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# Survey and characterization of oomycete species associated with root rot in UK red raspberry (*Rubus idaeus*)

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## Abstract

*Phytophthora rubi* has been reported as the primary causal agent of raspberry root rot (RRR) in the UK, resulting in severe economic losses. Recent observations suggest the RRR complex may be more diverse than previously thought. This study investigated the *Peronosporales* species present in UK raspberry plants and factors affecting their diversity and abundance. Eighty-six symptomatic root and 86 cane samples were collected from seven farms in the UK in 2020 and 2021. Five additional samples were collected from blackberry roots. Symptomatic tissue was plated onto selective media, and resultant isolates were identified by ribosomal internal transcribed spacer (ITS) barcoding. Twelve isolates of five species of *Phytophthora* (*P. citrophthora*, *P. pini*, *P. erythroseptica*, *P. cryptogea* and *P. pseudocryptogea*) and seven isolates of two *Phytopythium* species (*Pp. vexans* and *Pp. litorale*) were collected. Isolate pathogenicity was assessed on detached leaves, with *Pp. litorale* and *Pp. vexans* exhibiting significantly higher pathogenicity on raspberry leaves than an isolate of *P. rubi*. High-throughput sequencing of the ITS region of symptomatic root samples revealed farm location had a significant effect on *Peronosporales* community composition ($p = 0.001$), and both farm location and plant variety significantly affected the abundance of taxa ($p < 0.002$ and $p < 2 \times 10^{-16}$, respectively). This study presents *Pp. vexans* and *Pp. litorale* as new potential pathogens for further study. Furthermore, we recommend the inclusion of other *Peronosporales* species into resistance screens in raspberry breeding programmes to produce genotypes more resilient to community composition shifts in raspberry root pathospheres in the UK.

KEYWORDS
oomycete, metabarcoding, *Peronosporales*, *Phytophthora*, *Phytopythium*, blackberry

## 1 | INTRODUCTION

Raspberries are known for their high antioxidant and anthocyanin content and their associated health benefits. Raspberry production occurs throughout the UK, with centralized pockets of production in Scotland, the West Midlands and southeast England. Selective breeding of raspberries for larger berry size and better shelf life, as well as breeder and grower investment in new varieties and

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technologies, has been instrumental in the enhanced global uptake of the fruit and has enabled growers to increase productivity per hectare and improve turnover.

Root rot of the European red raspberry (*Rubus idaeus*), caused by a yet-uncharacterized consortium of *Phytophthora* species, is a recurring and destructive disease of this commodity fruit. Severe outbreaks of root rot in the UK in the 1980s prompted further investigation into the species present in the diseased plants (Duncan et al., 1987). Duncan et al. (1987) reported the pathogenicity of *Phytophthora megasperma*, *Phytophthora erythroseptica* and *Phytophthora dreschleri*, which is considered synonymous with *Phytophthora cryptogea*, on red raspberry in the UK (Cline et al., 2008; Duncan et al., 1987). Wilcox (1989) also investigated the pathogenicity of *P. megasperma*, *P. cryptogea*, *Phytophthora cactorum*, *Phytophthora citricola* (also known as *Phytophthora pini* in raspberry) and *Phytophthora fragariae* var. *rubi* (now known as *Phytophthora rubi*) on raspberry in New York, United States. Wilcox and Latorre (2002) observed *P. cryptogea*, *P. citricola*, *P. rubi*, *P. megasperma* and *Phytophthora gonopodyides* in Chilean raspberry plants. Furthermore, *Phytophthora bisheria* (now known as *Phytophthora bishii*) was reported in red raspberry in Australia (Abad et al., 2008). Following the disease outbreaks in soil-grown raspberries, novel production systems were implemented, such as pot-based production under greenhouse or tunnel cover, to increase substrate drainage and reduce disease spread (Dijkstra & Scholtens, 1993). Container growing allows for quick removal and destruction of diseased plants; however, to reduce the likelihood of pathogen spread, testing of stock prior to potting and identifying potential new pathogens through biomonitoring are critical steps in mitigating the effects of root rot.

