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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.

Key words: *volcanic deposits*, *pioneer grass*, *Miscanthus condensatus*, *culture-non-culture approaches, dark septate endophytic fungi*, *inoculation*, *plant growth promotion*

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 is significant in extreme conditions. As these fungal symbionts help plant survival mainly by: improving host nutrient uptake (Usuki and Narisawa, 2007; Yadav *et al*., 2009), defending against pathogens (Busby *et al*., 2016), promoting tolerance to abiotic stress (Rodriguez *et al*., 2008; Gill *et al*., 2016), and modifying 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 is 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 (Haruma *et al*., 2021; Yu *et al*., 2021). 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 adaptation to the hostile environment by: i) increasing resistance to heavy metal contamination and heat/drought stress via producing melanized cell walls (Li *et al*., 2018; Haruma *et al*., 2021) and, ii) facilitating uptake of nutrients such as nitrogen and phosphorous (Jumpponen *et al*., 1998; Surono and Narisawa, 2017).

Wild plant species may live in symbiosis with mycoflora that may have been lost during breeding of the cultivars used in agriculture (Yuan *et al*., 2010). Whilst, some of symbiotic fungi, that can assist plants to adapt to a given stress in a natural habitat, might increase tolerance of crop species to that stress in an agriculture system. Thus, from an agricultural point of view, the plant symbiotic fungi could be seen as an extended source for crop adaptation and growth in agronomy. In attempts to domesticate "wild" symbiotic fungi (associated with genetically wild type plant), some of these DSE species in natural system have been successfully transferred to agricultural species from their original host, providing benefits to the inoculated crops (Toju *et al*., 2018).

Rice (*Oryza sativa*) is the principal food grain crop (one of the four major food crops) for more than 3 billion people, and its consumption exceeds 100 kg per capita annually in many Asian countries (Yuan *et al*., 2010). During the last several decades, there have been major climatic events, including global warming, soil acidification, etc, that influenced agricultural productivity of rice around the world. Soil pH is a highly sensitive factor to determine plant survival, distribution, and interactions with microorganisms, which are vital for the availability of essential nutrients and plant growth (Luo *et al*., 2013). About 13% of the world's rice is produced in acid soil. Compared with other crops, rice has relatively stronger Al toxic resistance (Famoso *et al*., 2010), and is also the most complex cereal crop with Al resistance genes (Ma *et al*., 2002). Nevertheless, as for other crops, heavy metal toxicity in acid soil limits rice growth

95 and nutrients uptake, and subsequently reduces grain yield (Chen *et al*., 2020). The optimal pH
96 range for rice growth is 5.0-8.5, which shows the likely reduction of yield in the soil with the
97 extended pH range (Ma *et al*., 2002). To improve these soil acidity, liming is often used but is
98 practically difficult and unsustainable.

99 Microorganisms inoculation is a sustainable approach to potentially promote plant
100 resistance to acidic stress. For instance, plant-associated fungi, such as arbuscular mycorrhizal
101 fungi (AM fungi), reportedly play a key role in the protection of plants in acidic soils (Toju *et*
102 *al*., 2018). Yet, high concentrations of H^+ and Al^{3+} can inhibit hyphal growth and spore
103 germination in AM fungi, thereby decreasing the possibility of colonizing plant roots (Clark,
104 1997; Van Aarle *et al*., 2002; Postma *et al*., 2007). Comparably, DSE show marked potential
105 to help host plants resist acidity because of their higher H^+ tolerance than other colonizing fungi
106 (Postma *et al*., 2007). Still, there is a lack of reports of DSE improving host crop (e.g., rice)
107 growth under acidic conditions, especially an extremely acidic condition (pH 3.0).

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

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. This study, therefore, aimed to: i) reveal the fungal taxa associated with the roots of *M. condensatus* during vegetation

143 recovery by a combination of sequencing and culturing approaches, and ii) inoculate the major
144 food crop i.e., rice with these indigenous isolates (overlapped with sequencing-revealed taxa)
145 to evaluate their effects on rice growth, in particularly under low pH condition. We
146 hypothesized that prevalent colonization by DSE fungi occurs in the pioneer grass *M.*
147 *condensatus* inhabiting volcanic deposits near the crater of Miyake-jima, due to DSE's traits of
148 preferential colonization under oligotrophic and acidic conditions.
149
150 **Materials and Methods**
151 **Study site description and root sampling**
152 Miyake-jima (55.5 km^2 in area; highest point, 775 m), a basaltic volcanic island (34º05′ N,
153 139º31′E; Fig. 1), belongs to the Fuji volcanic southern zone in the East Japan volcanic belt.
154 As a model active basaltic volcanic island with an eruption in 2000, it ejected large amounts of
155 volcanic ash and gases such as sulfur dioxide and hydrogen sulfide (60% of vegetation on the
156 island was affected) (Yamanishi *et al*., 2003; Guo *et al*., 2014). As a result of SO_2 gas exposure,
157 volcanic ash deposits were acidified due to SO_4^{2-} absorption. They were characterized by strong
158 acidity, with high levels of exchangeable Ca^{2+} and Al^{3+} (Fujimura *et al*., 2016). Mount Oyama
159 is an active volcano, located in the center of the island. A large amount of volcanic SO_2 gas
160 (~54 kt d^{-1}) was ejected immediately from a newly created summit caldera after the latest
161 eruption in 2000 (Fujimura et al., 2016). The SO_2 gas exposure declined slowly after the
162 eruption, and as a result of this exposure, the volcanic ash deposits were acidified due to SO_4^{2-}
163 absorption. They were characterized by strong acidity [pH (H_2O), 3.1-4.0], with high levels of
164 exchangeable Ca^{2+} (33.5-115 cmol kg^{-1}) and Al^{3+} (0.8-10.2 cmol kg^{-1}) (Fujimura *et al*., 2016).
165 At 18 years after the eruption, the patchy vegetation of a pioneer grass, *Miscanthus condensatus*,
166 was established at site OY near the Miyake-jima summit crater (34º04′ N, 139º31′ E; 553m

