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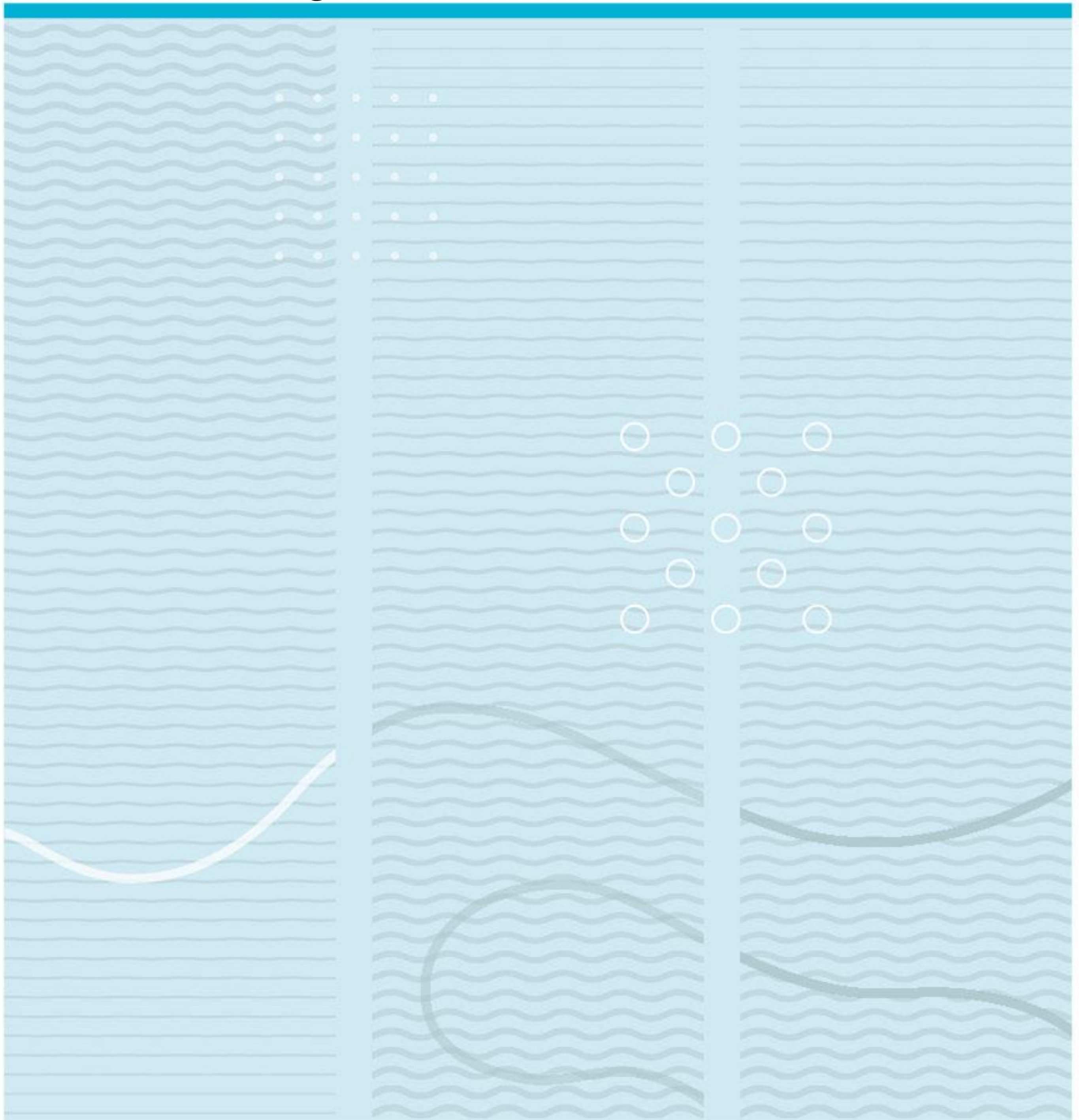
What's in the pot?

Identifying contaminant species in the ornamental plant trade with eDNA metabarcoding



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This thesis is worth 60 study points

Abstract

Monitoring alien species introduction pathways is a central strategy in early detection of alien species. eDNA metabarcoding is a potentially valuable method for detecting alien species and describing source populations of introduction pathways. In this study, eDNA metabarcoding was used to identify and assess source populations of contaminant species of vascular plants and fungi in ornamental plant imports to Norway. By filtering identified contaminant species to various records and databases, a range of native species, known alien species, and potentially emerging alien species to Norway were identified. Also, the richness and composition of contaminant species was found to vary between samples from different ornamental taxa and import containers. Further, estimates of contaminant species richness exceeded the number of observed species in sampled import containers. Lastly, increased sampling effort per sampled ornamental pot contributed to increased species detection. The findings of this study highlight the need for continued research and monitoring to assess and understand predictors of contaminant species source populations in ornamental imports, and the importance of appropriate study design and sampling effort for increasing species detection. The study concludes that metabarcoding is an valuable additional tool for identifying and assessing source populations of contaminant species in ornamental imports.

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Introduction

Impacts of alien species and biological invasions are among the leading factors driving global change and biodiversity loss (Díaz et al. 2019). Detrimental effects of alien species on native biodiversity and ecosystems are ecologically harmful (Pyšek et al. 2020) and economically costly to repair (Haubrock et al. 2021). Preventing alien species establishment is less ecologically harmful, more cost-effective, and more feasible, than efforts to eradicate or control established alien species (Pluess et al. 2012, Simberloff et al. 2013). Monitoring and managing alien species introduction pathways facilitate early detection and rapid response to prevent alien species establishment. Thus, managing and monitoring introduction pathways are considered central strategies in preventing biological invasions (Hulme et al. 2008, Essl et al. 2015).

Introduction pathways are characterized by the mechanisms and vectors facilitating them, such as global trade (Hulme et al. 2008, Harrower et al. 2018). Introduction pathways often have obvious associations to certain taxonomic groups, such as alien plants introduced through trade in ornamentals (van Kleunen et al. 2018). However, pathways may also facilitate unintended introductions of large quantities of diverse taxa as stowaways or contaminants in commodities (Pergl et al. 2017). The rate of first records of species typically introduced unintentionally have increased in the last decades (Seebens et al. 2017), and unintentional introductions of alien species have become more frequent than intentional introductions (McGrannachan et al. 2021). This is likely driven by access to new source populations of emerging alien species, as a result of increased globalization and trade (Seebens et al. 2018). Pathways delivering large quantities of diverse taxa are particularly important to manage because they are likely to facilitate introductions of emerging and high-risk alien species (Pergl et al. 2017).

The ornamental plant trade is a major introduction pathway for alien species (Hulme et al. 2018, van Kleunen et al. 2018). Trade in ornamental plants is the most important pathway for alien plant species worldwide (van Kleunen et al. 2018, Beaury et al. 2021). In addition to intentionally traded plants, ornamental imports serve as transportation vectors for a range of contaminant taxa. For instance, studies have found contaminant vascular plants and invertebrates (e.g., Conn et al. 2008, Bruteig et al. 2017), and pathogenic microorganisms (e.g., Rossmann et al. 2021), in soil samples from ornamental imports. During six years of sampling, researchers in Norway identified more than 500 species of contaminant invertebrates and vascular plants. A third of these species were non-native to Norway, and included known doorknocker species (i.e., alien species that may establish in Norway within 50 years; Sandvik

et al. 2020) (Westergaard et al. 2020a). Contaminant taxa in ornamental imports can be difficult or impossible to detect and identify morphologically for inspectors or even taxonomic experts (Liebhold et al. 2012). Effective and precise methods of species detection and identification are required to identify potential doorknocker species and document the diversity of contaminant species source populations in ornamental imports.

Genetic species identification through analysis of environmental DNA (eDNA) has become an important tool in surveying and monitoring biodiversity (Taberlet et al. 2018). eDNA refers to genetic material shed by organisms to their surrounding habitats, such as soil, that can be extracted from environmental samples. Through targeted DNA amplification and sequencing, eDNA can be used to identify specific species present in the sampled environment, including alien species (e.g., Kamoroff and Goldberg 2017, Valentin et al. 2020, Gargan et al. 2021). By targeting DNA regions specific to higher taxa, several species within taxonomic groups can be identified from a single environmental sample, known as eDNA metabarcoding (Deiner et al. 2017). Metabarcoding has been used to survey biodiversity of several taxonomic groups, such as vascular plants (Yoccoz et al. 2012, Coghlan et al. 2021) and fungi (Rosa et al. 2020, Tatsumi et al. 2021).

Metabarcoding has also been used to identify contaminant species in the ornamental plant trade. Rossmann et al. (2021) used metabarcoding to document a range of plant pathogenic oomycetes in soil samples from ornamental imports. Others reported that complementing morphological identifications with metabarcoding increased species identification of contaminant invertebrates, due to metabarcoding being more precise than conventional methods in identifying organisms in all life stages (Westergaard et al. 2020b). This demonstrates the utility of metabarcoding in identifying contaminant species in the ornamental plant trade.

