Sequence-based identification of soil fungi in different habitats on Surtsey

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ABSTRACT

Studies on primary succession and biological surveys in Surtsey have described the presence of different organisms including plants, soil fauna and fungi. However, since the last fungal census of 2008, based on visual observations, no further studies on fungal communities on the island have been published. In the present study, we aimed to expand the knowledge of the fungal communities of Surtsey using Internal Transcribed Spacer (ITS2) metabarcode sequencing to survey soil samples from 23 permanent plots, within and outside a gull colony. Additionally, we related fungal community composition to soil and ecosystem environmental variables that have been analyzed from these plots previously. We report at least 18 new genera and 44 new species for Iceland and 56 new species for Surtsey in this metabarcode survey, likely representing a combination of established organisms as well as those that may be present as dormant propagule bank. Our data indicate that fungal communities differ in areas with and without sea gulls as well as those that differ in soil substrate. Further, the community composition correlates with the number of gull nests, vegetation cover, ecosystem respiration, total N and exchangeable P. This survey provides new insights on the fungal community dynamics in relation to other biotic and abiotic factors. These findings complement what available data on soil biosphere on Surtsey and improve our understanding of primary succession.

INTRODUCTION

After Surtsey was formed between 1963 and 1967 primary succession has taken place and plants and sea birds have established in some areas contributing to soil formation and facilitating the arrival of other colonizers (Magnússon *et al.,* 2009). Several studies on primary succession and a variety of biological surveys have reported the presence of different taxonomic groups including lichens, vascular plants, soil microfauna and fungi (Magnússon *et al.,* 2009; Eyjólfsdóttir. 2008; Ilieva-Makulec *et al.,* 2015). For example, the funga on the island have been described by collecting sporocarps (Baldursson & Ingadöttir. 2007; Eyjólfsdóttir. 2008) and fungal spores (Greipsson & El-Mayas, 2000). In those studies, species representing different fungal phyla and occupying different ecological niches were observed. For example, ectomycorrhizal and arbuscular mycorrhizal fungi, which have an important role for plant colonization in the newly formed habitats, have been reported (Magnússon *et al.,* 2009). However, since the last fungal census in 2008 no additional studies on fungal communities have been published even though the research on colonization and expansion by flora and fauna has continued (Magnússon *et al.,* 2020).

Metabarcode sequencing provides means to study soil fungal communities in greater depth and resolution (Baalid *et al.,* 2012; Geml *et al.,* 2014) and has been a valuable in studying fungal primary succession (Baalid *et al.,* 2012; Brown and Jumpponen, 2014). In the present study, we

used metabarcode sequencing to analyze soil fungal communities in 23 permanent plots within and outside the gull colony in the southern part of Surtsey. We aimed to expand the knowledge of the species present on the island. Additionally, our goal was to correlate the fungal community structure with the environmental variables that have been measured in these plots over the years.

MATERIALS AND METHODS

The surveyed fungal communities in soils sampled from the 23 permanent plots established in 1990. These plots chosen to cover different substrate types and elucidate the influence of seagulls. The plots were located within and beyond the seagull colony. The two areas were then further subdivided into plots representing two substrates (Fig. 1): 1. Plots with no or shallow (≤10 cm) soils formed by windborne tephra sands that had covered the basaltic lava surfaces (Lava substrate) and 2. plots with deep soils (> 30 cm soil) in areas where the tephra sands had been deposited during the eruption (Sand substrate) (see Leblans *et al.,* 2014 for further details) (Table 1). Distinct plant communities have established within and beyond the seagull colony depending on the difference in the soil substrates (Table 1; Magnússon *et al*., 1996; Leblans *et al*., 2014).

