 g L^{-1}, Cornmeal 8.5 g L^{-1},
190 Bacto agar 7.5 g L^{-1}).

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


191

192 **Identification of fungal isolates and phylogenetic analysis**

193 Genomic DNA from each fungal isolate was extracted from mycelium using Prepman
194 Ultra Sample Preparation Reagent Protocol (Applied Biosystems, California, USA). The
195 universal primer pairs of ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') hybridize at the end of
196 18S rDNA, and the primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') that hybridizes at the
197 beginning of 28S rDNA was used to amplify fungal isolates (Mahmoud and Narisawa, 2013).
198 PCR amplification was carried out in a 50-µL reaction mixture containing 1 µL fungal genomic
199 DNA, 2.5 µL of each primer, 5 µL of 10 × Ex Taq buffer, 4 µL of dNTP, 0.25 µL of Ex *Taq*
200 DNA polymerase, and 34.75 µL of sterilized MilliQ water under thermal conditions of 4 min
201 at 94ºC, 35 cycles of 94ºC for 35 s, 52ºC for 55 s, and 72ºC for 2 min, and a final extension of
202 72ºC for 10 min using a Takara PCR Thermal Cycler Dice (Takara Bio INC., model TP 600,
203 Japan). The PCR products were purified and sequenced using an Applied Biosystems 3130*xl*
204 DNA sequencer. All sequences obtained were compared with similar DNA sequences retrieved
205 from the Genbank database using the NCBI BLASTN program.

206

207 **Illumina MiSeq sequencing for culture-independent identification**

208 Roots of November samples were added to 10-mL aliquots of sterile distilled water and
209 macerated with a pestle and mortar for DNA extraction with DNeasy Plant Mini Kit (Qiagen,
210 Hilden, Germany) following the manufacturer's protocol. DNA was purified using Ultra Clean
211 DNA Purification Kit (MOBIO, Carlsbad, CA, USA). Then, DNA was eluted in 50 µL of Tris
212 and EDTA buffer. A NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE,
213 USA) was used to quantify the DNA concentration. Finally, DNA samples were stored at -80ºC

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


before molecular analysis. The second nuclear ribosomal internal transcribed spacer (ITS2) region of the rRNA operon was targeted using the fungal-specific primer pairs ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Chen et al., 2021). PCR amplification was carried out in triplicate with 50-μL reactions containing 25 μL of Premix Taq (TaKaRa, Shiga, Japan), 23 μL of sterilized MilliQ water, 0.5 μL of both forward and reverse primers (125 pmol), and 1 μL of template DNA. The PCR program had the following thermocycling conditions: 35 cycles of denaturation at 94ºC for 30 s, annealing at 54ºC for 30 s, 72ºC for 45 s, and a final extension of 72ºC for 10 min. PCR products were pooled and their relative quantity was estimated by running 5 μL of amplicon DNA on 1.5% agarose gel, and products were purified with QIA Quick PCR Purification Kit (Qiagen, Shenzhen, China). The purified mixture was diluted and denatured to obtain an 8 pmol amplicon library and mixed with an equal volume of 8 pmol PhiX (Illumina) following the manufacturer's recommendations in the Illumina MiSeq reagent kit preparation guide (Illumina, San Diego, CA, USA). Finally, 600 μL of the amplicon mixtures were loaded with read 1, read 2, and the index sequencing primers, and paired-end sequencing (each 250 bp) was completed on a MiSeq platform (Illumina). The sequencing data were processed using the UPARSE pipeline (http://drive5.com/usearch/manual/uparse_pipeline.html). The raw sequences were subjected to quality control. The singleton and chimeric sequences were removed after dereplication, and the remaining sequences were categorized into operational taxonomic units (OUT) with 97% similarity and then assigned taxonomy using the UNITE database (https://unite.ut.ee/) .

Inoculation

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


The experiment was conducted as a complete randomized factorial design with two factors. The first factor had four levels: non-inoculation control or inoculation with three dominant isolates; and the second factor had three levels: pH 3, pH 4, and pH 5. Each treatment consisted of four replicates with two plants per pot/replicate, thus totaling 48 experimental pots. Fungal inoculates were prepared by aseptically growing three dominant DSE isolates on Petri dishes with oatmeal agar medium (10 g L^{-1} oatmeal and 15 g L^{-1} Bacto agar enriched with nutrients: 1 g L^{-1} $MgSO_4.7H_2O$, 1.5 g L^{-1} KH_2PO_4, and 1 g L^{-1} $NaNO_3$). Due to the host non-specific character of DSE, rice was chosen as a host plant in this study mostly for its important role in consumed cereal in the world and it is from the same family as *Miscanthus*. Rice seeds were surface- sterilized by immersion in 70% ethanol for 2 min, and a solution of 1% sodium hypochlorite for 5 min with agitation. The sterilized seeds were gently rinsed several times with sterilized distilled water, then dried overnight, and plated onto 1% water agar medium in Petri dishes for germination at 30ºC. Following pre-germination, 2-day-old seedlings (two seedlings per plate) were transplanted as growing fungal colonies on the medium at pH 3, pH 4, or pH 5. For DSE inoculation, two 5-mm plugs excised from the edge of an actively growing colony on culture medium were inoculated at a 1-cm range close to the rice seedlings. Seedlings transplanted onto non-inoculated medium were used as controls. The whole set was placed into sterile plastic culture bottles and incubated for 3 weeks at room temperature with an 18 h:6 h (L:D) regimen and intensity of 180 µmol $m^{-2}s^{-1}$. Assessed plants were harvested and oven-dried at 40ºC for 72 h. The shoot and root dry weights of treated plants were measured and compared with the control.

DSE root colonization observations

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


Root colonization by DSE fungal isolates was observed to confirm whether the selected DSE colonized the inner roots endophytically. Roots were harvested from plants after 3 weeks of cultivation. Root systems were washed thoroughly under running tap water to remove adhering agar, then rinsed with distilled water, and used for root staining. The root samples were cleared with 10% (v/v) potassium hydroxide in a water bath at 80ºC for 20 min. Subsequently, roots were acidified with 1% hydrochloric acid at room temperature for 5 min, then stained with 50% acetic acid solution containing 0.005% cotton blue at room temperature overnight. Root fragments were placed on a slide glass and covered with a cover glass. Fungal colonization was observed using a light microscope equipped with an Olympus DP25 digital camera.