While metabarcoding is increasingly being used in biodiversity surveys and monitoring, methodological choices are not standardized. Metabarcoding includes several steps requiring researchers to make decisions related to study design that can affect studies' results (Zinger et al. 2019). For instance, sampling method and effort can greatly influence the number of species detected (Dickie et al. 2018), and studies have reported that increased sampling effort resulted in higher levels of species detection (Mata et al. 2019, Macher et al. 2021). Species detection rates with varying sampling effort is an important consideration when applying metabarcoding to monitor ornamental imports, because source populations of contaminant species are highly diverse and largely unknown (Westergaard et al. 2020a).

The present study is part of a project ("the main project" from here) assigned to the Norwegian Institute for Nature Research (NINA) by the Norwegian Environment Agency to

monitor the ornamental plant trade as an introduction pathway for alien species to Norway since 2014 (Westergaard et al. 2021). The goal of the study was to use eDNA metabarcoding to identify species and assess source populations of contaminant vascular plants and fungi in soil samples from imported ornamental *Taxus* sp. and *Thuja* sp. in two import containers from the Netherlands to Norway.

From the detected species, I aimed to investigate the number of native, known alien, and potential alien species to Norway. I also surveyed potential alien species to assess whether they should be assessed as potential doorknocker species to Norway by taxonomic experts in accordance with revision of the Norwegian Alien Species List. For vascular plants, I also crosschecked identified species against those previously recorded in the main project. For this analysis, I included species identified morphologically after germination of seeds in soil samples from the same two import containers. Contaminant fungi have not been assessed by the main project in previous years and were therefore not subject to similar analysis. For fungi, I investigated the amounts of taxa belonging to different trophic modes.

Further, I intended to investigate patterns of contaminant species richness and composition between the two ornamental taxa and import containers. Next, I estimated the total number of contaminant species in each import container and the number of sampled pots required to detect all species. I hypothesized a difference between the number of observed species and estimated species richness, and predicted that estimated richness would exceed observed richness. Lastly, I investigated the number of species detected by taking one soil sample per pot compared to two samples per pot. I hypothesized that the number of species detected would differ between one and two samples per pot, predicting that more species would be detected when taking two samples per pot.

Methods

Soil sampling

Soil was sampled by staff from NINA on 7 April 2021 from two import containers ("Container 1" and "Container 2" from here) that arrived at Blomsterringen Engros AS (Sanitetsveien 15, 2013 Skjetten, Viken county, Norway) from Noviflora Holland B.V. (Venus 240, 2675 LN Honselersdijk, the Netherlands). Both containers contained *Taxus* sp. and *Thuja* sp., with an additional 12 ornamental taxa in one of them.

Soil samples for metabarcoding were taken only from *Taxus* sp. and *Thuja* sp. In each container, 15 pots of each taxon were sampled: ten pots were sampled once and five were sampled twice ("duplicate sampled pots" from here). This resulted in 20 samples per taxon per

container and 80 samples in total. Samples was taken by depositing approximately 20-25 grams of soil from around the plant's stems in Whirl-Pak bags using disposable gloves and plastic spoons that were changed between each sample. Bagged samples were marked with a serial number, date, and ornamental taxon name, before being frozen and sent to NINA's lab in Trondheim, Norway, for DNA extraction. In addition, soil samples for germination of contaminant plant seeds in the soil were taken from 10 pots in each container from various ornamental taxa. Procedures for sampling, vernalization and germination was carried out as described in (Westergaard et al. 2021).

Lab procedures

All lab procedures were carried out by staff at NINA. Lysis, homogenization, and DNA extraction was carried out in 2 ml volumes using the FastDNA Spin Kit for Soil (MP Biomedicals) according to the manufacturer's instructions. DNA was amplified in a two-step PCR following an Illumina protocol, using the ITS2_S2 and ITS4 primers to target plant DNA (Chen et al. 2010) and the fITS7 and ITS4 primers to target fungal DNA (White et al. 1990, Ihrmark et al. 2012). The first PCR run included primers with overhang-adaptor sequences and was conducted in 25 µL volumes containing 1X KAPA HiFi HotStart ReadyMix (Roche), 0.5 µM of each primer, and 2.5 µL of 10 ng/µL template DNA. The second PCR run included Illumina indices. For plants, PCR was conducted with an initial denaturation step at 95 °C for three minutes, and 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 56 °C for 30 seconds, extension at 72 °C for 30 seconds and a final step of extension at 72 °C for 10 minutes. For fungi, PCR was conducted with initial denaturation at 94 °C for three minutes and then at 94 °C for 35 cycles of 30 seconds, annealing at 56 °C for 35 cycles of 30 seconds, and extension at 72 °C for 35 cycles of 30 seconds, and then for seven minutes at 72 °C. Magnetic beads (Mag-Bind TotalPure NGS, Omega Bio-tek) were used to remove fragments shorter than 200 base pairs and longer than 600 base pairs, and the quality of the PCR products was inspected on an Agilent 4200 TapeStation system using a D1000 ScreenTape Assay. In the second PCR run, IDT for Illumina UD index adapters were added to the 5' and 3' ends of the amplicons according to the manufacturer's instructions. Magnetic beads were used to remove fragments shorter than 500 base pairs. Amplicons were then pooled in equimolar amounts and sequenced on an Illumina NovaSeq 6000 platform at the Norwegian Sequencing Centre, University of Oslo.

Bioinformatic procedures

Demultiplexing was conducted on the Illumina NovaSeq 6000 platform at the Norwegian Sequencing Centre, and bioinformatic procedures prior to data analyses were carried out by

staff at NINA. Primers were removed using cutadapt version 1.18 (Martin 2011), requiring a minimum length match of 17 base pairs with 0.15 expected errors. The DADA2 pipeline was used to generate ASVs (Callahan et al. 2016). Quality filtering was performed with the "filterAndTrim" function with a maximum of two expected errors for both forward and reverse reads, and a minimum read length of 50 base pairs after trimming. Error rates were estimated with the "learnError" function, reads were dereplicated with the "derepFastq" function, and the "dada" function was used to interpret the error rates, and correct erroneous sequences. Forward and reverse reads were merged with the "mergePairs" function to generate ASVs, and ASVs were mapped to each sample using the "makeSequenceTable" function, creating an ASV-by-sample matrix. Lastly, chimeras were removed with the "removeBimeraDenovo" function.

Plant taxonomy was assigned using the sintax algorithm (Edgar 2016) and PLANiTS database (Banchi et al. 2020), and fungal taxonomy with the RDP algorithm and the UNITE database (Abarenkov et al. 2010). Confidence was required to be higher than 80 % for successful assignment at a given taxonomic level. Non-target ASVs (non-Streptophyta for plants and non-fungal for fungi) were identified by Megablast comparisons to GenBank and removed.

Fungal ASVs were grouped according to ecological guilds with FUNGuild (available from: https://github.com/UMNFuN/FUNGuild). ASVs were classified as having one or more of three trophic modes; saprotrophic; symbiotrophic; and pathotrophic (Nguyen et al. 2016).

Data analysis

Separate objects were generated for vascular plants and fungi with the *phyloseq*-package v.1.38.0 (McMurdie and Holmes 2013) for analyses in R (R Core Team 2021). The number of sequences retained through bioinformatic filtering, from primer removal through each step of the DADA2-pipeline, was assessed visually with a line plot. The number of plant and fungal sequences retained per sample after bioinformatic filtering was assessed with bar plots. All three plots were generated with the R package *ggplot2* v.3.3.5 (Wickham 2016).

Contaminant species classification

Identified species of vascular plants and fungi were classified as native to Norway, known alien species to Norway, or potential alien species to Norway. To identify native species, I manually filtered species against the Norwegian Species Nomenclature Database (NSND) (Artsdatabanken 2015). Species not identified as native were filtered against the Norwegian Alien Species List (NASL) (Artsdatabanken 2018) to identify known alien species to Norway. Species that were neither native nor known aliens to Norway were looked up in the Global

Biodiversity Information Facility (GBIF 2022) to retrieve information about their geographic distribution. Species were classified by geographic prevalence, according to whether they were known in: Finland, Sweden, and/or Denmark ("Neighboring countries"); Europe; only outside of Europe ("Non-Europe").

I crosschecked vascular plant species identified from both metabarcoding and germination manually against species previously recorded by the main project by accessing the main project's database (NINA 2022).

The relative amounts of identified fungi belonging to different trophic modes was manually assessed for samples from *Taxus* and *Thuja* in the two containers separately and visualized with bar plots generated with *ggplot2*.

To identify potential doorknocker species to Norway, species that were neither native nor known aliens to Norway were manually crosschecked against five international alien species lists and databases. These were the European Union's List of Invasive Alien Species of Union Concern ("EU-list"; EU 2019); the European Network on Invasive Alien Species (NOBANIS 2022); the Global Register of Introduced and Invasive Species (GRIIS; Pagad et al. 2018); the Global Invasive Species Database (GISD; ISSG 2015); and the Inventory of alien species in Europe (DAISIE; Roy et al. 2020). I crosschecked species against the EU-list, NOBANIS, and GISD, online. I crosschecked species against GRIIS by accessing each species in GBIF, as data from GRIIS is made available there. Lastly, I downloaded the full DAISIE dataset from GBIF for manual crosschecking. Next, I surveyed species that were registered in one or more alien species databases with respect to the species' ecology and distribution. I performed literature searches in Plants of the World Online (POWO 2022), Mycobank (Robert et al. 2013), GBIF, and Google scholar to find relevant literature. Links to all databases and lists are provided in the Reference section.