In July 2014, we collected ten soil samples within each permanent plot along a 10 m. transect. The soil samples were pooled to one per plot. From this pool approx. 30 grams of soil was preserved in 25ml of 2X CTAB buffer and transported to the laboratory at Lund University where the material was freeze dried, ball-milled and the DNA was extracted using CTAB (hexadecyltrimethylammonium bromide) and EDTA (Ethylenediaminetetraacetic acid). The nucleic acids were purified from the cellular debris by chloroform and precipitated using isopropanol/ethanol. The precipitated extracts were further purified using the NucleoSpin Soil DNA extraction kit (Macherey-Nagel. Düren. Germany).

Aliquots of the extracted DNAs were shipped to Kansas State University and stored at -20°C until processed further. The DNA concentration

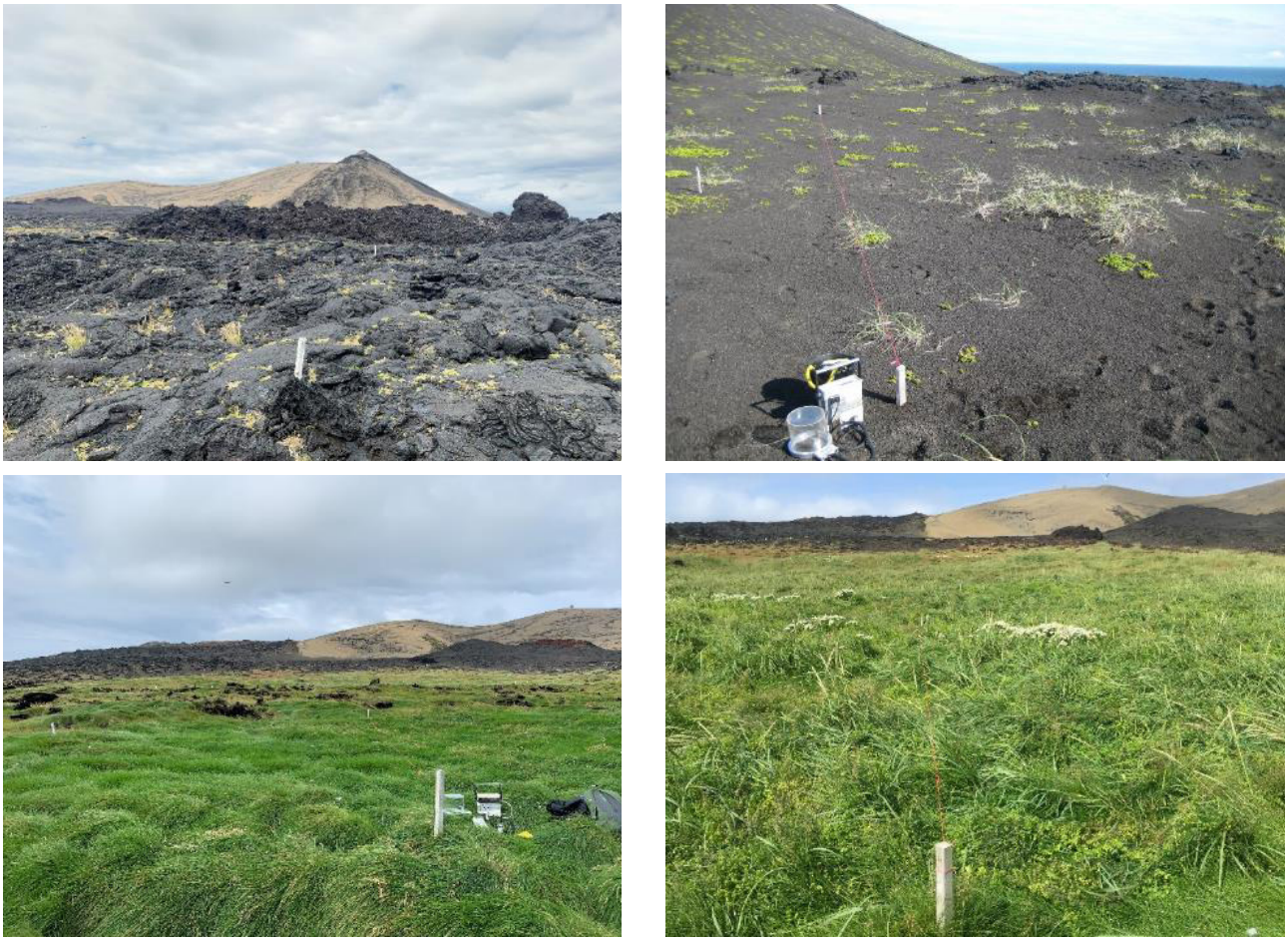

Figure 1. Photographs of the permanent plots formed on a lava substrate (to left) and sand substrate (to right) outside (top two) and inside (bottom two) the gull colony. Photos BDS.

Table 1. Characteristics of the permanent survey plots on Surtsey. Number of seabird nests during 2003-2015 within 1000 m^2, vascular plant and moss cover (%) in 2016, aboveground plant biomass (g m^{-2}) in 2018, Normalized Difference Vegetation Index (NDVI, unitless) 2016-2020, soil pH in 2004 and soil organic C and N concentration (%) in the 0-10 cm stratum in 2014, exchangeable total mineral N, P, Cu and Cd (µg of element 10 cm^{-2} 5 $days^{-1}$) in 2013 and ecosystem respiration (µmol CO_2 m^{-2} sec^{-1}) measured annually during 2015-2020.

	Inside the gull colony		Outside the gull colony	