Statistical analyses

All statistical analyses were performed in the R environment (version: V4.1.2). Homoscedasticiy was checked using Levene's test and normality using Shapiro-Wilk's test. The differences of mean dry biomass between the analyzed traits of the seedlings in different treatments in this study were calculated and analyzed statistically with two-way analysis of variance (ANOVA) and Tukey's honestly significant difference test at P-values<0.05.

Results and Discussion

The core fungal taxa identified by both culture-dependent and culture-independent methods

This study compared the culture-dependent isolates with the fungal taxa revealed by culture-independent methods. Based on 97% sequence similarity, all reads were clustered into 224 OTUs, and the valid sequences were classified into five phyla, including two major

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


281 dominant phyla of Ascomycota (71.5%) and Basidiomycota (17.1%), followed by
282 Mortierellomycota, Mucoromycota, and Calcarisporiellomycota, while the cultivable
283 endophytic fungi were classified into 2 different phyla of Ascomycota (97.5%) and
284 Basidiomycota (2.50%). Fifteen and four classes were detected by culture-independent and
285 culture-dependent approaches, respectively. Specifically, classes Sordariomycetes and
286 Leotiomycetes (both belonging to phylum Ascomycota) were the major classes in terms of the
287 number of OTUs. These data were in agreement with a previous study showing that
288 Leotiomycetes and Sordarimoycetes were the major classes of endophytic fungi associated with
289 plants (regardless of plant species, associated host tissue) in acidic, oligotrophic ecosystems
290 and nutrient-limiting boreal and arctic areas (Arnold *et al*., 2007; Yuan *et al*., 2010; Ghimire *et*
291 *al*., 2011; Luo *et al*., 2014; Knapp *et al*., 2019).

292 While looking at the lower level, 27 orders were found by Illumina-based sequencing
293 analysis, and 10 of them had an average abundance over 1%. Among these orders detected by
294 sequencing, 7 orders were identified via culture-dependent methods as well (Fig. 2).
295 Significantly higher proportions of Hypocreales (35.6%), Helotiales (21.2%), and Eurotiales
296 (13.2%) were observed by Illumina-based analysis (Fig. 2). Through culture-dependent
297 methods, an abundance of Helotiales (70.0%) occupied the whole community, followed by
298 Eurotiales (15.0%) and Hypocreales (8.75%). In general, the abundant orders of fungal isolates
299 also showed abundance in the OTU table generated by high-throughput sequencing. The
300 overlapping of taxa (Hypocreales and Helotiales) identified by both approaches suggests their
301 significance and dominance in *Miscanthus condensatus*-associated fungal communities.
302 Similarly, the key fungal and bacterial community in soils amended with wheat and oilseed
303 residues were identified via culture and non-culture approaches (Laval *et al*., 2021). Several
304 other studies also confirmed the feasibility to reveal major microbial taxa and showed the

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


305 marked potential of adopting the combination of both culture and non-culture approaches to
306 identify putative taxa (Laval *et al*., 2021; Bai *et al*., 2015; Zheng *et al*., 2021). Undoubtedly a
307 combination of culture-dependent and culture-independent methods might provide a powerful
308 strategy to identify and obtain novel endophytes.

309 The overlapping order Helotiales identified by both culture-dependent methods was
310 abundant in the *Miscanthus condensatus*-associated fungal community (Fig. 2). The isolates
311 including *P. fortinii*, *P. helvetica*, and *Phialocephala* sp. belonged to Helotiales species, which
312 are highly conserved and found to be co-occurring species in the root symbiont communities
313 based on Sanger sequencing (Walker *et al*., 2011; Bruzone *et al*., 2015). This study also found
314 these fungi, *i.e., Phialocephala* sp., *P. helvetica*, and *P. fortinii*, in all samples irrespective of
315 the sampling period (Table 1). Previous studies isolated *P. fortinii* from the root of *Pinus*
316 *resinosa* (Wang and Wilcox, 1985), *Vaccinium vitis-idaea*, *Betula platyphylla* var. *japonica*,
317 *Luetkea pectinate* (Addy *et al*., 2000), *Piceas abies*, *Betula pendula* (Menkis *et al*., 2004),
318 *Rhododendron* sp. (Grünig *et al*., 2008), *Chamaecyparis obtusa*, and *Rubus* sp. (Surono and
319 Narisawa, 2017). Yet, the phylogeny and ecological effects of *P. fortiniii* on plant quality still
320 remain largely unknown (Tedersoo *et al*., 2009). For example, *P. fortiniii* itself is genotypically
321 diverse and composed of at least 21 morphologically indistinguishable but genetically isolated
322 cryptic species (CSP) (Grünig *et al*., 2008). Up to seven isolates belonging to *P. fortinii* have
323 been formally described as CSP (Grünig *et al*., 2008). *Phialocephala helvetica* (sub-species of
324 *P. fortiniii*) associated with the root of *Picea abies* (Stroheker *et al*., 2021) and *Pinus sylvestris*
325 (Landolt *et al*., 2020), is regarded as one of the most common CSP. Yet, their functions in
326 promoting plant growth remain largely unknown.