Biomonitoring through novel diagnostic methods such as PCR, quantitative PCR (qPCR) and high-throughput sequencing (HTS) has prompted a revised understanding of the *Phytophthora* species composition in UK raspberry over the past 40 years (Duncan et al., 1987; Wedgwood et al., 2020). Through the integration of both traditional and molecular diagnostic methods, Wedgwood et al. (2020) tabulated six species of *Phytophthora* in UK raspberry plants including *P. rubi*, *Phytophthora idaei*, *Phytophthora citrophthora*, *P. citricola* (*P. pini*), *P. bishii* and *Phytophthora plurivora*—a first report of this species in raspberry.

Sapkota et al. (2022) published the first report of using HTS as a tool to assess the *Phytophthora* species associated with root rot in red raspberry. In their study, 128 oomycetes isolated in British Columbia from plants exhibiting root rot were identified using multiplex targeted sequencing on the Illumina platform. Eighty-five percent of isolates were identified as *P. rubi*, while the remaining 15% were *P. gonopodyides*, a species with moderate virulence on raspberry (Wilcox & Latorre, 2002).

HTS has yet to be investigated as a method of elucidating the complexity of the raspberry root pathosphere in the UK. Recent findings hint at a more diverse *Peronosporales* species composition in raspberry than currently reported; thus, metabarcoding using the ITS region on the Illumina NovaSeq platform was used in this study to investigate the identity, abundance and diversity of *Phytophthora* species present in the roots of raspberries displaying root rot symptoms from commercial UK raspberry farms (Browne et al., 2023).

To identify the causal *Phytophthora* species in a plant exhibiting the symptoms of root rot, pathologists usually sample symptomatic populations and perform laboratory-based isolations or molecular analysis on single samples. However, these techniques usually only detect a single species within a sample, and only those that have previously been reported. The optimization of HTS as a diagnostic tool has enabled researchers to identify a greater number of *Phytophthora* species present in a symptomatic plant, both known *Phytophthora* species and those that are yet undescribed (Riddell et al., 2019). HTS through metabarcoding has been particularly well used in studying *Phytophthora* species present in environmental samples and identifying factors that influence their diversity such as location, plant health status and substrate type (Bose et al., 2018; Burgess et al., 2017; Landa et al., 2021; Prigigallo et al., 2015; Redekar et al., 2019; Riddell et al., 2019; Sarker et al., 2023; Vélez et al., 2020).

Due to the deauthorization of many chemical control agents and, in some cases, their low efficacy, current disease management of raspberry root rot (RRR) relies on prevention through cultural practice and, to some extent, resistant germplasm. Infection prevention is employed through securing clean planting material, maintenance of freely draining soil and by choosing to grow cultivars with some level of tolerance. Most cultivars currently considered of commercial quality for the UK fresh market are, at best, moderately tolerant to RRR so further genetic improvement is still essential. Breeding programmes typically use *P. rubi* to test plant susceptibility to *Phytophthora*; however, changing species diversity in the RRR complex may impact the reliability of these genotypes in the field. We hypothesize that there are more species than *P. rubi* responsible for RRR in the UK and that *P. rubi* is less prevalent than previously reported. The primary objective of the current study was to characterize the *Phytophthora* and other *Peronosporales* composition in red raspberry plants exhibiting symptoms of RRR in the UK. Furthermore, the impact of substrate, plant genotype and farm location on the diversity and abundance of *Phytophthora* species in commercial red raspberry plants was assessed through metabarcoding analysis. The potential of a detached leaf assay as a method to preliminarily determine the pathogenicity of *Peronosporales* isolates and host susceptibility was also investigated.