Substrate	Shallow = lava	Deep = sand	Shallow = lava	Deep = sand
Seabird nests[1]	3.8	3.1	0.0	0.0
Flora				
Dominant vascular plant spp[1,2]	*Festuca rubra* > *Poa pratensis* > *Poa annua* > *Sagina procumbens*	*Poa pratensis* > *Stellaria media* > *Leymus arenarius*	*Sagina procumbens* > *Puccinellia distans* > *Cerastium fontanum* > *Silene uniflora*	*Honckenia peploides* > *Leymus arinarius* > *Rumex acitocella* > *Siline uniflora*
Vasc. pl. cover[1]	68%	177%	2%	5%
Moss cover[1]	1%	0%	5%	0%
Veg. biomass[1]	234	576	4	9
NDVI[5]	0.73	0.89	0.14	0.10
Soil				
pH[3]	6.5	6.8	7.2	7.8
Soil C[3]	8.9	1.4	0.24	0.04
Soil N[3]	0.52	0.11	0.02	0.01
Exch.able N[4]	21.3	111.2	6.6	5.3
Exch.able P[4]	20.2	20.5	0.5	0.4
Exch.able Cu[4]	0.06	0.08	0.24	0.13
Exch.able Cd[4]	0.01	0.01	0.06	0.04
Processes				
Ecos. Resp.[3,5]	1.65	2.24	0.08	0.10

1) Magnússon *et al.,* 2020. 2) Magnússon *et al.,* 2014. 3) Sigurdsson & Magnusson, 2010. 4) Sigurdsson & Leblans, 2020, 5) Sigurdsson *et al*., 2022

was measured using a Nanodrop ND2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA) and normalized to 5 ng/µL. For positive control, we constructed the fungal mock community from nine fungal pure cultures that broadly represent fungal taxa (Ascomycota: *Aspergillus niger*, *Chaetomium globosum*, *Penicilium griseoroseum*), *Saccharomyces cerevisiae*, *Sordaria fimicola*; Basidiomycota: *Coprinopsis cinerea;* Chytridiomycota: *Phlyctochytrium acuminatum* (synonym *Spizellomyces acuminatus*); Mucoromycota: *Phycomyces blakesleeanus*, *Rhizopus stolonifera*). Molecular grade RNA- and DNA-free H_2O was used as a negative control.