Because this study focused on contaminant species' status in Norway, species names were adopted from the NSND when available. Synonymous plant species names were derived from POWO and fungal species synonyms were retrieved from Mycobank and used throughout crosschecking species against the databases and lists mentioned above.

The taxonomic composition of identified taxa of vascular plants and fungi identified by metabarcoding was explored and visualized using Krona charts (Ondov et al. 2011). I generated separate Krona charts for vascular plants and fungi using an Excel template downloaded from GitHub (available from: https://github.com/marbl/Krona).

Observed contaminant species richness

For analysis of species richness, the number of sequences in all samples were normalized by rarefaction without replacement, using the “rarefy_even_depth” function in *phyloseq*. To retain as many samples and taxa as possible, vascular plant data was rarefied at a sample size of 21 000 sequences, while fungal data was rarefied at 198 000 sequences.

Mixed-effects models were chosen as appropriate to test for significant differences in species richness while accounting for the unequal number of soil samples taken from the pots (i.e., 20 of 60 pots were sampled twice). Prior to fitting the models, I investigated the distribution of the plant and fungal data visually with histograms, and tested the data’s goodness of fit using the functions “fitdist” and “gofstat” from the R package *fitdistrplus* v.1.1.6 (Delignette-Muller and Dutang 2015).

The plant data were not significantly different from a normal distribution, and I fitted a linear mixed-effects model with ornamental taxa and container, as well as their interaction (i.e., ornamental taxa within containers), as fixed effects, and sampled pot as a random effect to account for the uneven number of samples between pots. For this model, I used the package *lmerTest* v.3.1.3 (Kuznetsova et al. 2017). After fitting the model, I used the function “lsmeans” from the R package *lsmeans* (Russel 2016) for post-hoc pairwise comparison between groups.

The fungal data were log-transformed to fit a beta distribution, and the R package *glmmTMB* v.1.1.2.3 (Brooks et al. 2017) was used to fit a generalized linear mixed-effects model with ornamental taxa and container as fixed effects, and sampled pot as a random effect. P-values for the fungal model were generated with the “p_value” function in the R package *parameters* (Lüdecke et al. 2020). I visualized the models’ results with boxplots using *ggplot2*. I used the “simulateResiduals” function in the R package *DHARMa* v.0.4.5 (Hartig 2022) to validate that both models’ residuals did not deviate significantly from their respective distributions.

Contaminant species composition

Analysis of contaminant species composition was carried out using the same rarefied data as in the analysis of observed species richness. I assessed differences in species composition between samples visually by generating non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarities between samples, generated with the “plot_ordination” and “distance” functions in *phyloseq*, respectively. To test for statistically significant differences in species composition between samples from the two ornamental taxa and containers, and their interaction, I utilized PERMANOVA (Anderson 2017) models with the “adonis2” function in

the R package *vegan* v.2.5.7 (Oksanen et al. 2020). PERMONVAs were fitted equally for plant and fungal data with Bray-Curtis distances at 9999 permutations.

Estimated contaminant species richness

To investigate how many soil samples would be required to detect all contaminant species in the two import containers, estimates of plant and fungal species richness, respectively, were generated by extrapolation based on incidence data (i.e., the number of present species in each sample) (Chao et al. 2014). Only one sample was included from the duplicate sampled pots, therefore 60 samples were included in these estimates in total. Extrapolation was carried out using the *iNEXT* R package v.2.0.20 (Hsieh et al. 2016). Because *iNEXT* interpolates existing data to extrapolate species richness, I used unrarefied data for these estimates. The function "iNEXT" was used to conduct interpolation and extrapolation of species richness, with the number of knots set to 300. I used the function "ggINEXT" to plot corresponding interpolation/extrapolation curves to visually display the accumulation of new species detected per sampled pot.

Species detection in duplicate sampled pots

To compare the number of species detected in one and two samples per pot, I only used data from the 20 duplicate sampled pots. I manually compared species lists from the first and second samples taken from the pots and visualized the results with Euler diagrams generated with the R package *eulerr* (Larsson et al. 2021).

Results

Sequencing and bioinformatic filtering

From the vascular plant library, 63 131 949 raw sequences were produced from 80 soil samples (median per sample=672 006 ± 293 242.75 interquartile range (IQR)). After bioinformatic filtering and taxonomic assignment, 24 911 523 (39.45 % of raw sequences, median=233 702 ± 373 196.50 IQR) had been retained. The largest decline in sequence number during bioinformatic filtering was approximately 24 % during primer removal prior to input to DADA2, suggesting that approximately a fourth of sequences were of low quality. The number of sequences also declined from approximately 61 % to 39 % of raw sequences during taxonomic assignment, suggesting that a large portion of the sequences belonged to non-target taxa (Figure 1 A, B).

For the fungal library, 61 740 744 sequences were produced during sequencing (median=714 690 ± 408 475.75 IQR), and 40 505 859 (65.61 % of raw sequences, median=467 864 ± 257 099.80 IQR) sequences were retained after filtering and taxonomic assignment. The largest decrease in sequence number was from approximately 96 % to 78 % of raw sequences during quality filtering. Notably fewer sequences from the fungal library were discarded during taxonomic assignment than from the vascular plant library, suggesting that relatively more sequences belonged to target taxa in the fungal library (Figure 1 A, C).

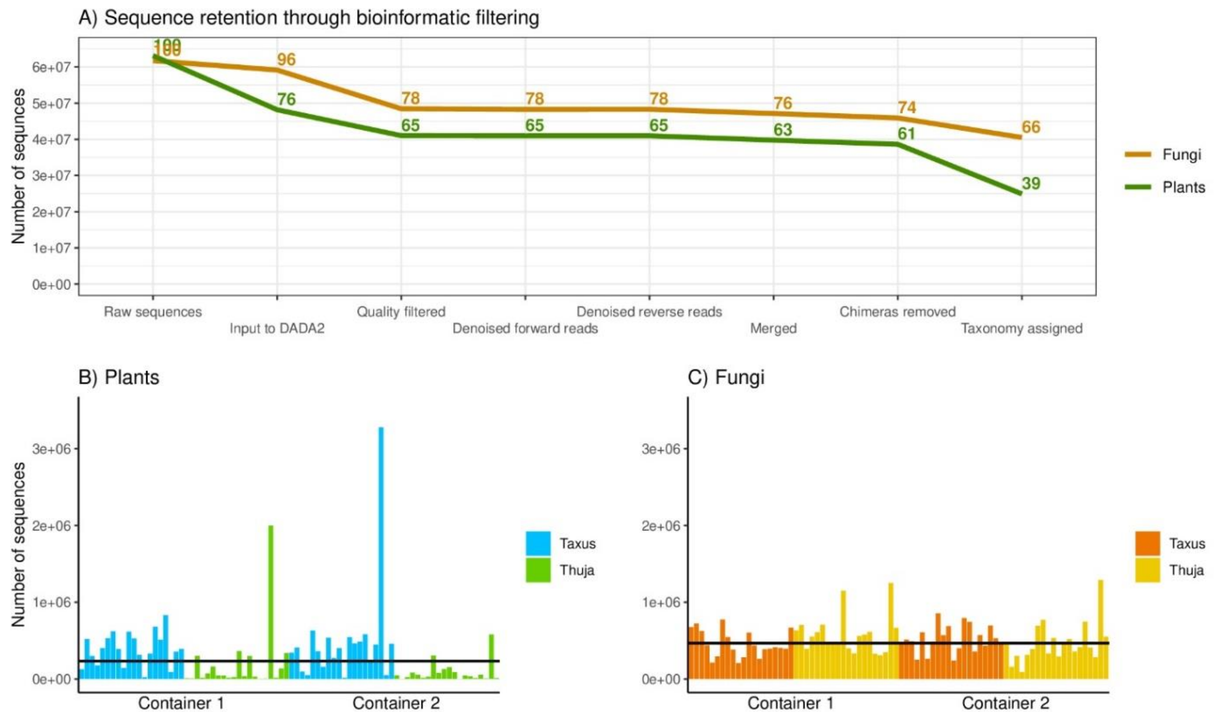

Figure 1: *A) Sequence retention through bioinformatic filtering and B) the number of sequences retained per sample after filtering in the vascular plant and C) fungal datasets. Annotated points in A) show the percentage of sequences retained after each step of bioinformatic filtering. Black horizontal lines in B) and C) show the median number of sequences per sample after bioinformatic filtering.*

Contaminant taxa and species origin

In 80 soil samples, 104 contaminant plant taxa were identified by metabarcoding. Nineteen (18.27 %) taxa that belonged to mosses (12 taxa), green algae (three taxa), *Taxus* sp. (two taxa), *Thuja* sp. (two taxa), and unassigned Streptophyta (one taxon), were removed (a full list of removed taxa is provided in the Supplementary material (Table S1)), leaving 85 vascular plant taxa ("plants" from here). Thirty-three (38.82 %) plants were identified to species level, 10 (30.30 %) of which were native and 15 (45.45 %) were known alien species in Norway. None of the remaining eight (24.24 %) plant species were known from any neighboring countries, while four (12.12 %) were known from Europe (Table 1, Figure 2). Considering all vascular

plant taxa identified by metabarcoding, the most abundant orders were Asterales (22.35 %), Caryophyllales (11.75 %), and Brassicales (10.58 %) (Table 1, Figure S1).