a.s.l; Fig. 1). The rhizosphere soils of *Miscanthus condensatus* were collected at site OY in November 2017, and March and September 2018. From each period, three healthy specimens of *M. condensatus* were collected, kept in sterile plastic bags, and immediately stored on ice. Samples were divided into two portions, and: 1) kept at 4ºC and processed within 48 h after collection for isolation, and 2) kept at -20 ºC until DNA extraction and molecular analysis.

Root surface sterilization and culturable endophytic fungal isolation

In order to remove adhering soil and free-living microbes, root surface sterilization was performed by modifying the method of Sahu, *et al*. (2022). Root samples were gently rinsed with tap water. Individual roots were severed aseptically in 1-cm-long sections with a sterile scalpel and put into 50-mL conical centrifuge tubes. Then, they were superficially sterilized with 0.005% Tween 20 and then rinsed with sterilized distilled water before the aseptic stepwise sterilization process was carried out. Root sections were treated with 70% ethanol for 1 min, with a further step in the above process, 1% sodium hypochlorite was added and sterilized for 5 min. Finally, sections were rinsed with sterilized distilled water three times (Sahu, *et al*., 2022).

After surface sterilization, the final wash was spread plated onto 1/2 Corn Meal agar medium (Cornmeal, Difco 25 g L^{-1}, Bacto agar, Difco 15 g L^{-1}) to confirm the disinfection and incubated for 2 weeks at 23ºC to examine for the presence of a growth colony. Root sections were dried with sterile filter paper overnight and then placed onto cornmeal agar medium containing 0.1 mg kg^{-1} streptomycin and incubated at 23ºC for 2 weeks. When endophytic fungal growth was observed, the mycelia were immediately transferred to a new plate. An isolate was transferred only when the probability of a good pure culture was considered high. Thus, when the strains originated very close to each other and in later stages, when they

overgrew earlier strains, they were left untransferred and not calculated into the total number of the isolates. After incubation, pure cultures were obtained by transferring single hyphae to cornmeal malt yeast agar medium (CMMY; Malt extract 10 g L^{-1}, Yeast extract 2 g L^{-1}, Cornmeal 8.5 g L^{-1}, Bacto agar 7.5 g L^{-1}).

Identification of fungal isolates and phylogenetic analysis

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

Illumina MiSeq sequencing for culture-independent identification

Roots of samples which collected in November were added to 10-mL aliquots of sterile distilled water and macerated with a pestle and mortar for DNA extraction with DNeasy Plant

Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA was purified using Ultra Clean DNA Purification Kit (MOBIO, Carlsbad, CA, USA). Then, DNA was eluted in 50 µL of Tris and EDTA buffer. A NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) was used to quantify the DNA concentration. Finally, DNA samples were stored at -80ºC 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. The 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

237 the remaining sequences were categorized into operational taxonomic units (OTU) with 97%
238 similarity and then assigned taxonomy using the UNITE database (https://unite.ut.ee/).
239
240 **Inoculation**
241 The experiment was conducted as a complete randomized factorial design with two factors.
242 The first factor had four levels: non-inoculation control or inoculation with three dominant
243 isolates (*Phialocephala fortinii, P. helvetica, and Phialocephala sp.*); and the second factor had
244 three levels: pH 3, pH 4, and pH 5. Each treatment consisted of four replicates with two plants
245 per pot, and thus totaling 48 experimental pots in the study. Fungal inoculates were prepared
246 by aseptically growing three dominant DSE isolates on Petri dishes with oatmeal agar medium
247 (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
248 L^{-1} KH_2PO_4, and 1 g L^{-1} $NaNO_3$). Due to the host non-specific character of DSE, rice was
249 chosen as a host plant in this study mostly for its important role in consumed cereal in the world
250 and it is from the same family as *Miscanthus*. Rice seeds were surface-sterilized by immersion
251 in 70% ethanol for 2 min, and a solution of 1% sodium hypochlorite for 5 min with agitation.
252 The sterilized seeds were gently rinsed several times with sterilized distilled water, then dried
253 overnight, and plated onto 1% water agar medium in Petri dishes for germination at 30ºC.
254 Following pre-germination, 2-day-old seedlings (two seedlings per plate) were transplanted as
255 growing fungal colonies on the medium at pH 3, pH 4, or pH 5. For DSE inoculation, two 5-
256 mm plugs excised from the edge of an actively growing colony on culture medium were
257 inoculated at a 1-cm range close to the rice seedlings. Seedlings transplanted onto non-
258 inoculated medium were used as controls. The whole set was placed into sterile plastic culture
259 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