We targeted Internal Transcribed Spacer 2 (ITS2) using forward fITS7 (Ihrmark et al. 2012) and reverse ITS4 (White et al. 1990) primers with 12bp barcodes as described in Narayanan et al. (2021). All PCR reactions were performed in triplicate 50µL reactions. Each PCR reaction included 10µL or 20ng of the template, 200 µM of each deoxynucleotide, 1µmol of forward and reverse primers, 10 µL of 5X Green HF PCR buffer (Thermo Scientific,Wilmington, Delaware, USA), 14.75 µL of molecular grade water and 0.5 units of the proofreading Phusion Green Hot Start II High-Fidelity DNA polymerase (Thermo Scientific, Wilmington, Delaware, USA). PCR amplification was performed using Eppendorf MasterCyclers (Eppendorf, Hamburg, Germany). The PCR reactions began with an initial denaturing step for 30 s (98°C) and were followed by 35 cycles of 10 s of denaturing (98°C); 30 s of annealing (54°C); 1 min of extension (72°C); and concluding with a 10 min final extension (72°C). Positive and negative controls were included in every PCR amplification.

The PCR products were visualized by agarose gel (1.5%) electrophoresis to ensure the successful amplification and correct amplicon sizes. The triplicate amplicons were combined into one per experimental unit and cleaned using Omega Mag-bind® RXNPure Plus system following a modified manufacturer protocol using 1:1 ration of magnetic beads to the

PCR volume and two rinse steps with 80% ethanol. The cleaned product was quantified using Nanodrop ND2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA) and 250ng of amplicons from each experimental unit pooled separately for sequencing. Illumina-specific primers and adapters were added in four PCR cycles with KAPA Hyper Prep Kit (Roche, Pleasenton, CA USA) and 0.5µg starting DNA. Libraries were sequenced (2 x 300 cycles) using Illumina MiSeq Personal Sequencing System at the Integrated Genomic Facility at Kansas State University. Sequence data are available at the Sequence Read Archive (BioProject PRJNA815675).

Sequence data processing

We processed 941,027 (37,641 ± 12,933) raw sequences using the mothur pipeline (v. 1.38.0; Schloss et al. 2009) as per the MiSeq standard operation protocol (Kozich et al. 2013) where possible. Sequences were extracted from paired-end .fastq files, reverse and forward reads contiged and any sequences with ambiguous bases, sequences with more than 1base pair (bp) mismatch with primer and any mismatches to the sample-specific 12 bp molecular identifiers (MIDs), or homopolymers longer than 9 bp were omitted. The >99% similar sequences were pre-clustered (Huse et al. 2008), screened for chimeras using UCHIME algorithm (Edgar et al. 2011), and putative chimeras removed. The remaining sequences were assigned to taxa using the UNITE reference data base (Abarenkov et al. 2020) and clustered to Operational Taxonomic Units (OTUs) at 97% similarity using vsearch (Rognes et al. 2016). This resulted in a total of 159,948 high quality target sequences (6,898 ± 3,434 sequences per sample). Rare OTUs (fewer than 10 in the dataset) and those that were detected in the negative controls were removed from the further analysis.

We iteratively (100 iterations) estimated bacterial and fungal richness and diversity for each sample using mothur (v. 1.38.0; Schloss et al. 2009). To minimize biases resulting from differences in sequencing depths among the libraries (Gihring et al. 2011), we rarefied the fungal data to 1,000 sequences per sample. We estimated observed (S_{Obs}) and extrapolated (Chao1) OTU richness, Shannon's diversity (H'), and evenness (E_H).

The statistical analyses for the fungal communities were performed using the VEGAN package (Oksanen *et al*., 2013) in R (R Core Team. 2013). Fungal communities were visualized with three-dimensional ordination using non-parametric multidimensional scaling (NMDS). To test for correlations between environmental variables previously measured in the permanent plots (i.e. sum of gull nests, soil nutrient amounts, vegetal cover) and the fungal community ordinations, the Envfit analysis was performed.

To detect if the fungal communities were significantly influenced by substrate type and the presence of gulls, permutational multivariate analysis of variance was done (PERMANOVA; Anderson, 2014). Differences between plots were tested by using the pairwise Adonis test with Bonferroni correction. To test if the abundance of a given fungal species is significantly associated with substrate type or the presence of gulls, the Indicator Species Analysis was performed (Cáceres *et al.*, 2012).