327

328 **Colonization of DSE fungal isolates in plant root**

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


329 Isolating and characterizing microorganisms could provide insights into their phylogenetic
330 identification, physiological properties, and metabolic potentials, which will help understand
331 the formation, persistence, adaptation mechanisms, and ecological functions of microbial
332 communities (Li *et al*., 2019). Therefore, these three most promising isolates of *Phialocephala*
333 sp., *P. fortinii*, and *P. helvetica*, as typical DSE, were further examined regarding their effects
334 on growth-promoting activity for plants. Based on the inoculation test, all rice seedlings
335 exhibited healthy growth throughout the experimental period by fungal isolate × agar pH
336 interaction (Fig. 3).
337 After harvesting, the roots were stained with 0.05% cotton blue to determine the endophytism
338 of DSE isolates. Microscopic observation revealed that all DSE isolates successfully colonized
339 hair roots of rice seedlings. The hair roots were coated with loose wefts of fungal hyphae. This
340 feature was identical to that previously described for typical DSE, *i.e.,* they are characterized
341 by microsclerotia, thick, and darkly pigmented septate hyphae in the hair roots. Non-inoculated
342 plants as a control showed no DSE colonization. The root colonization pattern was similar in
343 *P. fortinii* and *P. helvetica*, but the degree of fungal colonization of *Phialocephala* sp. was the
344 lowest compared with those two isolates. The images show the dense networks of hyphae of
345 DSE inter- and intracellularly colonizing rice roots (Fig. 4). Very few studies, however,
346 investigated the role and ecological significance of isolated DSE underlying plant growth.
347
348 **The role of isolated DSE in rice growth promotion**
349 As rice is one of the four major food crops for most Asian people, to domesticate these
350 isolated "wild" DSE can benefit agriculture production. Thus we transferred these DSE isolates
351 from their original hosts of *Miscanthus condensatus* to agricultural species (rice). DSE is widely
352 reported to be characterized with non-host specific, but different host (cross family) may have

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


353 different responses (in terms of morphology) to inoculated isolate. For example, *P. fortinii* is
354 frequently reported in roots and formed typical ectomycorrhizae with members of the Pinaceae
355 plants (Jumpponen *et al*., 1998). In contrast, for other family plants, *P. fortinii* is often found to
356 be an endophytic fungi. In addition, *C. chaetospira* was reported able to develop and form spiral
357 structures resembling ericoid mycorrhizas within the roots of ericaceous plants (Usuki and
358 Narisawa, 2005). Whilst *C. chaetospira* colonizing in other host family are characterized by
359 formation of microsleclerotia-aggregations of irregularly lobed hyphae and dark septate hyphae
360 growing inter- and intracellulary. Considering both plants, used in this study as a host (e.g.,
361 miscanthus and rice), belong to the same family of grass with similar host responses to DSE
362 (showing non-host specific trait), we aimed to test effects of these isolated DSE in crop (rice as
363 a proxy) growth promotion. Here, we found that shoot biomass of rice inoculated with DSE
364 isolates increased up to 7.6 times, compared with non-inoculated controls (Fig. 5). The greatest
365 shoot dry weight was recorded in plants treated with *P. fortinii*, followed by *P. helvetica* and
366 *Phialocephala* sp. Similarly, *P. fortinii* isolates were used to inoculate asparagus plants and
367 promote plant growth, e.g., shoot biomass increased by up to 53.5% (Surono and Narisawa,
368 2017). The beneficial effects of *P. fortinii* on enhancing plant yield have been reported
369 (Jumpponen *et al*., 1998; Jumpponen and Trappe, 1998).

370 This improvement in plant growth may be related to the ability of these isolates to use
371 organic nitrogen sources under nitrogen-deficient conditions. Low nitrogen uptake by plants is
372 associated with soil acidity. The presence of *P. fortinii* associated with plant tissues
373 demonstrated its ability to produce a variety of extracellular enzymes that break down complex
374 forms of organic matter containing nitrogen and phosphorus (Jumpponen *et al*., 1998). For
375 example, *Cladophialophora chaetospira* activates soil nitrogen and promotes aboveground
376 transfer in Chinese cabbage (Usuki and Narisawa, 2007). Therefore, the most abundant DSE

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


identified by both culture and non-culture approaches, acting as an important mycorrhizal symbiont via melanized septate hyphae formation that removed resource limitation, might promote plant growth. A labeled nitrogen study is required to validate this mechanism.

Rice growth was markedly different depending on the combination of DSE isolates and pH. Differences in dry weight of DSE inoculated rice compared with non-inoculated rice grown at pH 3.0 (as high as 7.6 fold) were significantly greater than for those DSE inoculated rice grown at pH 4.0 and 5.0 (as high as 1.6 fold and 1.2 fold, respectively). In particular, the root dry weight of *P. fortinii*-treated seedlings was the highest at pH 3.0 with respect to that of the control. Also, we observed that inoculated species of *Phialocephala* effectively promoted plant growth, particularly under acidic conditions. The enhanced shoot biomass via DSE isolate inoculation was most marked in acidic environments, *e.g.*, with 7.6, 1.6, and 1.2 times greater shoot biomass at pH 3, pH 4 and pH 5, respectively. Less promotion of plant growth by inoculation with *Phialocephala* at pH 5 compared with 4 and 3 agar indicated that these DSE isolates likely promote plant tolerance to soil acidity.

Many researchers have reported relatively narrow ranges of pH for the presence or activity of mycorrhizal fungi in soils (Clark, 1997; Postma *et al*., 2007). This is consistent with the observation that most colonized isolates associated with plants were found in acidic agar. Similarly, the colonization of investigated plants with DSE significantly decreased with increasing soil pH (Postma *et al*., 2007). The mechanisms underlying the promotion of plant growth by DSE fungal have been addressed. DSE fungal might help adaptability of crop to acid stress, *i.e.*, low soil pH, and subsequent support of plant growth. The relatively high abundance of DSE supports host survival in stress habitats mainly via high chitin contents and forming melanized septate hyphae and microsclerotia in plant roots (Likar and Regvar, 2013). Also, it

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.


might increase the concentration of Mg, known to ameliorate Al toxicity, in the roots of M.sinensis to decrease Al activity (Haruma et al., 2021).

Here, we validated the effects of these DSE isolates on rice growth, particularly under an extremely low pH condition, e.g., compared to control without inoculation, rice biomass enhanced by 7.6 times after inoculation of *P. fortinii*. DSE show great potential to help host crop resist acidity and thus enable crop cultivation, especially in acidic soil (Postma *et al*., 2007). Acidic soils occupy up to 50% of the arable worldwide, and around 13% of paddy is acid soil. While soil acidification can be a problem for crop yield, these DSE isolates might be used as a management strategy to reduce acidic harm to crops. This, yet, awaits field investigation.

Taken together, this study helps improve our understanding of the community of *Miscanthus condensatus*-associated DSE fungi and their functions. Our findings suggest that DSE have the ability to support rice growth under an extremely acidic conditions, and the formation of melanized septate hyphae and microsclerotia-associated rice tissues might promote increases in rice growth and root biomass via removing stress and resource limitations, and thus they show marked potential in not only re-vegetation of pioneer plants in post-volcanic ecosystems but also promotion of rice growth.