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 Discussions

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

While looking at the lower level, 27 orders were found by Illumina-based sequencing analysis, and 10 of them had an average abundance over 1%. Among these orders detected by sequencing, seven orders were identified via culture-dependent methods as well (Fig. 2). Significantly higher proportions of Hypocreales (35.6%), Helotiales (21.2%), and Eurotiales (13.2%) were observed by Illumina-based analysis (Fig. 2). Through culture-dependent methods, an abundance of Helotiales (70.0%) occupied the whole community, followed by Eurotiales (15.0%) and Hypocreales (8.75%). In general, the abundant orders of fungal isolates

also showed abundance in the OTU table generated by high-throughput sequencing. The overlapping of taxa (Hypocreales and Helotiales) identified by both approaches suggests their significance and dominance in *Miscanthus condensatus*-associated fungal communities. Similarly, the key fungal and bacterial community in soils amended with wheat and oilseed residues were identified via culture and non-culture approaches (Laval *et al*., 2021). Several other studies also confirmed the feasibility to reveal major microbial taxa and showed the marked potential of adopting the combination of both culture and non-culture approaches to identify putative taxa (Laval *et al*., 2021; Bai *et al*., 2015; Zheng *et al*., 2021). Undoubtedly a combination of culture-dependent and culture-independent methods might provide a powerful strategy to identify and obtain novel endophytes.

The overlapping order Helotiales identified by both culture-dependent methods was abundant in the *Miscanthus condensatus*-associated fungal community (Fig. 2). The isolates including *P. fortinii*, *P. helvetica*, and *Phialocephala* sp. belonged to Helotiales species, which are highly conserved and found to be co-occurring species in the root symbiont communities based on Sanger sequencing (Walker *et al*., 2011; Bruzone *et al*., 2015). This study also found these fungi, *i.e., Phialocephala* sp., *P. helvetica*, and *P. fortinii*, in all samples irrespective of the sampling period (Table 1). Previous studies isolated *P. fortinii* from the root of *Pinus resinosa* (Wang and Wilcox, 1985), *Vaccinium vitis-idaea*, *Betula platyphylla* var. *japonica*, *Luetkea pectinate* (Addy *et al*., 2000), *Piceas abies*, *Betula pendula* (Menkis *et al*., 2004), *Rhododendron* sp. (Grünig *et al*., 2008), *Chamaecyparis obtusa*, and *Rubus* sp. (Surono and Narisawa, 2017). Yet, the phylogeny and ecological effects of *P. fortiniii* on plant quality still remain largely unknown (Tedersoo *et al*., 2009). For example, *P. fortiniii* itself is genotypically diverse and composed of at least 21 morphologically indistinguishable but genetically isolated cryptic species (CSP) (Grünig *et al*., 2008). Up to seven isolates belonging to *P. fortinii* have

329 been formally described as CSP (Grünig *et al*., 2008). *Phialocephala helvetica* (sub-species of
330 *P. fortiniii*) associated with the root of *Picea abies* (Stroheker *et al*., 2021) and *Pinus sylvestris*
331 (Landolt *et al*., 2020), is regarded as one of the most common CSP. Yet, their functions in
332 promoting plant growth remain largely unknown.
333
334 **Colonization of DSE fungal isolates in plant root**

335 Isolating and characterizing microorganisms could provide insights into their phylogenetic
336 identification, physiological properties, and metabolic potentials, which will help understand
337 the formation, persistence, adaptation mechanisms, and ecological functions of microbial
338 communities (Li *et al*., 2019). Therefore, these three most promising isolates of *Phialocephala*
339 sp., *P. fortinii*, and *P. helvetica*, as typical DSE, were further examined regarding their effects
340 on growth-promoting activity for plants. Based on the inoculation test, all rice seedlings
341 exhibited healthy growth throughout the experimental period by fungal isolate × agar pH
342 interaction (Fig. 3).
343 After harvesting, the roots were stained with 0.05% cotton blue to determine the
344 endophytism of DSE isolates. Microscopic observation revealed that all DSE isolates
345 successfully colonized hair roots of rice seedlings. The hair roots were coated with loose wefts
346 of fungal hyphae. This feature was identical to that previously described for typical DSE, *i.e.,*
347 they are characterized by microsclerotia, thick, and darkly pigmented septate hyphae in the hair
348 roots. Non-inoculated plants as a control showed no DSE colonization. The root colonization
349 pattern was similar in *P. fortinii* and *P. helvetica*, but the degree of fungal colonization of
350 *Phialocephala* sp. was the lowest compared with those two isolates. The images show the dense
351 networks of hyphae of DSE inter- and intra-cellularly colonizing rice roots (Fig. 4). Very few