In addition to the 33 plant species identified by metabarcoding, 30 species were identified morphologically after germination. Only five species (8.62 %) were identified by both methods, adding up to a total of 58 plant species identified in the two import containers. Of the 25 species only identified morphologically, four (16.00 %) had not been recorded previously in the main project. In contrast, 27 (96.43 %) of the 28 species identified exclusively by metabarcoding had not been previously recorded. All five species that were identified by both methods had been recorded in previous years. A full overview of plant species detected by metabarcoding and germination and their detection history in the main project is provided in Table S2.

Of the 4618 fungal taxa identified, 1232 (26.68 %) were identified to species. Of these, 487 (39.53 %) were native to Norway, and only 10 (0.81 %) were known alien species to Norway. Further, 275 (22.32 %) species were known from at least one neighboring country, 282 (22.89 %) were known from Europe, and 134 (10.88 %) were only known from outside Europe. Data on prevalence and distribution was unavailable in GBIF for 44 (3.57 %) of the fungal species (Table 1, Figure 2). Considering all fungal taxa, the most abundant class was Agaricomycetes (21.13 %), followed by Sordariomycetes (17.35 %) and Dothideomycetes (12.42 %) (Table 1, Figure S1). A full list of all fungal species is provided in Table S3.

Data on trophic modes was available for 2557 of the identified fungal taxa. The distribution of trophic modes was similar across ornamental taxa and containers, with saprotrophic fungi being most abundant (>30 % across ornamental taxa and containers) and pathotrophic-symbiotrophic being least abundant (≤5 %). Symbiotrophs were slightly more abundant in samples from *Taxus* than in samples from *Thuja* (Figure 3). A full overview of FUNGuild-results for all relevant taxa is provided in Table S4.

Table 1: *List of contaminant vascular plants and fungi identified with eDNA metabarcoding. The total number of identified taxa is sorted by class and order for plants, and by phylum and class for fungi. For both groups, taxa identified to species are sorted according to whether they were: Native to Norway; Known alien species to Norway ("Alien to Norway"); known in Sweden, Finland, and/or Denmark ("Swe., Fin., Den."); known in Europe; known only outside of Europe ("Non-Europe"). Species that were non-native and not known alien species to Norway with no available data on geographic prevalence in GBIF are listed in the column "NA".*

Plants

Identified taxa			Species origin						
Class	**Order**	**Number of taxa**	**Native to Norway**	**Alien to Norway**	**Swe., Fin., Den.**	**Europe**	**Non-Europe**	**NA**	**Sum of species**
Magnoliopsida	Apiales	4	1			1	1		3
	Aquifoliales	1				1			1
	Asparagales	2		1					1
	Asterales	19	1	5			1		7
	Brassicales	9	1	3					4
	Caryophyllales	10	1	1		1			3
	Cucurbitales	1		1					1
	Ericales	2				1	1		2
	Fabales	2		1					1
	Fagales	4							
	Geraniales	2	1						1
	Lamiales	4					1		1
	Malpighiales	3		1					1
	Malvales	2							
	Myrtales	3							
	Poales	7	3						3
	Proteales	1							
	Rosales	3	1						1
	Sapindales	2		1					1
	Solanales	3	1						1
Pinopsides	Cupressales	1		1					1
	Sum of plants	85	10	15		4	4		33

Fungi

Identified taxa			Species origin						
Phylum	**Class**	**Number of taxa**	**Native to Norway**	**Alien to Norway**	**Swe., Fin., Den.**	**Europe**	**Non-Europe**	**NA**	**Sum of species**
Aphelidiomycota	Aphelidiomycetes	11							
	NA	3							
Ascomycota	Archaeorhizomycetes	3							
	Ascomycota_cls_ Incertae_sedis	1							
	Dothideomycetes	573	11		29	57	29	6	132
	Eurotiomycetes	258	9		23	34	11	3	80
	Geoglossomycetes	1				1			1
	GS37	2							
	Laboulbeniomycetes	5			1	1			2

	Lecanoromycetes	25	10		1		2		13
	Leotiomycetes	273	12		18	16	2	1	49
	Orbiliomycetes	40			1	4	1	2	8
	Pezizomycetes	65	7		1	2	2		12
	Pezizomycotina_cls_ Incertae_sedis	5			1		1		2
	Saccharomycetes	37	1		9	8		1	19
	Sareomycetes	1	1						1
	Sordariomycetes	801	37	1	44	86	37	16	221
	Taphrinomycetes	11	2			1	2	2	7
	NA	295							
Basidiobolomycota	Basidiobolomycetes	7							
	NA	7							
Basidiomycota	Agaricomycetes	976	385	9	60	23	13	3	493
	Agaricostilbomycetes	39				3			3
	Atractiellomycetes	3	2				1		3
	Classiculomycetes	1					1		1
	Cystobasidiomycetes	46			3	9	1		13
	Dacrymycetes	2							
	Exobasidiomycetes	17	1		3	3	1		8
	Geminibasidiomycetes	4							
	Malasseziomycetes	6			3		1		4
	Microbotryomycetes	78	2		11	9	2	1	25
	Pucciniomycetes	12	1						1
	Spiculogloeomycetes	5							
	Tremellomycetes	115	2		27	2	13	2	46
	Ustilaginomycetes	9	1		3	2			6
	Wallemiomycetes	6	2		1	1			4
	NA	44							
Blastocladiomycota	Blastocladiomycetes	3				1			1
Calcarisporiellomycota	Calcarisporiellomycetes	1							
Chytridiomycota	Chytridiomycetes	8					4		4
	GS13	1							
	Lobulomycetes	2							
	Rhizophlyctidomycetes	8			1		1		2
	Rhizophydiomycetes	55			2		1	2	5
	Spizellomycetes	83			2	4	6	1	13
	NA	38							
Entomophthoromycota	Entomophthoromycetes	5						1	1
Glomeromycota	Archaeosporomycetes	9			2				2
	Glomeromycetes	80			3	2	1	2	8
	Paraglomeromycetes	2			1				1
	NA	1							
Kickxellomycota	Kickxellomycetes	10							
Monoblepharomycota	Monoblepharidomycetes	9							
	NA	6							
Mortierellomycota	Mortierellomycetes	93			16	8	1	1	26
	NA	12							
Mucoromycota	Endogonomycetes	26							
	Mucoromycetes	17	1		6	3			10
	Umbelopsidomycetes	4			2				2
Neocallimastigomycota	NA	10							

Olpidiomycota	GS17	1							
	GS18	1							
	Olpidiomycetes	4			1				1
	NA	3							
Rozellomycota	Rozellomycotina_cls_ Incertae_sedis	26							
Zoopagomycota	Zoopagomycetes	13				2			2
	NA	12							
NA	NA	288							
	Sum of fungi	4618	487	10	275	282	134	44	1232

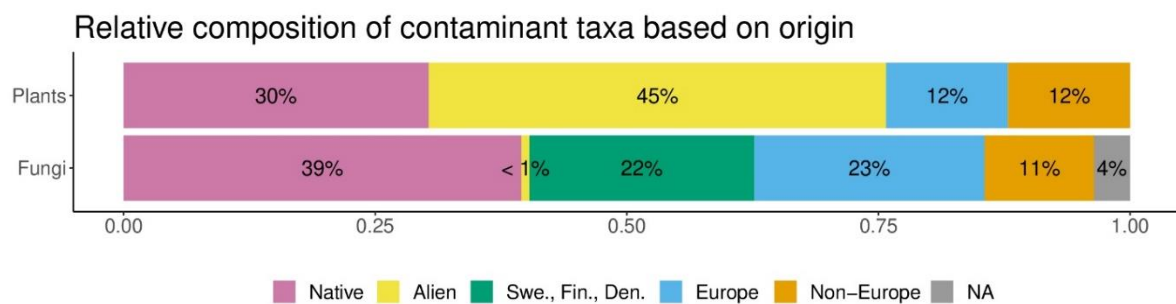

Figure 2: *Relative composition of plants and fungi identified by metabarcoding, categorized by native or alien status in Norway ("Native"; "Alien"), and geographic prevalence outside of Norway: Species registered in Sweden, Finland, and/or Denmark ("Swe., Fin., Den."); species known elsewhere in Europe ("Europe"); species known only outside Europe ("Non-Europe"); species without available information about geographic prevalence ("NA").*