Conclusion

The present study provided detailed insights into the diversity and function of the endophytic fungal community in *Miscanthus condensates*, using both culture-dependent and -independent approaches. Here, we showed that the fungal community was dominated by isolates of *Phialocephala*, which were abundant and widely distributed in the volcanic deposits. Additionally, we validated the functions of these DSE in rice growth, particularly under acidic conditions, by adopting the approach of isolation-inoculation. Considering that these fungal

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.

424 isolates promote plant adaptation to acidic soil, the identified DSE, e.g., *Phialocephala. fortinii,*
425 *P. helvetica*, *and Phialocephala* sp., might be potential candidates as plant growth-promoting
426 fungi for either restoring vegetation or promoting rice growth under extreme conditions.
427
428 **Acknowlegements**
429 This research was supported by a Grant-in-Aid for Scientific Research (B) (No.21H02191)
430 from the Japan Society for the Promotion of Science (JSPS), and a Grant-in-Aid for Challenging
431 Exploratory Research (No.22K19164) from JSPS.
432
433
434 **References**

435 Addy, H.D., Hambleton, S., Currah, R.S., 2000. Distribution and molecular characterization of
436 the root endophyte Phialocephala fortinii along an environmental gradient in the boreal forest
437 of Alberta. Mycological Research 104, 1213–1221.
438 https://doi.org/10.1017/S0953756200002896
439 An, G.-H., Miyakawa, S., Kawahara, A., Osaki, M., Ezawa, T., 2008. Community structure of
440 arbuscular mycorrhizal fungi associated with pioneer grass species *Miscanthus sinensis* in acid
441 sulfate soils: Habitat segregation along pH gradients. Soil Science and Plant Nutrition 54, 517–
442 528. https://doi.org/10.1111/j.1747-0765.2008.00267.x
443 Arnold, A.E., Henk, D.A., Eells, R.L., Lutzoni, F., Vilgalys, R., 2007. Diversity and
444 phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and
445 environmental PCR. Mycologia 99, 185–206.
446 https://doi.org/10.1080/15572536.2007.11832578
447 Bai, Y., Müller, D.B., Srinivas, G., Garrido-Oter, R., Potthoff, E., Rott, M., Dombrowski, N.,
448 Münch, P.C., Spaepen, S., Remus-Emsermann, M., Hüttel, B., McHardy, A.C., Vorholt, J.A.,
449 Schulze-Lefert, P., 2015. Functional overlap of the Arabidopsis leaf and root microbiota. Nature
450 528, 364–369. https://doi.org/10.1038/nature16192
451 Bruzone, M.C., Fontenla, S.B., Vohník, M., 2015. Is the prominent ericoid mycorrhizal fungus
452 Rhizoscyphus ericae absent in the Southern Hemisphere's Ericaceae? A case study on the
453 diversity of root mycobionts in Gaultheria spp. from northwest Patagonia, Argentina.
454 Mycorrhiza 25, 25–40. https://doi.org/10.1007/s00572-014-0586-3
455 Bultman, T.L., McNeill, M.R., Goldson, S.L., 2003. Isolate-dependent impacts of fungal
456 endophytes in a multitrophic interaction. Oikos 102, 491–496. https://doi.org/10.1034/j.1600-
457 0706.2003.11477.x
458 Busby, P.E., Ridout, M., Newcombe, G., 2016. Fungal endophytes: modifiers of plant disease.
459 Plant Mol Biol 90, 645–655. https://doi.org/10.1007/s11103-015-0412-0

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.

Chen, Y., Liu, F., Kang, L., Zhang, D., Kou, D., Mao, C., Qin, S., Zhang, Q., Yang, Y., 2021. Large-scale evidence for microbial response and associated carbon release after permafrost thaw. Glob Change Biol 27, 3218–3229. https://doi.org/10.1111/gcb.15487

Clark, R.B., 1997. Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. Plant and Soil 192, 15–22. https://doi.org/10.1023/A:1004218915413

Clay, K., 1996. Interactions among fungal endophytes, grasses and herbivores. Res Popul Ecol 38, 191–201. https://doi.org/10.1007/BF02515727

Deram, A., Languereau-Leman, F., Howsam, M., Petit, D., Haluwyn, C.V., 2008. Seasonal patterns of cadmium accumulation in Arrhenatherum elatius (Poaceae): Influence of mycorrhizal and endophytic fungal colonisation. Soil Biology and Biochemistry 40, 845–848. https://doi.org/10.1016/j.soilbio.2007.09.023

Ezaki, B., Nagao, E., Yamamoto, Y., Nakashima, S., Enomoto, T., 2008. Wild plants, Andropogon virginicus L. and Miscanthus sinensis Anders, are tolerant to multiple stresses including aluminum, heavy metals and oxidative stresses. Plant Cell Rep 27, 951–961. https://doi.org/10.1007/s00299-007-0503-8

Famoso, A.N., Clark, R.T., Shaff, J.E., Craft, E., McCouch, S.R., Kochian, L.V., 2010. Development of a Novel Aluminum Tolerance Phenotyping Platform Used for Comparisons of Cereal Aluminum Tolerance and Investigations into Rice Aluminum Tolerance Mechanisms. Plant Physiology 153, 1678–1691. https://doi.org/10.1104/pp.110.156794

Fujimura, R., Kim, S.-W., Sato, Y., Oshima, K., Hattori, M., Kamijo, T., Ohta, H., 2016. Unique pioneer microbial communities exposed to volcanic sulfur dioxide. Scientific Reports 9.