## 2 | MATERIALS AND METHODS

### 2.1 | Sampling

Raspberry plants exhibiting symptoms of RRR were sampled on commercial farms in Kent, Herefordshire/Staffordshire and Scotland in November 2020 and September 2021 (Figure 1). Sites sampled in 2020 are coded I–IV while those taken in 2021 are coded 1–9. Varieties are coded A–D (BB for blackberry) to preserve grower confidentiality. Samples were collected only from plants exhibiting symptoms of RRR; sites had varying levels of disease meaning 5–15 samples were taken per farm with an average of 10 samples per farm. Due to variation in cultivar susceptibility

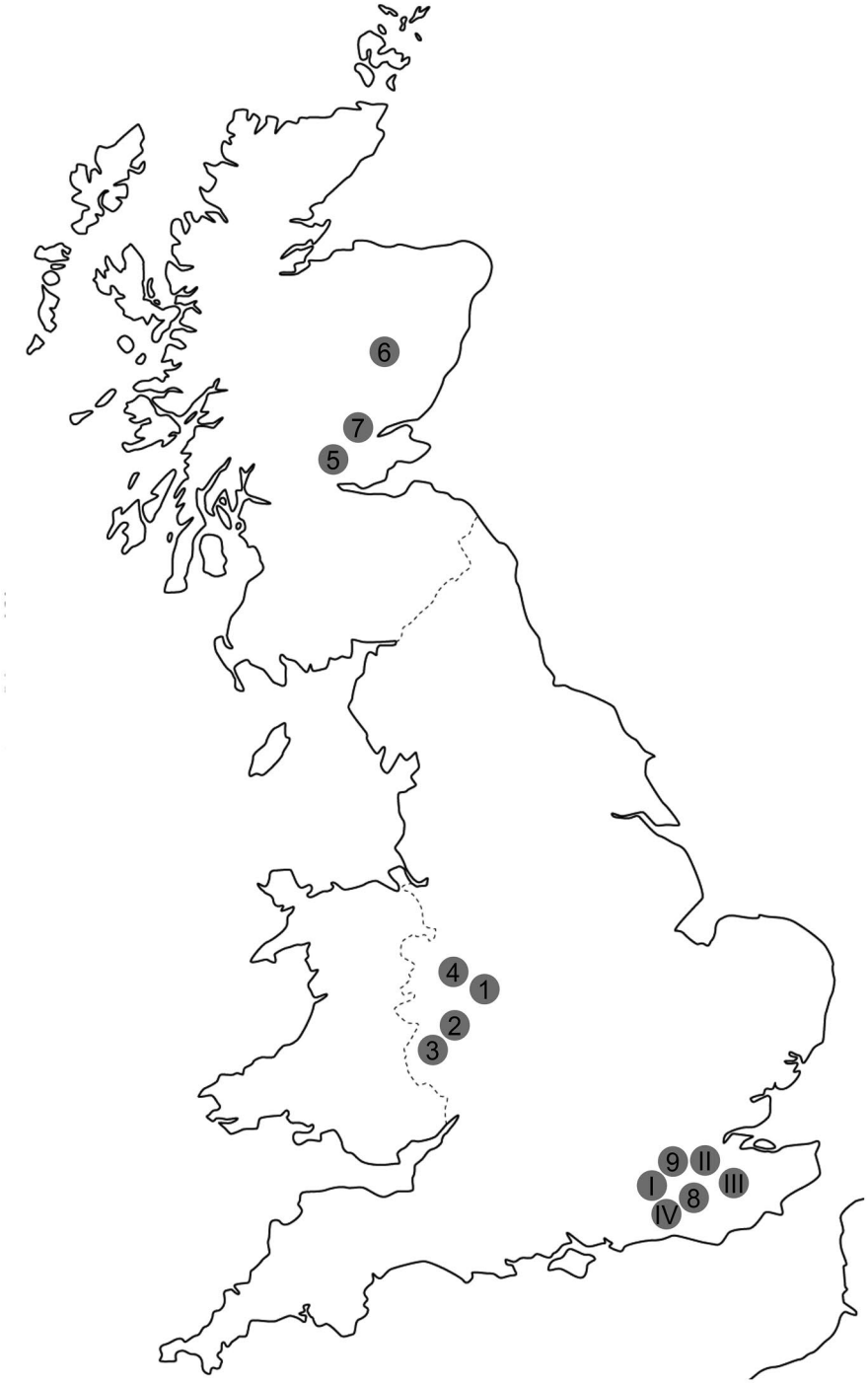


**FIGURE 1** Approximate location of farms where raspberry plants showing symptoms of raspberry root rot were sampled as part of this study.

to RRR and some growers having a primary cultivar with an additional smaller crop of a secondary cultivar, each cultivar was not sampled equally. Sites I–IV were visited in 2020. Two or three canes and roots with rhizosphere soil or substrate attached were taken and placed in 1-L plastic bags. Samples were held in a 4°C cold store until processing within 4 days of collection. Equal numbers of symptomatic and asymptomatic samples were taken in 2020; only symptomatic samples were taken in 2021. Out of the 86 samples analysed in this study, 51 were from plants grown in coconut coir, and 19 were from compost. Nine samples were taken from soil-grown plants.