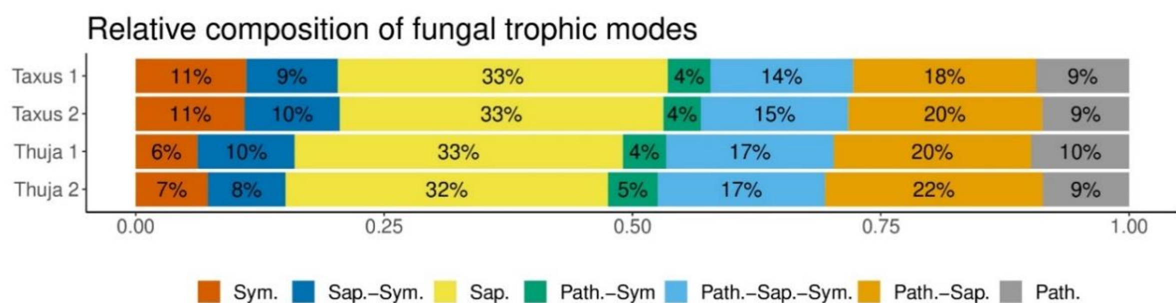

Figure 3: *Relative composition of fungal taxa (n=2557) belonging to one or more trophic modes ("Sym."=symbiotrophic, "Sap."=saprotrophic, "Path."=pathotrophic) in soil samples from ornamental* Taxus *sp. and* Thuja *sp. in two import containers from the Netherlands to Norway in 2021. "Taxus 1" and "Thuja 1" refer to samples from the respective ornamental taxa in Container 1, while "Taxus 2" and "Thuja 2" refer to samples from Container 2.*

Known alien and potential doorknocker species

Fifteen known alien plant species to Norway were identified by metabarcoding, 11 (73.33 %) of which were either not risk assessed (NR) nor have any known impacts (NK), while two (13.33 %) were classified as low impact (LO). Two species (13.33 %), *Conyza canadensis* and *Acer pseudoplatanus*, were classified as having potentially high (PH) and severe impacts (SE)

in Norway, respectively. Two plant species, *Diospyros virginiana* and *Taraxacum sinicum*, were neither registered as native nor alien to Norway, but were listed as alien elsewhere in several alien species databases and should be assessed as potential doorknocker species to Norway (Table 2). An overview of all known alien plant species to Norway and their assessment from the NASL is included in Table S2.

Of the 10 known alien fungal species to Norway, three (30.00 %) were not risk assessed (NR) or have any known impacts (NK), while five (50.00 %) have been assessed as having low impact (LO). *Cryptostroma corticale* has been assessed as having potentially high impact (PH), and *Mutinus ravenelii* has been assessed as having a high impact (HI) in Norway. Three species, *Akanthomyces lecanii*, *Ganoderma pfeifferi* and *G. resinaceum*, were identified as potential doorknocker species to Norway (Table 2). An overview of all known alien fungal species to Norway and their assessment from the NASL is included in Table S3.

Table 2: *List of plants and fungi that were non-native and not known alien species to Norway while being listed in one or more alien species databases and lists (DAISIE, NOBANIS, GRIIS).*

Species	DAISIE	NOBANIS	GRIIS
PLANTS			
Diospyros virginiana	X		X
Taraxacum sinicum	X	X	X
FUNGI			
Akanthomyces lecanii			X
Ganoderma pfeifferi	X	X	X
Ganoderma resinaceum	X	X	X

Observed contaminant species richness

After rarefaction, 80 plant taxa in 66 soil samples from 51 pots were left in the dataset used for fitting the mixed-effects model. Observed contaminant plant species richness was significantly higher in samples from *Taxus* (n samples=39, median species per sample=7 ± 4.50 IQR) than from *Thuja* (n=27, median=5 ± 4.50 IQR) (P<0.001). There was also a significant interaction between ornamental taxa and container (P=0.006), and samples from *Thuja* in Container 1 (n=14, median=3 ± 2.75 IQR) had significantly lower species richness than samples from *Taxus* in Container 1 (n=19, median=9 ± 6 IQR) (P<0.001) and 2 (n=20, median=6.50 ± 4.75 IQR) (P=0.030), while *Thuja* samples in Container 2 (n=13, median=6 ± 4 IQR) were not significantly different from *Taxus* samples in either container or *Thuja* in Container 1 (Table 3 A, Figure 4 A).

In the fungal dataset, 4503 taxa from 78 samples in 60 pots remained after rarefaction. Observed contaminant fungal species richness was significantly higher in samples from *Taxus* (n=40, median=597.5 ± 140.50 IQR) than from *Thuja* (n=38, median=470.50 ± 135.25 IQR) (P<0.001) (Table 3 B, Figure 4 B). Fungal richness was also significantly higher in samples from Container 2 (n=40, median=549 ± 179.5 IQR) than Container 1 (n=38, median=484.50 ± 140.75 IQR) (P=0.029) (Table 3 B, Figure 4 C).

Table 3: *Results from A) a linear mixed-effects model testing the effect of ornamental taxa, container, and their interaction ("Ornamental taxa × Container"), on observed species richness of vascular plants, and B) a generalized linear mixed-effects model testing the effect of ornamental taxa and container on observed species richness of fungi.*

A) Vascular plant LMM (66 samples from 51 pots)

	Estimate	SE	T	P
(Intercept)	*8. 4875*	*0. 7392*	*11. 482*	*<0.001*
Ornamental taxa (Thuja)	*-4. 7663*	*1. 1258*	*-4.234*	*<0.001*
Container (2)	*-1. 5608*	*1. 0303*	*-1.515*	*0.138*
Ornamental taxa × Container	*4. 5705*	*1. 5916*	*2.872*	*0.006*

B) Fungal GLMM (78 samples from 60 pots)

	Estimate	SE	Z	P
(Intercept)	*0.5232*	*0.0182*	*28.701*	*<0.001*
Ornamental taxa (Thuja)	*-0.0997*	*0.0212*	*-4.706*	*<0.001*
Container (2)	*0.0462*	*0.0212*	*2.181*	*0.029*

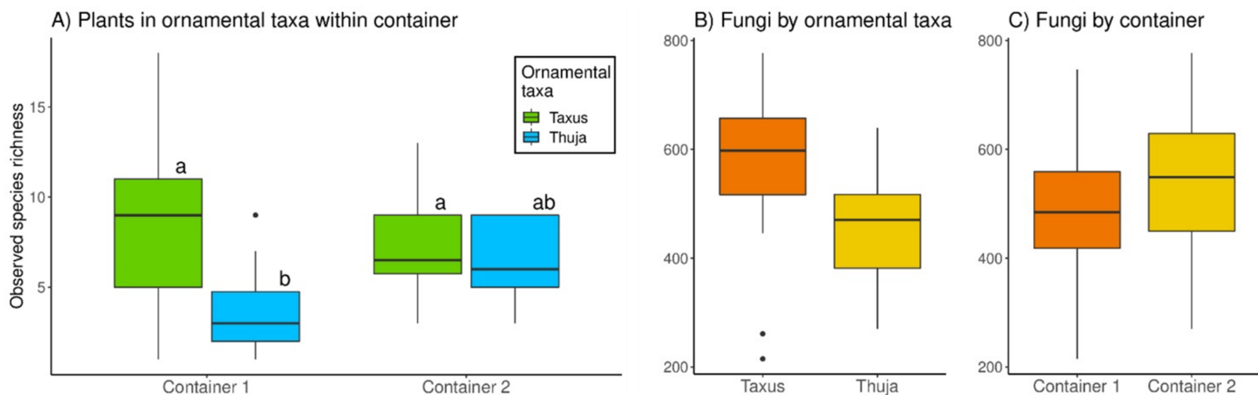

Figure 4: *Boxplots displaying observed species richness of A) vascular plants by ornamental taxa within containers and fungi by B) two ornamental taxa and C) containers, identified by metabarcoding. Different annotated letters ("a" or "b") in A) indicate statistically significant differences in observed species richness between groups.*

Contaminant species composition

Contaminant plant species composition in 66 soil samples was significantly affected by ornamental taxa (P<0.001) and container (P=0.006). Ornamental taxa had the largest effect on

species composition, explaining more than 20 % of the variation between samples, while the effect of container explained less than 4 %. The interaction between ornamental taxa and container also had a significant effect on species composition, explaining more than 4 % of the observed variation, and samples from *Taxus* were more similar across the two containers than samples from *Thuja* were (Table 4, Figure 5 A).

Contaminant fungal composition was also significantly affected by ornamental taxa (P<0.001) and container (P<0.001), as well as their interaction (P<0.001). Ornamental taxa explained more than 20 % and container explained more than 7 % of the observed variation. As was the case for plants, samples from *Thuja* were less similar across containers than samples from *Taxus*, and this interaction explained nearly 7 % of the observed variation in species composition (Table 4, Figure 5 B).