352 studies, however, investigated the role and ecological significance of isolated DSE underlying
353 plant growth.
354
355 **The role of isolated DSE in rice growth promotion**
356 As rice is one of the four major food crops for most Asian people, to domesticate these
357 isolated "wild" DSE can benefit agriculture production. Thus we transferred these DSE isolates
358 from their original hosts of *Miscanthus condensatus* to agricultural species (rice). DSE is widely
359 reported to be characterized with non-host specific, but different host (cross family) may have
360 different responses (in terms of morphology) to inoculated isolate. For example, *P. fortinii* is
361 frequently reported in roots and formed typical ectomycorrhizae with members of the Pinaceae
362 plants (Jumpponen *et al*., 1998). In contrast, for other family plants, *P. fortinii* is often found to
363 be an endophytic fungi. In addition, *C. chaetospira* was reported able to develop and form spiral
364 structures resembling ericoid mycorrhizas within the roots of ericaceous plants (Usuki and
365 Narisawa, 2005). Whilst *C. chaetospira* colonizing in other host family are characterized by
366 formation of microsleclerotia-aggregations of irregularly lobed hyphae and dark septate hyphae
367 growing inter- and intracellulary. Considering both plants, used in this study as a host (e.g.,
368 miscanthus and rice), belong to the same family of grass with similar host responses to DSE
369 (showing non-host specific trait), we aimed to test effects of these isolated DSE in crop (rice as
370 a proxy) growth promotion. Here, we found that shoot biomass of rice inoculated with DSE
371 isolates increased up to 7.6 times, compared with non-inoculated controls (Fig. 5). The greatest
372 shoot dry weight was recorded in plants treated with *P. fortinii*, followed by *P. helvetica* and
373 *Phialocephala* sp. Similarly, *P. fortinii* isolates were used to inoculate asparagus plants and
374 promote plant growth, e.g., shoot biomass increased by up to 53.5% (Surono and Narisawa,

2017). The beneficial effects of *P. fortinii* on enhancing plant yield have been reported (Jumpponen *et al*., 1998; Jumpponen and Trappe, 1998).

This improvement in plant growth may be related to the ability of these isolates to use organic nitrogen sources under nitrogen-deficient conditions. Low nitrogen uptake by plants is associated with soil acidity. The presence of *P. fortinii* associated with plant tissues demonstrated its ability to produce a variety of extracellular enzymes that break down complex forms of organic matter containing nitrogen and phosphorus (Jumpponen *et al*., 1998). For example, *Cladophialophora chaetospira* activates soil nitrogen and promotes aboveground transfer in Chinese cabbage (Usuki and Narisawa, 2007). Therefore, the most abundant DSE 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 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 isolates promote plant adaptation to acidic soil, the identified DSE, e.g., *Phialocephala. fortinii, P. helvetica*, *and Phialocephala* sp., might be potential candidates as plant growth-promoting fungi for either restoring vegetation or promoting rice growth under extreme conditions.

Acknowlegements

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References

Addy, H. D., Hambleton, S., and Currah, R. S.: Distribution and molecular characterization of the root endophyte *Phialocephala fortinii* along an environmental gradient in the boreal forest of Alberta, Mycol Res., 104, 1213–1221, https://doi.org/10.1017/S0953756200002896, 2000.

446 An, G.-H., Miyakawa, S., Kawahara, A., Osaki, M., and Ezawa, T.: Community structure of
447 arbuscular mycorrhizal fungi associated with pioneer grass species *Miscanthus sinensis* in
448 acid sulfate soils: Habitat segregation along pH gradients, Soil Sci Plant Nutr., 54, 517–
449 528, https://doi.org/10.1111/j.1747-0765.2008.00267.x, 2008.

450 Arnold, A. E., Henk, D. A., Eells, R. L., Lutzoni, F., and Vilgalys, R.: Diversity and
451 phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing
452 and environmental PCR, Mycologia., 99, 185–206,
453 https://doi.org/10.1080/15572536.2007.11832578, 2007.

454 Bai, Y., Müller, D. B., Srinivas, G., Garrido-Oter, R., Potthoff, E., Rott, M., Dombrowski, N.,
455 Münch, P. C., Spaepen, S., Remus-Emsermann, M., Hüttel, B., McHardy, A. C., Vorholt,
456 J. A., and Schulze-Lefert, P.: Functional overlap of the Arabidopsis leaf and root
457 microbiota, Nature., 528, 364–369, https://doi.org/10.1038/nature16192, 2015.

458 Bruzone, M. C., Fontenla, S. B., and Vohník, M.: Is the prominent ericoid mycorrhizal fungus
459 *Rhizoscyphus ericae* absent in the Southern Hemisphere's Ericaceae? A case study on the
460 diversity of root mycobionts in *Gaultheria* spp. from northwest Patagonia, Argentina,
461 Mycorrhiza., 25, 25–40, https://doi.org/10.1007/s00572-014-0586-3, 2015.

462 Bultman, T. L., McNeill, M. R., and Goldson, S. L.: Isolate-dependent impacts of fungal
463 endophytes in a multitrophic interaction, Oikos., 102, 491–496,
464 https://doi.org/10.1034/j.1600-0706.2003.11477.x, 2003.

465 Busby, P. E., Ridout, M., and Newcombe, G.: Fungal endophytes: modifiers of plant disease,
466 Plant Mol Biol., 90, 645–655, https://doi.org/10.1007/s11103-015-0412-0, 2016.

467 Chen, Y., Liu, F., Kang, L., Zhang, D., Kou, D., Mao, C., Qin, S., Zhang, Q., and Yang, Y.:
468 Large-scale evidence for microbial response and associated carbon release after
469 permafrost thaw, Glob Change Biol., 27, 3218–3229, https://doi.org/10.1111/gcb.15487,
470 2021.

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

474 Clay, K.: Interactions among fungal endophytes, grasses and herbivores, Res Popul Ecol., 38,
475 191–201, https://doi.org/10.1007/BF02515727, 1996.

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

480 Famoso, A. N., Clark, R. T., Shaff, J. E., Craft, E., McCouch, S. R., and Kochian, L. V.:
481 Development of a Novel Aluminum Tolerance Phenotyping Platform Used for
482 Comparisons of Cereal Aluminum Tolerance and Investigations into Rice Aluminum

Tolerance Mechanisms, Plant Physiol., 153, 1678–1691, https://doi.org/10.1104/pp.110.156794, 2010.