RESULTS

Of the 50 most abundant OTUs present in the permanent plots (Figure 2), 13 are may represent new reports for Iceland, 30 new fungal species for Iceland and 45 new species for Surtsey (compare to Hallgrímsson & Eyjólfsdóttir, 2022). We used indicator taxon analyses to highlight those that were disproportionately more abundant in one habitat type than in the others. In these analyses, 19 OTUs were disproportionately more abundant in the gull plots with lava substrate; 15 in the gull plots with sand substrate; 3 in plots beyond the gull colony and with lava substrate; and, 3 in plots beyond the gull colony with sand substrate (Table 2).

The substrate type and the presence of gull colonies affected the fungal communities (PERMANOVA: $p<0.0001$; $F=1.9$; $R^2=0.2$) (Figure 3). The pairwise comparisons indicated that plots inside the gull colonies differed from those outside the gull colonies. The two substrates (lava and sand) also differed within the gull colonies (pairwise Adonis, $p < 0.05$; $F=1.7$, $R^2=0.2$) but not outside them.

Several environmental variables previously measured in these permanent plots significantly correlated with the soil fungal community composition: the average and the sum of gull nests; the vegetation cover (sum of vascular and non-vascular plant cover) and vegetation biomass; NDVI; ecosystem respiration; soil pH; P exchangeable; Cu exchangeable; Cd exchangeable; total C and total N were significantly correlated with the NMDS ordinations (Figure 3).