Table 4: *Results from PERMANOVA models testing the effect of ornamental taxa, import container, and their interaction ("Ornamental taxa × Container"), on the composition of contaminant vascular plants and fungi identified by metabarcoding.*

	Variable	Degrees of freedom	Sum of squares	Coefficient of variation	Pseudo F-statistic	P-value
Plants	Ornamental taxa	*1*	*5.0921*	*0.21896*	*19.4729*	*<0.001*
	Container	*1*	*0.9294*	*0.03996*	*3.5541*	*0.006*
	Ornamental taxa × Container	*1*	*1.0214*	*0.04392*	*3.9061*	*0.003*
	Residuals	*62*	*16.2127*	*0.69715*		
	Sum	*65*	*23.2556*	*1.00000*		
Fungi	Ornamental taxa	*1*	*4.4413*	*0.22531*	*26.5073*	*<0.001*
	Container	*1*	*1.4970*	*0.07594*	*8.9346*	*<0.001*
	Ornamental taxa × Container	*1*	*1.3746*	*0.06973*	*8.2040*	*<0.001*
	Residuals	*74*	*12.3988*	*0.62901*		
	Sum	*77*	*19.7117*	*1.00000*		

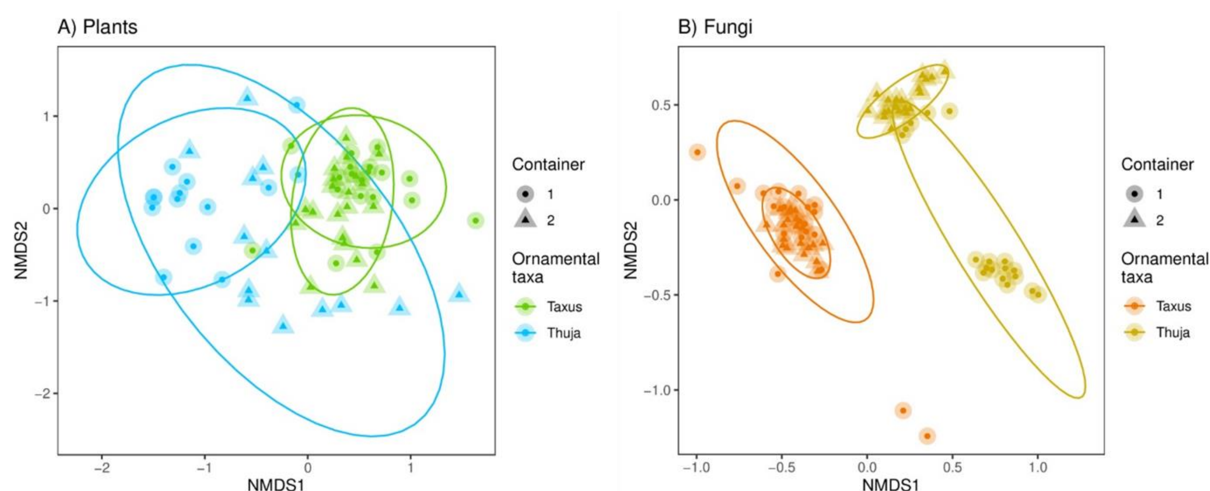

Figure 5: *NMDS plots based on Bray-Curtis dissimilarities displaying differences in contaminant species composition of A) plants and B) fungi identified by metabarcoding.*

Estimated contaminant species richness

Plant species richness for both containers combined was estimated to 105 species, indicating that the interpolated observed species richness (79 species) covered 75.22 % of total estimated species richness. For the two containers separately, the estimated species richness in Container 1 was also 105 species, while it was 142 species in Container 2, and interpolated species richness made up 55.04 % and 38.73 % of estimated richness in the two containers, respectively. According to the estimates, sampling approximately 70 pots would be required to detect 80 % of species in Container 1 and the containers combined, while 130 pots would be required for Container 2 alone. Sampling around 250 pots was required for detecting 100 % of species in Container 1 and the containers combined, while nearly 500 pots would have to be sampled to detect all species in Container 2 (Table 5, Figure 6 A).

The estimate of total contaminant fungal species richness in both containers combined was 6964 species, while the respective estimates for the two containers were 4973 species in Container 1 and 5075 species in Container 2. The interpolated observed species richness for both containers (4248 species) covered more than 60 % of the estimated asymptotic species richness, and estimates were similar for the two containers separately. For both containers combined, more than 120 pots would have to be sampled to detect 80 % of species, while more than 1000 pots would have to be sampled to detect 100 % of species. For both containers separately, approximately 60 pots would have had to be sampled to detect 80 % of species, and more than 400 pots to detect 100 % of present species (Table 5, Figure 6 B).

Table 5: *Interpolated species richness and extrapolated asymptotic species richness ("Asymptotic estimate ± SE"), along with 95 % lower (LCL) and upper confidence limits (UCL), of contaminant vascular plants and fungi identified by metabarcoding. The column "Pct. of estimate" shows how many percent of the estimated species richness was covered by the interpolated species richness. The approximate estimated number of sampled pots required for detecting 80 % and 100 % of estimated species is also provided in columns "80 %" and "100 %", respectively.*

	Container	**Interpolated richness**	**Asymptotic estimate ± SE**	**95 % LCL**	**95 % UCL**	**Pct. of estimate**	**80 %**	**100 %**
Plants	1 & 2	*79*	*105.03 ± 12.50*	*89.66*	*142.56*	*75.22*	*70*	*265*
	1	*58*	*105.37 ± 25.46*	*75.65*	*185.16*	*55.04*	*70*	*240*
	2	*55*	*142.00 ± 51.09*	*84.94*	*307.83*	*38.73*	*130*	*480*
Fungi	1 & 2	*4248*	*6963.62 ± 186.30*	*6622.34*	*7353.94*	*61.00*	*125*	*1000*
	1	*3121*	*4973.09 ± 142.28*	*4714.55*	*5273.58*	*62.76*	*60*	*410*
	2	*3094*	*5075.24 ± 156.28*	*4791.85*	*5405.93*	*60.96*	*60*	*450*

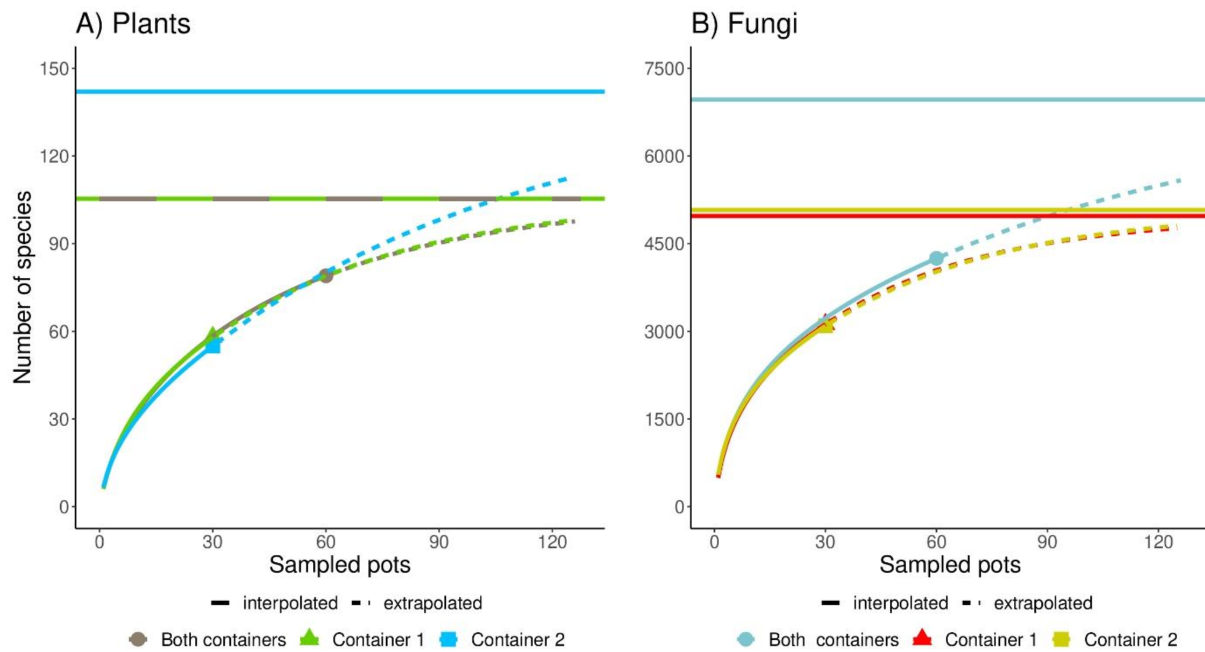

Figure 6: *Interpolated and extrapolated species accumulation curves of A) vascular plants and B) fungi identified by metabarcoding in soil samples.*

Species detection in duplicate sampled pots

The mean percent of shared plant taxa between the first and second samples was 42.32 % (± 9.34 % standard deviation (SD)), while the mean percent of unique taxa was 29.71 % (± 11.43 % SD) and 27.97 % (± 12.52 % SD) for the first and second samples, respectively. Shared taxa made up less than 50 % in samples from both *Taxus* and *Thuja* in Container 2, and in *Thuja* samples from Container 1. The largest percentage of unique vascular plant taxa was in *Taxus* samples from Container 2, where 12 of 27 (44.44 %) taxa were only identified in the second samples (Figure 7 A). Across both ornamental taxa and containers, known alien species to Norway found in duplicate sampled pots were only detected in either the first or second sample. *Conyza canadensis* (PH) was found in either the first or the second sample in three pots. Similarly, *Acer pseudoplatanus* (SE) was detected in five pots, and in only one pot was it detected in both the first and second samples.