Ghimire, S.R., Charlton, N.D., Bell, J.D., Krishnamurthy, Y.L., Craven, K.D., 2011. Biodiversity of fungal endophyte communities inhabiting switchgrass (Panicum virgatum L.) growing in the native tallgrass prairie of northern Oklahoma. Fungal Diversity 47, 19–27. https://doi.org/10.1007/s13225-010-0085-6

Gill, S.S., Gill, R., Trivedi, D.K., Anjum, N.A., Sharma, K.K., Ansari, M.W., Ansari, A.A., Johri, A.K., Prasad, R., Pereira, E., Varma, A., Tuteja, N., 2016. Piriformospora indica: Potential and Significance in Plant Stress Tolerance. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.00332

Gonzalez Mateu, M., Baldwin, A.H., Maul, J.E., Yarwood, S.A., 2020. Dark septate endophyte improves salt tolerance of native and invasive lineages of Phragmites australis. ISME J 14, 1943–1954. https://doi.org/10.1038/s41396-020-0654-y

Grünig, C.R., Queloz, V., Sieber, T.N., Holdenrieder, O., 2008. Dark septate endophytes (DSE) of the *Phialocephala fortinii* s.l. – *Acephala applanata* species complex in tree roots: classification, population biology, and ecology. Botany 86, 1355–1369. https://doi.org/10.1139/B08-108

Haruma, T., Yamaji, K., Masuya, H., 2021. *Phialocephala fortinii* increases aluminum tolerance in *Miscanthus sinensis* growing in acidic mine soil. Lett Appl Microbiol 73, 300–307. https://doi.org/10.1111/lam.13514

Hirata, M., Hasegawa, N., Nogami, K., Sonoda, T., 2007. Evaluation of forest grazing as a management practice to utilize and control Miscanthus sinensis in a young tree plantation in southern Kyushu, Japan. Grassland Science 53, 181–191. https://doi.org/10.1111/j.1744-697X.2007.00091.x

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.

505 Huusko, K., Ruotsalainen, A.L., Markkola, A.M., 2017. A shift from arbuscular mycorrhizal to
506 dark septate endophytic colonization in Deschampsia flexuosa roots occurs along primary
507 successional gradient. Mycorrhiza 27, 129–138. https://doi.org/10.1007/s00572-016-0736-x
508 Jeewani, P.H., Luo, Y., Yu, G., Fu, Y., He, X., Van Zwieten, L., Liang, C., Kumar, A., He, Y.,
509 Kuzyakov, Y., Qin, H., Guggenberger, G., Xu, J., 2021. Arbuscular mycorrhizal fungi and
510 goethite promote carbon sequestration via hyphal-aggregate mineral interactions. Soil Biology
511 and Biochemistry 162, 108417. https://doi.org/10.1016/j.soilbio.2021.108417
512 Jingguang, C., Qi, L., Baiquan, Z., Longbiao, G., Guoyou, Y., 2020. Progress on Molecular
513 Mechanism of Aluminum Resistance in Rice. Rice Science 27, 454–467.
514 https://doi.org/10.1016/j.rsci.2020.09.003
515 Jumpponen, A., Mattson, K.G., Trappe, J.M., 1998. Mycorrhizal functioning of Phialocephala
516 fortinii with Pinus contorta on glacier forefront soil: interactions with soil nitrogen and organic
517 matter. Mycorrhiza 7, 261–265. https://doi.org/10.1007/s005720050190
518 Jumpponen, A., Trappe, J.M., 1998. Dark septate endophytes: a review of facultative biotrophic
519 root-colonizing fungi. New Phytologist 140, 295–310. https://doi.org/10.1046/j.1469-
520 8137.1998.00265.x
521 Khastini, R.O., Jannah, R., 2021. Potential Contribution of Dark-Septate Endophytic Fungus
522 Isolated From Pulau Dua Nature Reserve, Banten on Growth Promotion of Chinese Cabbage:
523 Presented at the 2nd and 3rd International Conference on Food Security Innovation (ICFSI
524 2018-2019), Banten, Indonesia. https://doi.org/10.2991/absr.k.210304.015
525 Knapp, D.G., Imrefi, I., Boldpurev, E., Csíkos, S., Akhmetova, G., Berek-Nagy, P.J.,
526 Otgonsuren, B., Kovács, G.M., 2019. Root-Colonizing Endophytic Fungi of the Dominant
527 Grass Stipa krylovii From a Mongolian Steppe Grassland. Front. Microbiol. 10, 2565.
528 https://doi.org/10.3389/fmicb.2019.02565
529 Landolt, M., Stroheker, S., Queloz, V., Gall, A., Sieber, T.N., 2020. Does water availability
530 influence the abundance of species of the Phialocephala fortinii s.l. – Acephala applanata
531 complex (PAC) in roots of pubescent oak (Quercus pubescens) and Scots pine (Pinus
532 sylvestris)? Fungal Ecology 44, 100904. https://doi.org/10.1016/j.funeco.2019.100904
533 Laval, V., Kerdraon, L., Barret, M., Liabot, A.-L., Marais, C., Boudier, B., Balesdent, M.-H.,
534 Fischer-Le Saux, M., Suffert, F., 2021. Assessing the Cultivability of Bacteria and Fungi from
535 Arable Crop Residues Using Metabarcoding Data as a Reference. Diversity 13, 404.
536 https://doi.org/10.3390/d13090404
537 Li, A.-Z., Han, X.-B., Zhang, M.-X., Zhou, Y., Chen, M., Yao, Q., Zhu, H.-H., 2019. Culture-
538 Dependent and -Independent Analyses Reveal the Diversity, Structure, and Assembly
539 Mechanism of Benthic Bacterial Community in the Ross Sea, Antarctica. Front. Microbiol. 10,
540 2523. https://doi.org/10.3389/fmicb.2019.02523
541 Likar, M., Regvar, M., 2013. Isolates of dark septate endophytes reduce metal uptake and
542 improve physiology of Salix caprea L. Plant Soil 370, 593–604.
543 https://doi.org/10.1007/s11104-013-1656-6
544 Luo, J., Walsh, E., Naik, A., Zhuang, W., Zhang, K., Cai, L., Zhang, N., 2014. Temperate Pine
545 Barrens and Tropical Rain Forests Are Both Rich in Undescribed Fungi. PLoS ONE 9, e103753.
546 https://doi.org/10.1371/journal.pone.0103753
547 Luo, Y., Durenkamp, M., Nobili, M.D., Lin, Q., Devonshire, B.J., Brookes, P.C., 2013.
548 Microbial biomass growth, following incorporation of biochars produced at 350 C or 700 C,
549 in a silty-clay loam soil of high and low pH. Soil Biology 11.

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.