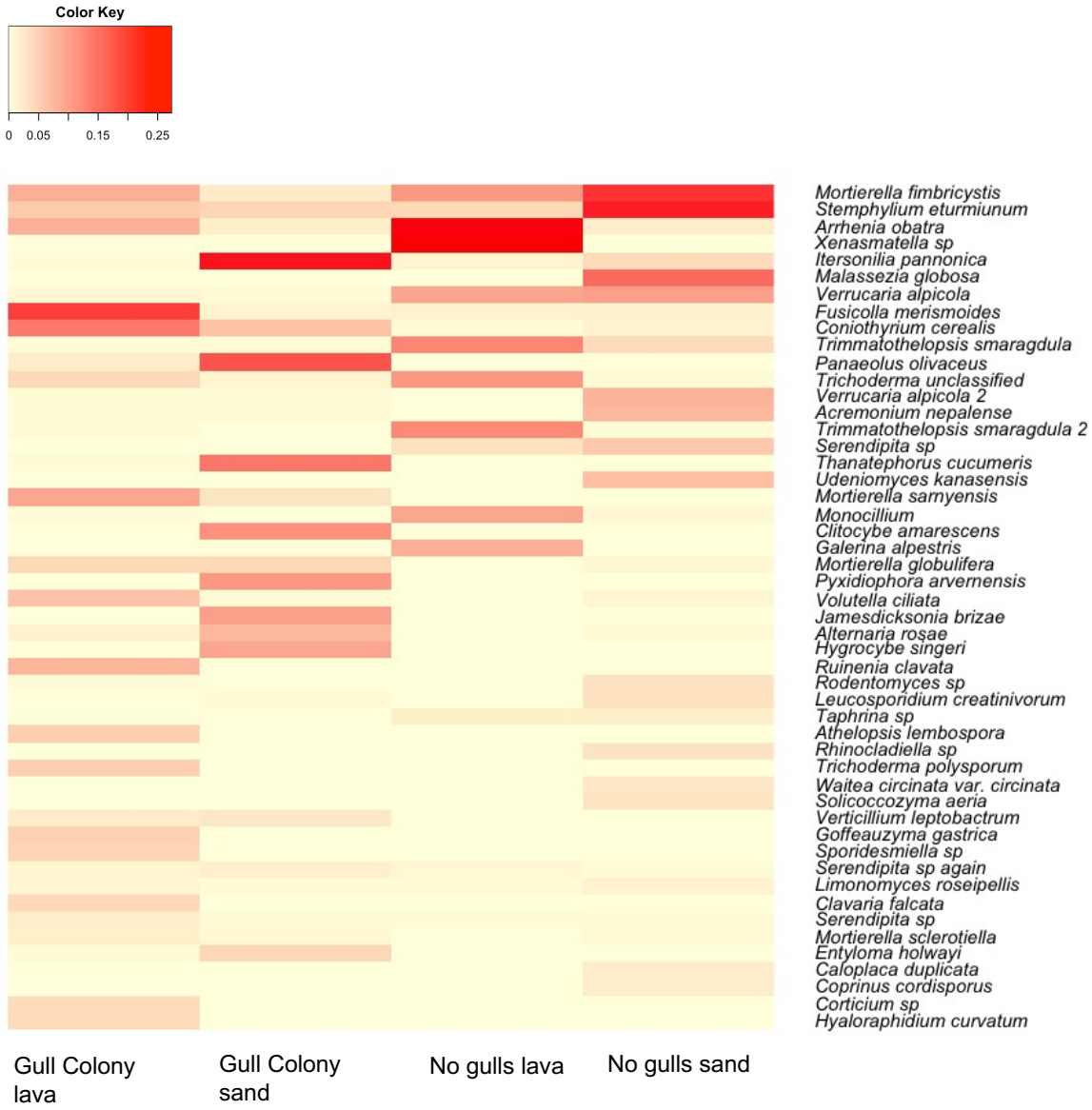

Figure 2. Heat map of the 50 more abundant fungal species in the permanent plots. The values in the color key correspond to the average relative abundance of the species for each treatment plot.

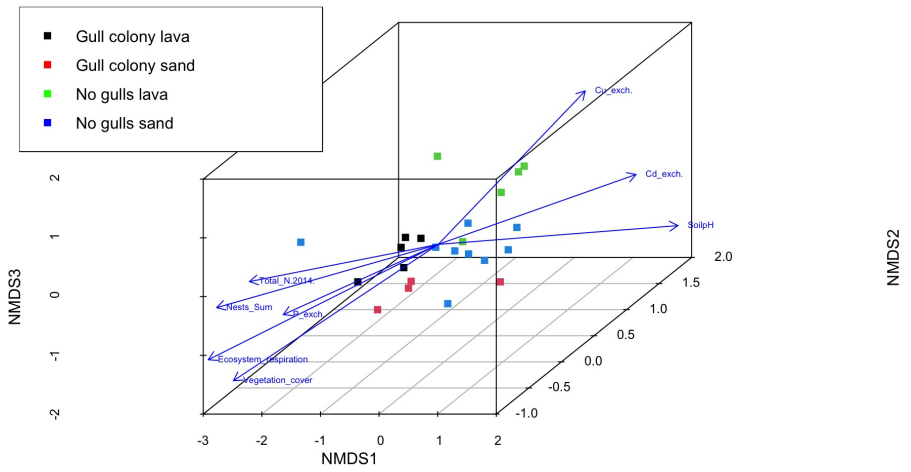

Figure 3. Three-dimensional NMDS ordination of the fungal communities from the permanent plots. The blue arrows represent the environmental variables that were significantly correlated with the ordination axis. The vector corresponding to the amount of total C overlapped with the vector corresponding to Total N. The vegetation biomass and NDVI (not showed in this graph) formed vectors with similar directions as the vegetation cover and the ecosystem respiration respectively.

Table 2. List of OTUs significantly associated with substrate type or the presence of gulls. Fidelity index = 1 means that the species occurs only in that group. Sensitivity index=1 means that the species is present in all replicates from that group.