For fungi, the mean percent of shared taxa was 49.22 % (± 1.96 % SD), and the mean percent of unique taxa was 26.56 % (± 2.66 % SD) for the first samples and 24.22 % (± 2.03 % SD) for the second samples. Shared taxa made up more than 50 % of taxa only in *Taxus* samples from Container 2. The largest percentage of unique fungal taxa was in the first samples from *Thuja* in Container 2, with 460 of 1535 (29.97 %) of the total number of taxa in those samples (Figure 7 B). *Cryptostroma corticale* (PH) was identified in seven duplicate sampled pots and

was identified in both samples in only one pot. Contrarily, *Mutinus ravenelii* (HI) was identified in both the first and second samples in 10 of 11 pots where it was found.

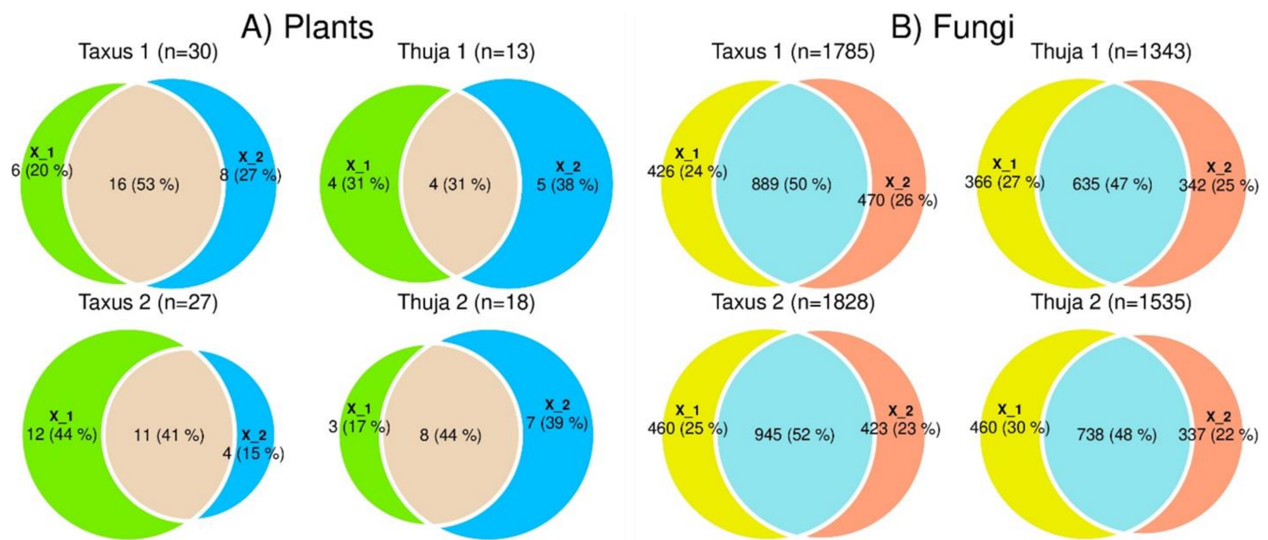

Figure 7: *Euler diagrams displaying the number and percent of unique and shared taxa of A) vascular plants B) and fungi between duplicate soil samples taken from the same pots. Each diagram represents five sampled pots. Diagram titles refer to the ornamental taxa and container that samples were taken from, and the number of species detected in those samples (e.g., "Taxus 1 (n=30)" represents the 30 plant species detected in samples from* Taxus *sp. in Container 1). Areas marked "X_1" and "X_2" refer to the number of species detected only in the first and second samples, respectively, while the overlap represents the number of species detected in both the first and second samples.*

Discussion

eDNA metabarcoding was successfully used to identity contaminant species in ornamental imports to Norway. Native, known alien, and potential alien species to Norway were all among the detected species. Also, contaminant species richness and composition varied between samples from the two ornamental taxa and import containers. Further, the estimated contaminant species richness exceeded the observed richness in both containers. Lastly, two soil samples per duplicate sampled pot contributed to increased species detection compared to one sample.

Contaminant species

Among 58 plant species identified by metabarcoding and morphologically after germination, only five were detected by both methods. Most of the species detected exclusively by metabarcoding had not been previously recorded by the main project, while the opposite was true for plants identified morphologically. Divergence between species detected with metabarcoding and conventional methods is not unusual and has been reported in studies

comparing methods (e.g., van der Heyde et al. 2020, Johnson et al. 2021). A direct comparison between plants identified by metabarcoding and germination cannot be made in this study because soil samples for the two methods were not taken systematically from the same pots. However, these results indicate that most of the plant species identified by metabarcoding were detected from traces of DNA rather than viable seeds. This suggests that metabarcoding may be insufficient as a sole method of monitoring ornamental imports, and that morphological identifications are required to confirm the presence of viable contaminant seeds. Nevertheless, the results illustrate that metabarcoding is useful for assessing source populations of emerging alien species (Seebens et al. 2018) by shedding light on species that can be present in ornamental imports. In sum, these results support that metabarcoding and germination should be used as complementary methods for monitoring ornamental imports, to assess contaminant species source populations while detecting viably present species in this introduction pathway.

The relative amounts of known alien species to Norway identified by metabarcoding was much higher for plants than for fungi (Figure 2). However, fungi are relatively underrepresented compared to vascular plants in several alien species records, such as the NASL (less than 150 fungi compared to more than 2000 vascular plants) and the GISD (19 fungi to 468 vascular plants). Fungi are inherently more difficult to study than plants, leading to fungi historically receiving less attention than plants in the context of alien species (Thakur et al. 2019). Poor knowledge about alien fungi is likely also due to a similar lack of knowledge about native fungal diversity and distribution. For instance, a recent report estimated that more than 3000 native species of fungi have yet to be documented in Norway (Elven and Søli 2021). I identified 275 fungal species that were non-native to Norway while being known in one or more of Norway's neighboring countries. Several of these species may be native to Norway, though they may also be potential or even established alien species to Norway. Increasing our knowledge about native biodiversity would contribute to increasing our knowledge about alien biodiversity, which is pivotal for informed and effective management of alien species and introduction pathways. Combined efforts between programs surveying native biodiversity and introduction pathways could benefit both types of programs, and eDNA metabarcoding is a potentially valuable tool in this context (Comtet et al. 2015, Westergaard et al. 2020b).

Potential doorknocker species to Norway

Two plants and three fungi identified by metabarcoding were neither native nor known alien to Norway while being registered in alien species databases. These species should be assessed by

taxonomic experts as doorknocker species to Norway. A summary of their ecology and distribution will be presented here.

Diospyros virginiana is a large tree species native to the southeastern USA, commonly known as American persimmon. The species grows in areas of temperate deciduous forests (Wallnöfer 2001) in subtropical climate (Kottek et al. 2006), and is a registered as alien in Israel (Dufour-Dror et al. 2020). One specimen of *D. virginiana* was germinated in a private arboretum in Hardanger, Norway in 2010, and was still alive at the time of writing. However, the specimen was no taller than 1 meter and had yet to bear fruit (Geir Flatabø, personal communication). Considering the climatic conditions in its native and alien range, it may be unlikely that *D. virginiana* could establish outside of cultivation in Norway.

Taraxacum sinicum is a species of dandelion native to northern and northwestern China, Mongolia, and southern Siberia and the Altai region in Russia (Kirschner et al. 2006). These areas are mostly characterized by arid and dry climates, although the Altai region is characterized by humidity and cool summers, similar to large parts of Norway (Kottek et al. 2006). Further, it is reported as alien in Khabarovsk and northwestern parts of European Russia (Petrosyan et al. 2020). The species grows between 800 and 4500 meters above sea level, in open habitats such as meadows and steppes (Kirschner et al. 2006) and may be able to establish in in similar habitats in Norway, where hayfields are classified as critically endangered (CR) on the Norwegian Red List for Ecosystems and Habitats (Hovstad et al. 2018). This should be considered in an assessment of *T. sinicum* a potential doorknocker species to Norway.

Ganoderma pfeifferi is a polypore native and common in south and central Europe north to Denmark (Ryvarden et al. 2017). The northernmost registrations of the species are in southern parts of Sweden, where it is classified as endangered (EN) on the Swedish Red List (SLU Artdatabanken 2020). *G. pfeifferi* is registered as alien in Poland (Solarz et al. 2020) and Slovenia (De Groot et al. 2020). It grows on living beech (*Fagus*) and other deciduous trees, where it acts as a parasite and saprotroph (Ryvarden et al. 2017). Limited distribution of beech and Norwegian climate may limit the establishment potential of *G. pfeifferi* in Norway. However, European beech (*Fagus sylvatica*) is expanding in Norway (Solstad et al. 2021), and combined with climate change and warming temperatures, this could make conditions more favorable for *G. pfeifferi*. This should be considered in further assessment of *G. pfeifferi* as a potential doorknocker species to Norway.