Ma, J.F., Shen, R., Zhao, Z., Wissuwa, M., Takeuchi, Y., Ebitani, T., Yano, M., 2002. Response of Rice to Al Stress and Identification of Quantitative Trait Loci for Al Tolerance. Plant and Cell Physiology 43, 652–659. https://doi.org/10.1093/pcp/pcf081

Mahmoud, R.S., Narisawa, K., 2013. A New Fungal Endophyte, Scolecobasidium humicola, Promotes Tomato Growth under Organic Nitrogen Conditions. PLoS ONE 8, e78746. https://doi.org/10.1371/journal.pone.0078746

Menkis, A., Allmer, J., Vasiliauskas, R., Lygis, V., Stenlid, J., Finlay, R., 2004. Ecology and molecular characterization of dark septate fungi from roots, living stems, coarse and fine woody debris. Mycological Research 108, 965–973. https://doi.org/10.1017/S0953756204000668

Omacini, M., Chaneton, E.J., Ghersa, C.M., Müller, C.B., 2001. Symbiotic fungal endophytes control insect host–parasite interaction webs. Nature 409, 78–81. https://doi.org/10.1038/35051070

Porcel, R., Aroca, R., Ruiz-Lozano, J.M., 2012. Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agron. Sustain. Dev. 32, 181–200. https://doi.org/10.1007/s13593-011-0029-x

Postma, J.W.M., Olsson, P.A., Falkengren-Grerup, U., 2007. Root colonisation by arbuscular mycorrhizal, fine endophytic and dark septate fungi across a pH gradient in acid beech forests. Soil Biology and Biochemistry 39, 400–408. https://doi.org/10.1016/j.soilbio.2006.08.007

Regvar, M., Likar, M., Piltaver, A., Kugonič, N., Smith, J.E., 2010. Fungal community structure under goat willows (Salix caprea L.) growing at metal polluted site: the potential of screening in a model phytostabilisation study. Plant Soil 330, 345–356. https://doi.org/10.1007/s11104-009-0207-7

Rodriguez, R.J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., Kim, Y.-O., Redman, R.S., 2008. Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2, 404–416. https://doi.org/10.1038/ismej.2007.106

Stewart, J.R., Toma, Y., Fernández, F.G., Nishiwaki, A., Yamada, T., Bollero, G., 2009. The ecology and agronomy of *Miscanthus sinensis* , a species important to bioenergy crop development, in its native range in Japan: a review. GCB Bioenergy 1, 126–153. https://doi.org/10.1111/j.1757-1707.2009.01010.x

Stroheker, S., Dubach, V., Vögtli, I., Sieber, T.N., 2021. Investigating Host Preference of Root Endophytes of Three European Tree Species, with a Focus on Members of the Phialocephala fortinii—Acephala applanata Species Complex (PAC). JoF 7, 317. https://doi.org/10.3390/jof7040317

Surono, Narisawa, K., 2017. The dark septate endophytic fungus Phialocephala fortinii is a potential decomposer of soil organic compounds and a promoter of Asparagus officinalis growth. Fungal Ecology 28, 1–10. https://doi.org/10.1016/j.funeco.2017.04.001

Tedersoo, L., Pärtel, K., Jairus, T., Gates, G., Põldmaa, K., Tamm, H., 2009. Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the *Helotiales*. Environmental Microbiology 11, 3166–3178. https://doi.org/10.1111/j.1462-2920.2009.02020.x

Usuki, F., Narisawa, K., 2007. A mutualistic symbiosis between a dark septate endophytic fungus, *Heteroconium chaetospira,* and a nonmycorrhizal plant, Chinese cabbage. Mycologia 99, 175–184. https://doi.org/10.1080/15572536.2007.11832577

Usuki, F., Narisawa, K., 2005. Formation of structures resembling ericoid mycorrhizas by the root endophytic fungus Heteroconium chaetospira within roots of Rhododendron obtusum var. kaempferi. Mycorrhiza 15, 61–64. https://doi.org/10.1007/s00572-004-0333-2

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.

596 Van Aarle, I.M., Olsson, P.A., Söderström, B., 2002. Arbuscular mycorrhizal fungi respond to
597 the substrate pH of their extraradical mycelium by altered growth and root colonization. New
598 Phytologist 155, 173–182. https://doi.org/10.1046/j.1469-8137.2002.00439.x
599 Walker, J.F., Aldrich-Wolfe, L., Riffel, A., Barbare, H., Simpson, N.B., Trowbridge, J.,
600 Jumpponen, A., 2011. Diverse Helotiales associated with the roots of three species of Arctic
601 Ericaceae provide no evidence for host specificity. New Phytologist 191, 515–527.
602 https://doi.org/10.1111/j.1469-8137.2011.03703.x
603 Wang, C.J.K., Wilcox, H.E., 1985. New Species of Ectendomycorrhizal and
604 Pseudomycorrhizal Fungi: Phialophora finlandia, Chloridium paucisporum, and Phialocephala
605 fortinii. Mycologia 77, 951. https://doi.org/10.2307/3793308
606 Watanabe, T., Jansen, S., Osaki, M., 2006. Al?Fe interactions and growth enhancement in
607 Melastoma malabathricum and Miscanthus sinensis dominating acid sulphate soils. Plant Cell
608 Environ 29, 2124–2132. https://doi.org/10.1111/j.1365-3040.2006.001586.x
609 Wu, Q.-S., Xia, R.-X., 2006. Arbuscular mycorrhizal fungi influence growth, osmotic
610 adjustment and photosynthesis of citrus under well-watered and water stress conditions. Journal
611 of Plant Physiology 163, 417–425. https://doi.org/10.1016/j.jplph.2005.04.024
612 Yadav, R.L., Shukla, S.K., Suman, A., Singh, P.N., 2009. Trichoderma inoculation and trash
613 management effects on soil microbial biomass, soil respiration, nutrient uptake and yield of
614 ratoon sugarcane under subtropical conditions. Biol Fertil Soils 45, 461–468.
615 https://doi.org/10.1007/s00374-009-0352-4
616 Yuan, Z., Zhang, C., Lin, F., Kubicek, C.P., 2010. Identity, Diversity, and Molecular Phylogeny
617 of the Endophytic Mycobiota in the Roots of Rare Wild Rice (*Oryza granulate*) from a Nature
618 Reserve in Yunnan, China. Appl Environ Microbiol 76, 1642–1652.
619 https://doi.org/10.1128/AEM.01911-09
620 Zheng, H., Yang, T., Bao, Y., He, P., Yang, K., Mei, X., Wei, Z., Xu, Y., Shen, Q., Banerjee,
621 S., 2021. Network analysis and subsequent culturing reveal keystone taxa involved in microbial
622 litter decomposition dynamics. Soil Biology and Biochemistry 157, 108230.
623 https://doi.org/10.1016/j.soilbio.2021.108230
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.