Fujimura, R., Kim, S.-W., Sato, Y., Oshima, K., Hattori, M., Kamijo, T., and Ohta, H.: Unique pioneer microbial communities exposed to volcanic sulfur dioxide, Sci Rep., 6, 19687, https://doi.org/10.1038/srep19687, 2016.

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

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., and Tuteja, N.: *Piriformospora indica*: Potential and Significance in Plant Stress Tolerance, Front. Microbiol., 7, 332, https://doi.org/10.3389/fmicb.2016.00332, 2016.

Gonzalez Mateu, M., Baldwin, A. H., Maul, J. E., and Yarwood, S. A.: 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, 2020.

Grünig, C. R., Queloz, V., Sieber, T. N., and Holdenrieder, O.: 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, 2008.

Guo, Y., Fujimura, R., Sato, Y., Suda, W., Kim, S., Oshima, K., Hattori, M., Kamijo, T., Narisawa, K., and Ohta, H.: Characterization of Early Microbial Communities on Volcanic Deposits along a Vegetation Gradient on the Island of Miyake, Japan, Microb. Environ., 29, 38–49, https://doi.org/10.1264/jsme2.ME13142, 2014.

Haruma, T., Yamaji, K., and Masuya, H.: *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, 2021.

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

Huusko, K., Ruotsalainen, A. L., and Markkola, A. M.: A shift from arbuscular mycorrhizal to dark septate endophytic colonization in *Deschampsia flexuosa* roots occurs along primary successional gradient, Mycorrhiza., 27, 129–138, https://doi.org/10.1007/s00572-016-0736-x, 2017.

Jeewani, P. H., Luo, Y., Yu, G., Fu, Y., He, X., Van Zwieten, L., Liang, C., Kumar, A., He, Y., Kuzyakov, Y., Qin, H., Guggenberger, G., and Xu, J.: Arbuscular mycorrhizal fungi and

goethite promote carbon sequestration via hyphal-aggregate mineral interactions, Soil Biol Biochem., 162, 108417, https://doi.org/10.1016/j.soilbio.2021.108417, 2021.

Jingguang, C., Qi, L., Baiquan, Z., Longbiao, G., and Guoyou, Y.: Progress on Molecular Mechanism of Aluminum Resistance in Rice, Rice Sci., 27, 454–467, https://doi.org/10.1016/j.rsci.2020.09.003, 2020.

Jumpponen, A. and Trappe, J. M.: Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi, New Phytol., 140, 295–310, https://doi.org/10.1046/j.1469-8137.1998.00265.x, 1998.

Jumpponen, A., Mattson, K. G., and Trappe, J. M.: Mycorrhizal functioning of *Phialocephala fortinii* with *Pinus contorta* on glacier forefront soil: interactions with soil nitrogen and organic matter, Mycorrhiza., 7, 261–265, https://doi.org/10.1007/s005720050190, 1998.

Khastini, R. O. and Jannah, R.: Potential Contribution of Dark-Septate Endophytic Fungus Isolated From Pulau Dua Nature Reserve, Banten on Growth Promotion of Chinese Cabbage, in: 2nd and 3rd International Conference on Food Security Innovation (ICFSI 2018-2019), Banten, Indonesia, https://doi.org/10.2991/absr.k.210304.015, 2021.

Knapp, D. G., Imrefi, I., Boldpurev, E., Csíkos, S., Akhmetova, G., Berek-Nagy, P. J., Otgonsuren, B., and Kovács, G. M.: Root-Colonizing Endophytic Fungi of the Dominant Grass Stipa krylovii From a Mongolian Steppe Grassland, Front. Microbiol., 10, 2565, https://doi.org/10.3389/fmicb.2019.02565, 2019.

Landolt, M., Stroheker, S., Queloz, V., Gall, A., and Sieber, T. N.: Does water availability influence the abundance of species of the *Phialocephala fortinii* s.l. – *Acephala applanata* complex (PAC) in roots of pubescent oak (*Quercus pubescens*) and Scots pine (*Pinus sylvestris*)?, Fungal Ecol., 44, 100904, https://doi.org/10.1016/j.funeco.2019.100904, 2020.

Laval, V., Kerdraon, L., Barret, M., Liabot, A.-L., Marais, C., Boudier, B., Balesdent, M.-H., Fischer-Le Saux, M., and Suffert, F.: Assessing the Cultivability of Bacteria and Fungi from Arable Crop Residues Using Metabarcoding Data as a Reference, Diversity., 13, 404, https://doi.org/10.3390/d13090404, 2021.

Li, A.-Z., Han, X.-B., Zhang, M.-X., Zhou, Y., Chen, M., Yao, Q., and Zhu, H.-H.: Culture-Dependent and -Independent Analyses Reveal the Diversity, Structure, and Assembly Mechanism of Benthic Bacterial Community in the Ross Sea, Antarctica, Front. Microbiol., 10, 2523, https://doi.org/10.3389/fmicb.2019.02523, 2019.

Li, X., He, X., Hou, L., Ren, Y., Wang, S., and Su, F.: Dark septate endophytes isolated from a xerophyte plant promote the growth of *Ammopiptanthus mongolicus* under drought condition, Sci Rep., 8, 7896, https://doi.org/10.1038/s41598-018-26183-0, 2018.