Ganoderma resinaceum is another parasitic and saprotrophic polypore, usually growing on living oak (*Quercus*) and other deciduous trees. The species has similar European distribution as *G. pfeifferi*, but is also common in tropical areas and known from North America,

Africa, and Asia (Ryvarden et al. 2017). Similarly to *G. pfeifferi*, *G. resinaceum* is northmost known from southern Sweden and is endangered there (SLU Artdatabanken 2020). In Sweden, the species is exclusively associated with old oak trees (Knutsson 2019) which may be a limiting factor for establishment in Norway, while further south in Europe, it is also associated with other deciduous tree species (Læssøe and Petersen 2019). Thus, climate change may be an important consideration in an expert assessment of *G. resinaceum*.

Akanthomyces lecanii (formerly *Lecanicillium lecanii*) is an entomopathogenic fungus and the anamorph phase of the teleomorph *Torrubiella confragosa* in the family *Cordycipitaceae* (Zare and Gams 2001 (as *L. lecanii*)). It is reported as primarily distributed in tropical areas of the Americas, southeast Asia, and Turkey (Zare and Gams 2001), though occurrences in Europe are reported in GBIF under various species synonyms (GBIF 2021). It is registered as alien in the Galápagos Islands (Pagad 2020). *A. lecanii* is an important species in biocontrol of pests in horticulture, particularly of thrips (*Thysanoptera*), soft scales (*Coccidae*), and mites (*Acari*) (Shinde et al. 2010, Medina et al. 2021, Subramaniam et al. 2021), which could explain its detection in this study. Biocontrol species can have negative impacts on native biodiversity in areas where they are introduced (e.g., the harlequin ladybird (*Harmonia axyridis*); Roy et al. 2016), and knowledge of the diversity and ecology of soft scales and mites in Norway is generally poor (Elven and Søli 2021). Introduced pathogens such as *A. lecanii* can potentially have detrimental effects on the populations of these native taxa. Therefore, *A. lecanii* should be further assessed by taxonomic experts as a potential doorknocker species to Norway.

In sum, establishment in Norway may be unlikely for these five potential doorknocker species due to climatic differences between their current known distribution and Norway, and a lack of suitable habitats and hosts. However, this may change in the future, with warming climates and alterations of Norwegian ecosystems (Gjershaug et al. 2009). Therefore, the potential for establishment and ecological impacts of these species should be properly assessed by taxonomic experts in accordance with future revisions of the NASL (Sandvik et al. 2020).

Contaminant species richness and composition

Patterns in richness and composition of contaminant species were similar for plants and fungi detected by metabarcoding in this study, and ornamental taxa was the most important factor affecting both richness and composition. Contaminant plant communities are likely products of several factors, such as immediate neighboring ornamentals in nurseries, seed contamination, and non-ornamental plants from outside nursery facilities, such as weeds. In turn, contaminant

fungal communities may be influenced by contaminant plant composition. Franić et al. (2019) reported that contaminant fungal composition in traded tree seeds varied significantly based on the seeds' country of origin and taxonomic grouping. In another recent study, Oskay et al. (2022) found a strong correlation between the number of analyzed Scots pine (*Pinus sylvestris*) seeds and alpha diversity estimates of contaminant fungi. This indicates that richness and composition of contaminant plants may be an important predictor of contaminant fungal diversity and composition in ornamental imports, which could contribute to explain the similar patterns in contaminant plants and fungi between ornamental taxa and import containers found in this study. Future studies could analyze species associations and cooccurrence between contaminant plants and fungi in ornamental imports to investigate this.

Focus on cooccurrence of contaminant plants and fungi when monitoring ornamental plant imports is relevant in the context of co-invasion. While alien plants serve as transportation vectors for alien fungi, alien fungi may increase alien plants' establishment potential in novel ecosystems through commensal relationships with alien species and antagonistic relationships with native species (Bongard 2012, Dickie et al. 2017). In the present study, both symbiotic and pathogenic fungi were identified (Figure 3), which could potentially increase establishment potential for emerging aliens and doorknocker species to Norway. Further monitoring and assessments of contaminant species source populations in the ornamental plant trade is required to uncover potential patterns of co-invasion between alien plants and fungi introduced through this introduction pathway.

Optimizing study design and sampling effort is necessary to uncover patterns and drivers of species richness and composition in ornamental imports. According to estimates of species richness, several hundred pots would have had to be sampled to detect all contaminant plants and fungi in this study (Table 5). However, sampling may be time sensitive if ornamental plants are set to be distributed to dealers shortly after arrival at logistic centers. Thus, with limited resources and personnel, sampling an adequate number of pots to detect all species may not be feasible. In the present study, roughly 50 % of contaminant taxa identified in duplicate sampled pots were only detected in either the first or second samples (Figure 7). Previous metabarcoding studies have also found that increasing the number of replicates per sampled unit can contribute to higher levels of species detection (e.g., Mata et al. 2019, Macher et al. 2021, Oskay et al. 2022). This suggests that increasing the number of soil samples per pot can, to an extent, compensate for a low number of sampled pots. In addition, other methodological decisions regarding technical replication (Mata et al. 2019), choice of DNA markers (Fahner et al. 2016), and bioinformatic procedures (Pauvert et al. 2019), can affect the number and

composition of species detected. These are important considerations for optimizing species detection when monitoring ornamental imports with metabarcoding, to understand drivers of richness and composition in contaminant species source populations.

Factors determining the richness and composition of contaminant species in ornamental imports are likely related to the ornamentals' life histories, including the soil used in nurseries. Soils are highly diverse habitats that can, as demonstrated in this and previous studies (e.g., Bruteig et al. 2017, Rossmann et al. 2021), harbor large amounts of diverse contaminant taxa. Information about the origin and history of soils used for nursing and transporting ornamental plants would be highly useful in assessing source populations and drivers of contaminant species diversity and composition (Westergaard et al. 2020a). While restrictions on soil import are in place in Norway (Regulations on plant health 2019), phytosanitary certificates for ornamental imports provide no information on soil origin and history, and records of imported soil are equally poorly documented (IUCN 2019, Westergaard et al. 2020a). Increased attention and documentation regarding soil used in the ornamental plant trade would aid in understanding factors driving contaminant species richness and composition in ornamental imports. In turn, this could raise the quality of information used to inform management of this introduction pathway.

Conclusion

This study demonstrated the utility of eDNA metabarcoding to identify and assess source populations of contaminant species in soil samples from ornamental imports. The value of combining metabarcoding with conventional methods of species detection was demonstrated by the many plant species identified only by metabarcoding, that had not previously been recorded by the main project. Metabarcoding also enabled identifying contaminant fungi, which has previously not been in focus for the main project. This shows that metabarcoding facilitates identification of potential emerging alien species to Norway, allowing expert assessments and implementation of measures prior to species establishment.

It was also shown that contaminant species richness and composition can vary between ornamental taxa and import containers. Identifying drivers of contaminant species diversity and composition could potentially facilitate measures to limit the number of contaminant species introduced with traded ornamentals. This requires additional monitoring of ornamental imports, as well as documentation and transparency from the ornamental plant industry about the history of ornamental plants and soil used for nursing. Lastly, this study also emphasized the

importance of appropriate sampling effort and study design to detect as many contaminant species as possible, which is important to achieve the goals addressed above.

In sum, the findings of this study are useful for further improving monitoring programs of ornamental imports. This is important for early detection of emerging alien species and rapid response to prevent their establishment. In turn, this is crucial to prevent biological invasions and negative impacts of alien species.

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Supplementary material

All supplementary materials are provided in different sheets in an Excel file submitted with this thesis. Supplementary tables and figures are provided in the same order as they were first referenced in the thesis.

Table S1 provides a list of 19 non-target contaminant plant taxa identified by eDNA metabarcoding in soil samples from ornamental *Taxus* sp. and *Thuja* sp. imported from the Netherlands to Norway in 2021.

Figure S1 provides Krona charts displaying the relative abundance of A) orders and families of contaminant vascular plants and B) phyla and classes of contaminant fungi. All taxa were identified with eDNA metabarcoding in soil samples from ornamental *Taxus* sp. and *Thuja* sp. in two import containers from the Netherlands to Norway in 2021.

Table S2 provides a list of 58 contaminant vascular plant species identified in soil samples from ornamental *Taxus* sp. and *Thuja* sp. imported from the Netherlands to Norway in 2021. Species are sorted according to their method of detection; whether they have been previously recorded by the main project; and their status as native, known alien, or potential alien species to Norway. assessments from the Norwegian Alien Species List are provided for known alien species.

Table S3 provides a list of 1232 contaminant fungal species identified in soil samples from ornamental *Taxus* sp. and *Thuja* sp. imported from the Netherlands to Norway in 2021. Species identified as native to Norway are marked with an "X" in column "Native", and assessments from the Norwegian Alien Species List are provided for relevant species in the column "Known alien".

Table S4 provides a list of 2558 contaminant fungal taxa identified with eDNA metabarcoding in soil samples from ornamental *Taxus* sp. and *Thuja* sp. imported from the Netherlands to Norway in 2021. Species are listed along with their trophic modes derived from FUNGuild.