Taxonomic assignment	Fidelity index	Sensitivity index	Stat	p value
Gull colony with lava substrate				
Goffeauzyma gastrica	0.99	1.00	0.99	0.005
Fusicolla merismoides	0.84	1.00	0.92	0.005
Ruinenia clavata	0.98	0.80	0.89	0.015
Neoascochyta tardicrescens	0.75	1.00	0.86	0.015
Kurtzmanomyces sp.	0.91	0.80	0.86	0.02
Rhinocladiella sp.	0.86	0.80	0.83	0.05
*Brachyphori*s sp.	0.84	0.80	0.82	0.01
Serendipita sp.	0.61	1.00	0.78	0.015
Parastagonospora avenae	0.76	0.80	0.78	0.015
Flagelloscypha sp.	1.00	0.60	0.78	0.02
Alfaria terrestris	1.00	0.60	0.78	0.025
Beauveria bassiana	0.96	0.60	0.76	0.035
Acaulospora nivalis	0.72	0.80	0.76	0.03
Schizothecium glutinans	0.71	0.80	0.75	0.045
Serendipita sp.	0.68	0.80	0.74	0.045
Dominikia sp.	0.87	0.60	0.72	0.03
Apiotrichum	0.86	0.60	0.72	0.045
Cladophialophora sp.	0.52	1.00	0.72	0.035
Drechslera sp.	0.41	1.00	0.64	0.045
Gull colony with sand substrate				
Jamesdicksonia brizae	0.97	1.00	0.98	0.005
Entyloma dahliae	0.87	1.00	0.94	0.01
Clitocybe amarescens	0.99	0.75	0.86	0.01
Pyxidiophora arvernensis	0.99	0.75	0.86	0.015
Sarocladium summerbellii	0.85	0.75	0.80	0.035
Cortinarius fulvescens	0.75	0.75	0.75	0.02
Parastagonospora novozelandica	0.73	0.75	0.74	0.045
Dominikia aurea	1.00	0.50	0.71	0.015
Papiliotrema frias	1.00	0.50	0.71	0.025
Lepista sordida	1.00	0.50	0.71	0.015
Filobasidium wieringae	1.00	0.50	0.71	0.025
Pyxidiophora arvernensis	1.00	0.50	0.71	0.015
Drechslera poae	0.98	0.50	0.70	0.04
Rhizoctonia alpina	0.88	0.50	0.66	0.025
Pseudoseptoria obscura	0.78	0.50	0.63	0.045
No gulls lava substrate				
Trimmatothelopsis smaragdula	0.74	1.00	0.86	0.02
Serendipita sp.	0.82	0.80	0.81	0.015
Cotylidia undulata	0.96	0.60	0.76	0.025
No gulls sand substrate				
Hirsutella rhossiliensis	0.85	0.67	0.75	0.02
Powellomyces sp.	0.71	0.78	0.74	0.03
Powellomyces sp.	0.98	0.56	0.74	0.05

DISCUSSION

In the current survey new genera and species for Surtsey (and even for Iceland) are reported. From the 50 more abundant OTUs up to 45 species were new registers for the island. However, this does not necessarily mean all those species are actively growing. The DNA detected for some of them could belong to dormant structures like spores from propagule banks. From the species that were significantly more abundant in the gull colonies plots with lava as substrate, only two arbuscular mycorrhizal fungal (AMF) species were found *Acaulospora nivalis* and *Dominikia* sp. In the gull colony plots with sand as substrate *Dominikia aurea* was significantly more abundant. No more AMF species were significantly represented in the gull plots and the rest of significantly abundant species belonged to yeasts, saprotrophic, phytopathogenic and entomopathogenic fungi (*Beauveria bassiana*). *Serendipita* sp. was found to be significantly abundant in the lava substrates from both inside and outside the gull colonies. *Serendipita* is a genus of root endophyte associated with AMF plants and whose species have been reported to be beneficial to its plant host in terms of nutrition and drought tolerance (Hallasgo *et al.,* 2020 ; Zhang *et al.,* 2017). It could be relevant to further study the effect of this endophyte and AMF species on the plant communities' adaptation to the different substrates and nutrient regimes in the permanent plots. In this context it should be pointed out that Delavaux *et al.* (2021) recently showed that AMF plant species have more limited dispersal ability than other mycorrhizal and non-mycorrhizal plant species and decrease relatively more on islands and with distance to continents.