Likar, M. and Regvar, M.: Isolates of dark septate endophytes reduce metal uptake and improve physiology of *Salix caprea* L., Plant Soil., 370, 593–604, https://doi.org/10.1007/s11104-013-1656-6, 2013.

Luo, J., Walsh, E., Naik, A., Zhuang, W., Zhang, K., Cai, L., and Zhang, N.: Temperate Pine Barrens and Tropical Rain Forests Are Both Rich in Undescribed Fungi, PLoS One., 9, e103753, https://doi.org/10.1371/journal.pone.0103753, 2014.

Luo, Y., Durenkamp, M., Nobili, M. D., Lin, Q., Devonshire, B. J., and Brookes, P. C.: Microbial biomass growth, following incorporation of biochars produced at 350ºC or 700º C, in a silty-clay loam soil of high and low pH, Soil Biol Biochem., 11, 513-523, https://doi.org/10.1016/j.soilbio.2012.10.033, 2013.

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

Mahmoud, R. S. and Narisawa, K.: 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, 2013.

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

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

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

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

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

Sahu, P. K., Tilgam, J., Mishra, S., Hamid, S., Gupta, A., Verma, S. K., and Kharwar, R. N.: Surface sterilization for isolation of endophytes: Ensuring what (not) to grow, J Basic Microbiol., 62, 647-668, https://doi.org/10.1002/jobm.202100462, 2022.

Stewart, J. R., Toma, Y., Fernández, F. G., Nishiwaki, A., Yamada, T., and Bollero, G.: 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, 2009.

Stroheker, S., Dubach, V., Vögtli, I., and Sieber, T. N.: 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, 2021.

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

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

Toju, H., Peay, K. G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K., Fukuda, S., Ushio, M., Nakaoka, S., Onoda, Y., Yoshida, K., Schlaeppi, K., Bai, Y., Sugiura, R., Ichihashi, Y., Minamisawa, K., and Kiers, E. T.: Core microbiomes for sustainable agroecosystems, Nat Plants., 4, 247–257, https://doi.org/10.1038/s41477-018-0139-4, 2018.

Usuki, F. and Narisawa, K.: 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, 2005.

Usuki, F. and Narisawa, K.: 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, 2007.

Van Aarle, I. M., Olsson, P. A., and Söderström, B.: Arbuscular mycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered growth and root colonization, New Phytol., 155, 173–182, https://doi.org/10.1046/j.1469-8137.2002.00439.x, 2002.

Walker, J. F., Aldrich-Wolfe, L., Riffel, A., Barbare, H., Simpson, N. B., Trowbridge, J., and Jumpponen, A.: Diverse Helotiales associated with the roots of three species of *Arctic Ericaceae* provide no evidence for host specificity, New Phytol., 191, 515–527, https://doi.org/10.1111/j.1469-8137.2011.03703.x, 2011.

Wang, C. J. K. and Wilcox, H. E.: New Species of Ectendomycorrhizal and Pseudomycorrhizal Fungi: *Phialophora finlandia*, *Chloridium paucisporum*, and *Phialocephala fortinii*, Mycologia., 77, 951, https://doi.org/10.2307/3793308, 1985.

Watanabe, T., Jansen, S., and Osaki, M.: Al-Fe interactions and growth enhancement in *Melastoma malabathricum* and *Miscanthus sinensis* dominating acid sulphate soils, Plant Cell Environ., 29, 2124–2132, https://doi.org/10.1111/j.1365-3040.2006.001586.x, 2006.

Wu, Q.-S. and Xia, R.-X.: Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions, J Plant Physiol., 163, 417–425, https://doi.org/10.1016/j.jplph.2005.04.024, 2006.

Yadav, R. L., Shukla, S. K., Suman, A., and Singh, P. N.: Trichoderma inoculation and trash management effects on soil microbial biomass, soil respiration, nutrient uptake and yield of ratoon sugarcane under subtropical conditions, Biol Fertil Soils., 45, 461–468, https://doi.org/10.1007/s00374-009-0352-4, 2009.

Yu, Y., Teng, Z., Mou, Z., Lv, Y., Li, T., Chen, S., Zhao, D., and Zhao, Z.: Melatonin confers heavy metal-induced tolerance by alleviating oxidative stress and reducing the heavy metal accumulation in *Exophiala pisciphila*, a dark septate endophyte (DSE), BMC Microbiol., 21, 40, https://doi.org/10.1186/s12866-021-02098-1, 2021.

Yuan, Z., Zhang, C., Lin, F., and Kubicek, C. P.: Identity, Diversity, and Molecular Phylogeny of the Endophytic Mycobiota in the Roots of Rare Wild Rice (*Oryza granulate*) from a Nature Reserve in Yunnan, China, Appl Environ Microb., 76, 1642–1652, https://doi.org/10.1128/AEM.01911-09, 2010.

Zheng, H., Yang, T., Bao, Y., He, P., Yang, K., Mei, X., Wei, Z., Xu, Y., Shen, Q., and Banerjee, S.: Network analysis and subsequent culturing reveal keystone taxa involved in microbial litter decomposition dynamics, Soil Biol Biochem., 157, 108230, https://doi.org/10.1016/j.soilbio.2021.108230, 2021.