Biogeosciences Discussions Open Access EGU

Tables

Table 1. Summary of the endophytic fungal isolates among three months of sampling in *Miscanthus condensatus*

Phylum	Class	Blast top-hit	Sequence similarity (%)	Accession number in NCBI	Total number		
					Nov	Mar	Sep
Ascomycota	*Sordariomycetes*	*Acremonium sp.*	98	KT192555.1	4	2	0
		Sarocladium sp.	99	MG649463.1	3	2	0
		Xylariaceae sp.	97	AB741591.1	1	1	0
		Arthrinium phaeospermum	99	MH857420.1	0	2	2
	Leotiomycetes	*Phialocephala fortinii*	97	KJ817297.1	24	17	16
		Phialocephala helvetica	97	MT107593.1	21	36	37
		Phialocephala sp.	99	KT323172.1	11	14	16
		Pezicula ericae	99	NR155653.1	0	5	2
	Eurotiomycetes	*Talaromyces verruculosus*	97	MG748649.1	9	2	2
		Penicillium funiculosum	97	JQ724527.1	3	0	0
	Dothideomycetes	*Pyrenochaetopsis setosissima*	97	LT623227.1	2	2	1
Basidiomycota	*Agaricomycetes*	*Tulasnella calospora*	98	JQ713577.1	1	0	0
		Hypochnicium cremicolor	97	KP814161.1	1	0	0
		Phaeophlebiopsis peniophoroides	98	KP135417.1	0	0	3
		Phlebiopsis gigantea	98	MH114867.1	0	0	3
Dikarya	*Polyporus*	*Polyporus arcularius*	99	KP283489.1	0	1	0
Mucoromycota	*Mortierellomycotina*	*Mortierellales sp.*	97	JQ272348.1	0	2	1
					80	86	83

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.

Figure legends

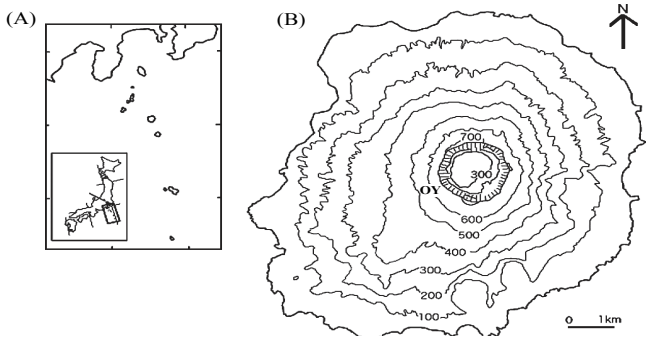


Fig. 1. (A) Map showing the location of Miyake-jima in the western rim of the Pacific Ocean. (B) Map showing study site OY near the summit crater in Miyake-jima

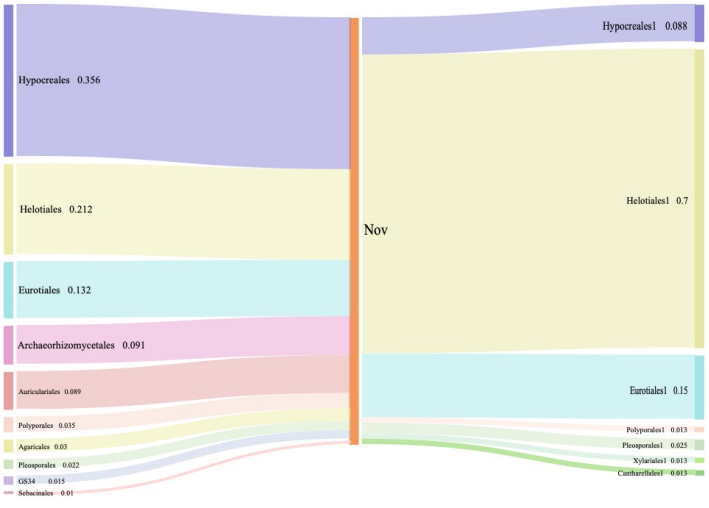
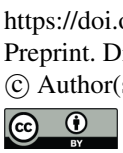
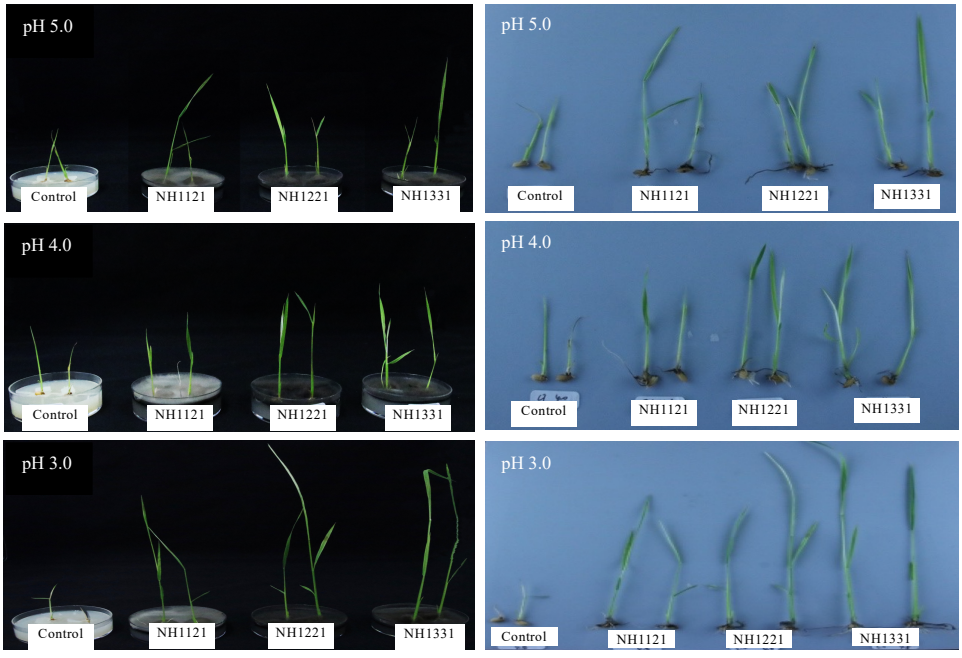


Fig. 2. Composition and relative abundance of endophytic fungi at order level by culture-independent (left) and culture-dependent methods (right)

https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.