In 2000 Greipsson & El-Mayas collected AMF spores belonging to the species *Glomus hoi* and *Scutellospora calospora* in dunes colonized by *Leymus arenarius* in Surtsey. In the survey of the present study *G. hoi* and *S. calospora* were not detected but the genera *Glomus, Paraglomus*, *Claroideoglomus* and *Rhyzoglomus* were found. However, they were not significantly influenced by the habitat types (according to the Species indicator analysis) and their abundance was low (less that 1 % of the species relative abundance). A higher abundance of AMF species (especially the *Glomus* spp.) should be expected in the gull colony plots where *Leymus arenarius* is co-dominating the plant community. The more abundant species registered in this study were saprotrophic fungi and plant pathogens. Since saprotrophic species produce more biomass per soil volume than the AMF species (as reported by Jiang *et al.,* 2020) it is possible that they earlier contributed with more DNA and diluted the signal of the AMF species. The use of ingrowth meshbags that select for extramatrical mycorrhizal growth (Wallander *et al.*, 2001) could be useful to increase the resolution of AMF signal and to study the differences in AMF composition in the permanent plots.

Ectomycorrhizal species were also registered in the present survey. For example *Cortinarius fulvescens* was significantly associated with the plots inside the gull colonies with sand substrate. This fungus is probably forming association with Salix plants that have established here; *Salix herbacea*. Eyjolfsdottir 2009 found an unidentified *Cortinairus* species which probably was *C. fulvescens*, and also several other ectomycorrhizal genera in her survey of sporocarps, namely *Hebeloma, Inocybe* and *Laccaria*. From these genera only *Hebeloma* was found in the present study, but at low numbers (less that 1 % of the species relative abundance).

Fungal communities in the plots within the seagull colony differed from those beyond it. Moreover, the number of seagull nests, vegetation cover, ecosystem respiration, total nitrogen and exchangeable P exchangeable were correlated with the ordination of the fungal communities from the Gull plots. Taken together, this indicates that the N and P inputs from the seagull deposits that have led to greater C accumulation, vegetation cover and plant biomass (Leblans *et al,.* 2014; Magnússon *et al.,* 2020) may also drive fungal community dynamics. Our data confirm that the presence of the seagulls is the key factor in shaping the soil biosphere on the island as has been reported for other soil organisms including microfauna (Magnússon *et al.,* 2014 ; Ilieva-Makulec *et al.,*2015) and bacteria (Marteinsson *et al.,* 2015).

The substrate (lava or sand) also affected the fungal communities but only within the seagull colony. The sand plots (>30 cm of tephra sand) contain deeper soil than the lava plots. Under these conditions there is a lesser desiccation risk when soil temperatures fluctuate providing thus better environmental conditions for plant roots and soil microorganisms (e.g. Sigurdsson 2009; Sigurdsson & Stefansdottir, 2015). These different substrates could have shaped the composition of fungal communities as they also differ in the plant cover, plant biomass and plant community composition between

the plots (Magnússon *et al.,* 2020). For plots outside the gull colony on the other hand, the two different substrates were not enough to cause differences in the fungal communities indicating that both vegetal cover and plant species composition are the more important factors influencing fungal communities. This is consistent with other studies showing that the soil fungal community varies along with changes in the plant community (Zinger *et al.,* 2011; De Bellis *et al.,* 2007).

By using next generation sequencing the aim of the present study was to expand the knowledge of the funga of Surtsey. Indeed, we have provided new species registers (including AMF) that were not described before with other survey methods. Moreover, we found that the fungal community structure is strongly influenced by the nutrients brought from the sea by marine birds as has been described for other soil organisms. Since soil fungi play a key role for plant nutrition and carbon sequestration these results are key to understand primary succession. Further studies on the effect of fungal endophytes on plant colonization to the new habitats are advised.

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