669 **Tables**

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

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Figure legends

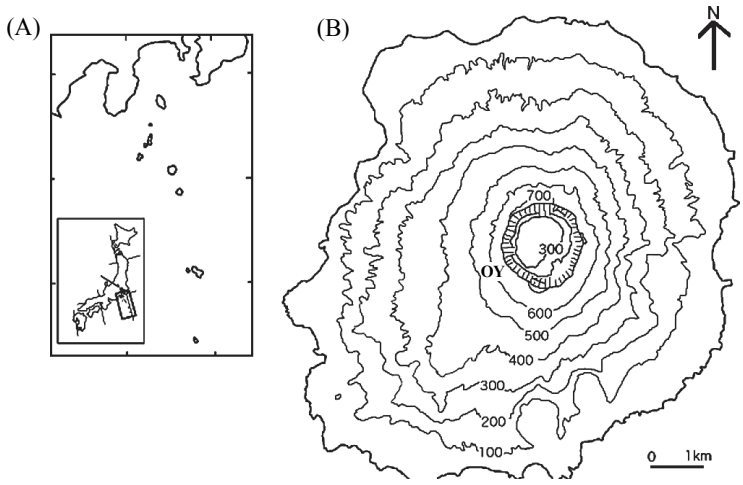

Fig. 1. (A) DEM Map showing the location of Miyake-jima in the western rim of the Pacific Ocean. (B) DEM Map showing study site OY near the summit crater in Miyake-jima

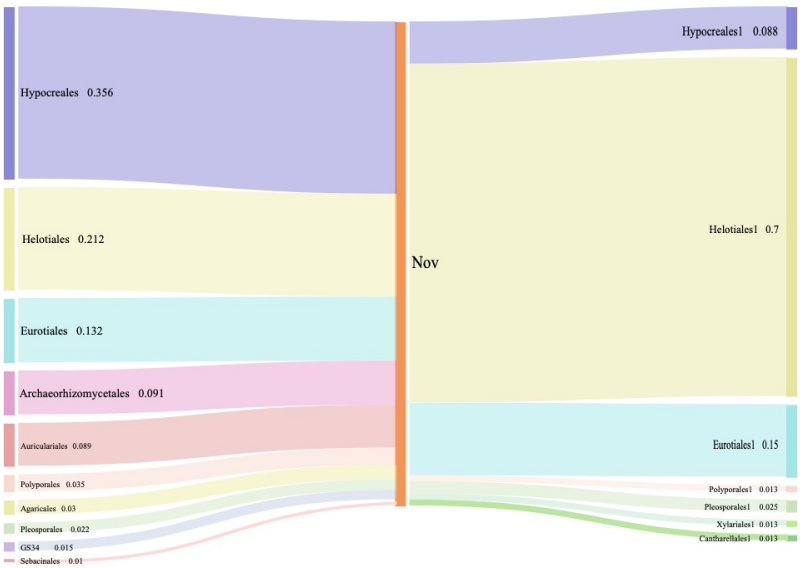

Fig. 2. Composition and relative abundance of endophytic fungi at order level by culture-independent (left) and culture-dependent methods (right)

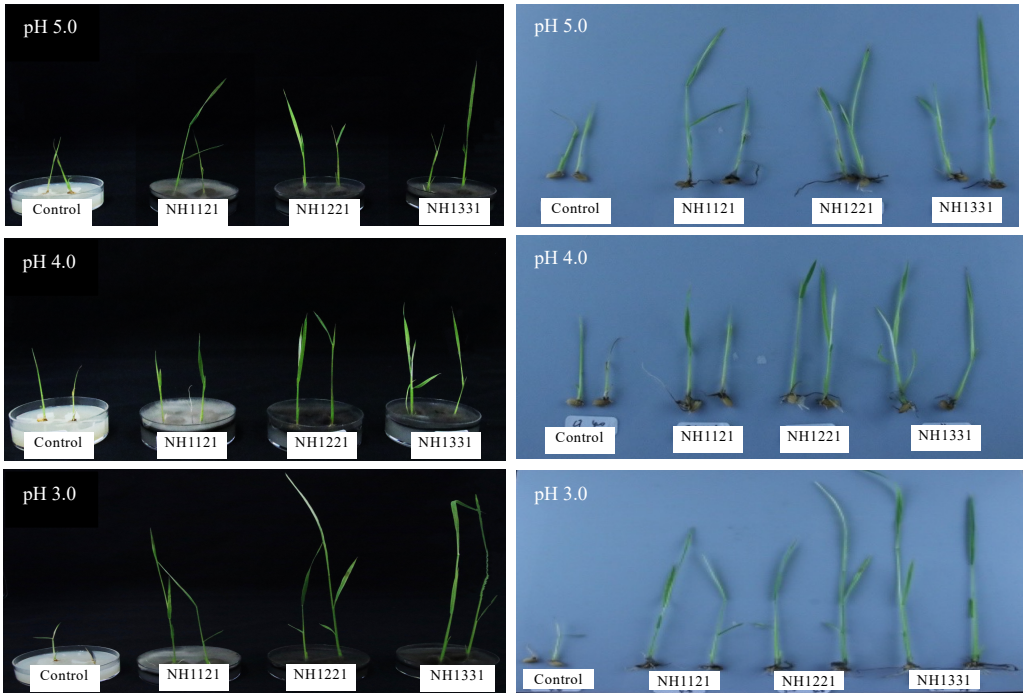

Fig. 3. Growth and development of rice plants inoculated with DSE fungal isolates under different pH conditions.