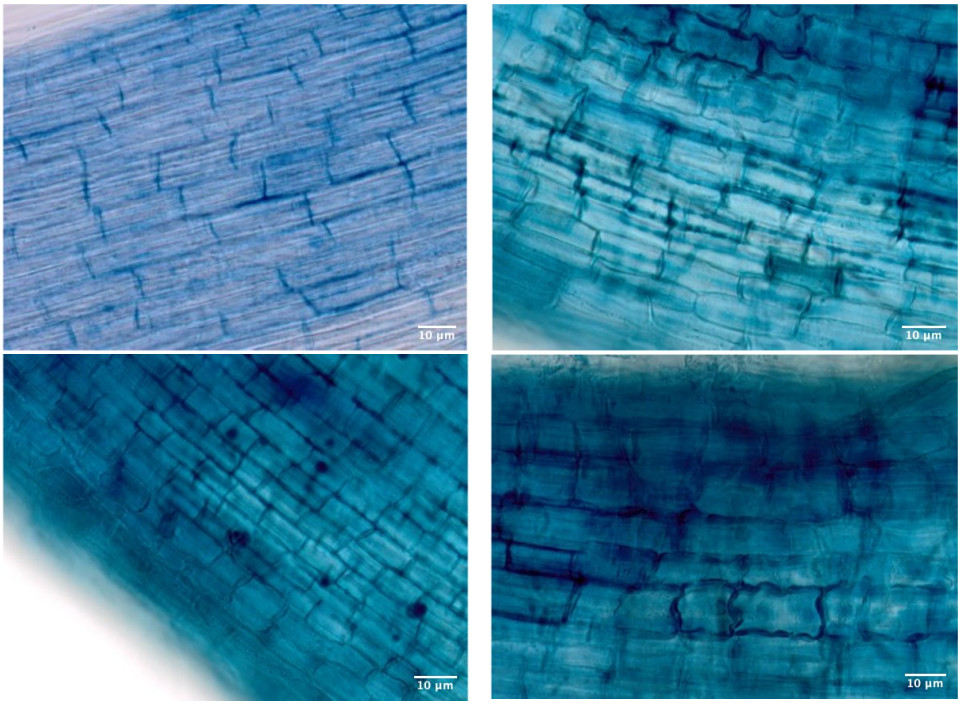


Biogeosciences
Discussions
Open Access
EGU



693 **Fig. 3.** Growth and development of rice plants inoculated with DSE fungal isolates under different pH conditions.

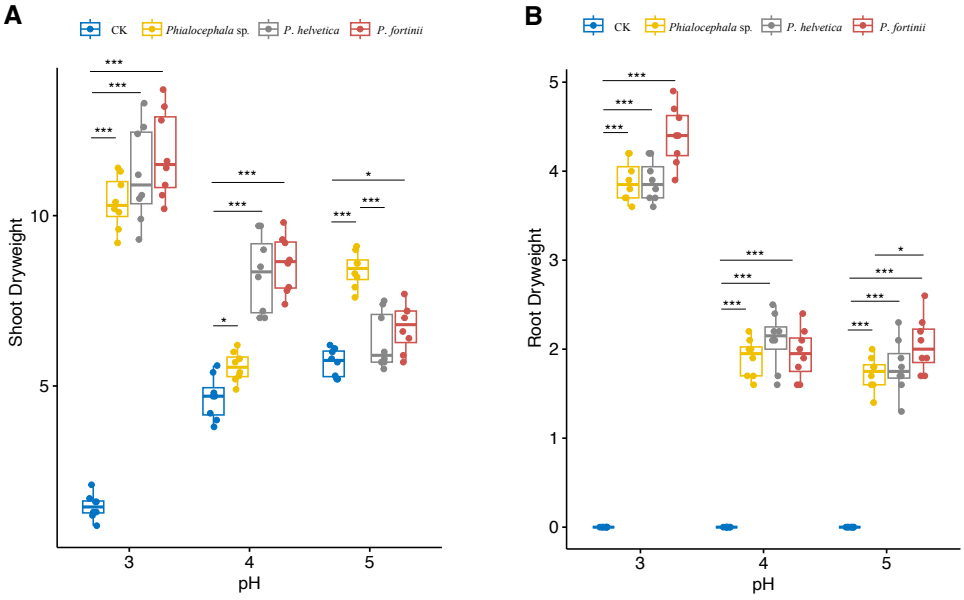
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708



https://doi.org/10.5194/bg-2023-133
Preprint. Discussion started: 14 August 2023
© Author(s) 2023. CC BY 4.0 License.

Biogeosciences
Discussions
Open Access
EGU

709 **Fig. 4.** (A) Non-treated DSE as control roots. (B) *Phialocephala* sp. (NH1121)-treated roots. (C) *Phialocephala helvetica*
710 (NH1221)-treated roots. (D) *Phialocephala fortinii* (NH1331)-treated roots.
711



712

713 **Fig. 5.** Shoot and root dry weights of rice seedlings inoculated with NH1121 (*Phialocephala* sp.), NH1221 (*Phialocephala*
714 *helvetica*), and NH1331 (*Phialocephala fortinii*) after three weeks of growth on oatmeal agar either at pH 3, pH 4, or pH 5
715 (acidic conditions). There are biological replicates (n=8). Median values are lines across the box with lower and upper boxes
716 indicating the 25th to 75th percentiles, respectively. Whiskers represent the maximum and minimum values. Significance was
717 determined by ANOVA.
718