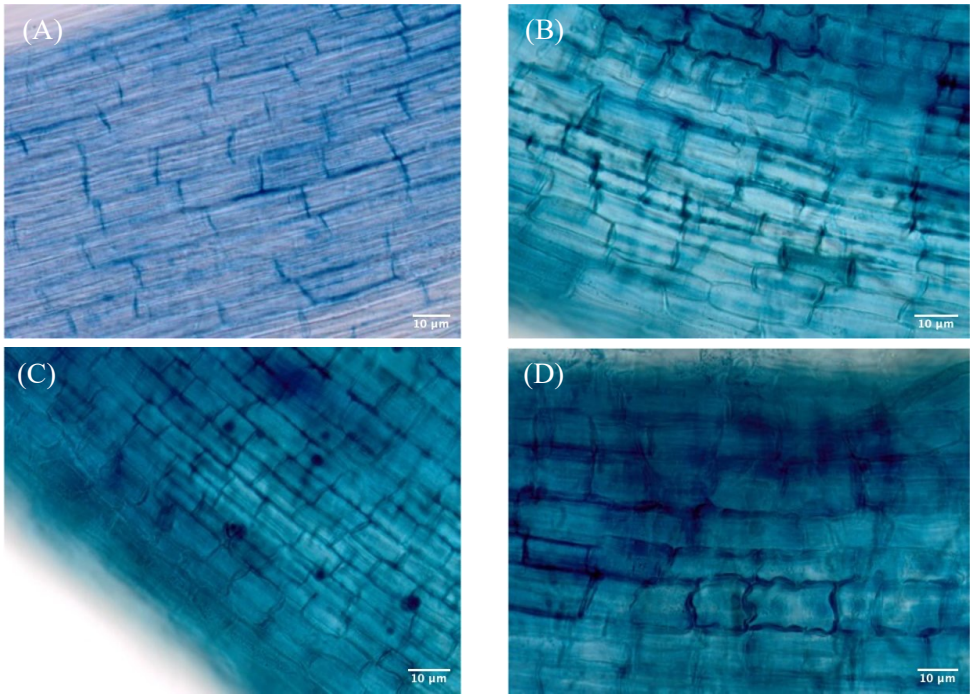

Fig. 4. (A) Non-treated DSE as control roots. (B) *Phialocephala* sp. (NH1121)-treated roots. (C) *Phialocephala helvetica* (NH1221)-treated roots. (D) *Phialocephala fortinii* (NH1331)-treated roots.

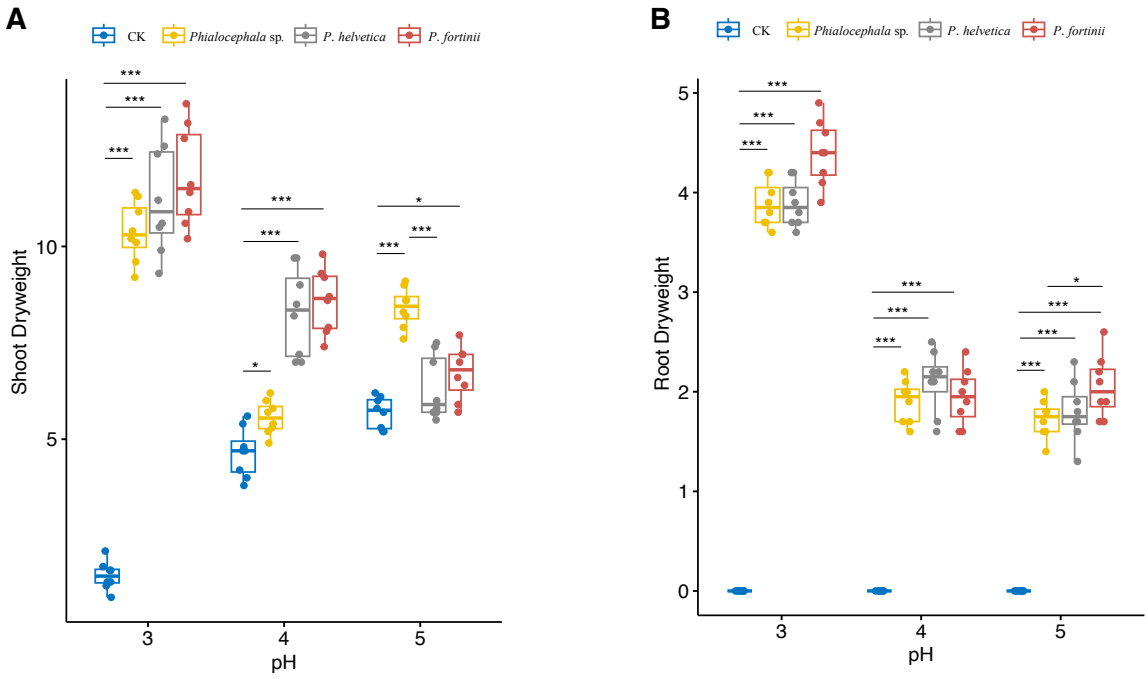

Fig. 5. Shoot and root dry weights of rice seedlings inoculated with NH1121 (*Phialocephala* sp.), NH1221 (*Phialocephala helvetica*), and NH1331 (*Phialocephala fortinii*) after three weeks of growth on oatmeal agar either at pH 3, pH 4, or pH 5 (acidic conditions). There are biological replicates (n=8). Median values are lines across the box with lower and upper boxes indicating the 25th to 75th percentiles, respectively. Whiskers represent the maximum and minimum values. Significance was determined by ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.