## 2.2 | DNA extraction, molecular identification and sequencing of isolates

Isolation was performed according to a modified version of the method outlined in Stewart et al. (2014). Diseased roots were placed in a sieve and rinsed in running tap water to remove soil/substrate. The roots were cut into 10 mm sections and transferred to 70% ethanol for 1 min and rinsed twice in sterile distilled water. After rinsing, roots were placed on sterile filter paper to dry for 30 s. Five root sections per sample were aseptically transferred to 9 cm Petri dishes containing cornmeal agar (Difco) amended with pimaricin (0.4 mL/L of a 2.5% wt/vol stock),

ampicillin (250 mg/L), rifampicin (0.1 mg/L) and pentachloronitrobenzene (PCNB; 5 mg/L) (CMA-PARP; Jeffers & Martin, 1986). Diseased cane tissue was cut into 5 mm sections, soaked in sodium hypochlorite (1.2% available chlorine) for 2 min and rinsed in sterile distilled water three times. Four pieces of cane, per sample, were carefully submerged in Petri dishes containing CMA-PARP. The plates were incubated in the dark at 20°C until mycelial growth was observed (5–7 days).

To determine the identity of cultures, a rapid fungal DNA extraction was performed using the Extract-N-Amp Plant extraction and dilution buffers (Sigma-Aldrich), following the manufacturer's protocol. The extracted DNA was stored at −20°C in preparation for downstream analysis. For Sanger sequencing, the internal transcribed spacer 1 (ITS1), 5.2S and ITS2 region (>900 bp) were amplified from the DNA using the ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers (White et al., 1990). Sterile Milli-Q water was used as a negative control, and genomic DNA extracted from stock cultures of *P. fragariae* and *Cladosporium cladosporioides* were used as oomycete and fungal positive controls, respectively. PCR amplifications were carried out in 25 μL reaction volumes. Each reaction tube contained 2.5 μL of each primer at 10 μM, 2.5 μL of sterile Milli-Q water, 12.5 μL of 2× PCR MyTaq Red Mix (containing 5 mM dNTPs and 15 mM $MgCl_2$) (Bioline), and 5 μL of DNA template or Milli-Q water. The thermocycling reaction was carried out in a C1000 Touch thermal cycler (Bio-Rad) with the following programme: 34 cycles of denaturing at 94°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 30 s and a final step at 72°C for 10 min. The PCR amplification products were separated by electrophoresis in 1.5% agarose gels stained with GelRed (Biotium) in TAE (40 mM Tris acetate pH 8.0, 1 mM EDTA) buffer at 100 V for 1 h and visualized under a UV transilluminator (ChemiDoc MP Imaging System; Bio-Rad). Images were taken with Image Lab (v. 5.2) image acquisition and analysis software. Amplification product size was determined by comparison with a 500 bp Hyper DNA ladder (Bioline). Sanger sequencing using both forward and reverse primers was performed by Eurofins Genomics, Ebersberg, Germany.

## 2.3 | HTS of root samples

HTS was performed on root samples from each farm. Freeze-dried root samples (50 mg each) were sent to Novogene (UK) Ltd for DNA extraction, library preparation and ITS amplicon sequencing. Amplicon PCR was conducted using the *Phytophthora*-specific nested primer pairs 18Ph2F (5′-GGATAGACTGTTGCAATTTTCAGT-3′) and 5.8S-1R in the first round and ITS6 (5′-GAAGGTGAAGTCGTAACAAGG-3′) and 5.8S-1R (5′-GCARRGACTTTCGTCCCYRC-3′) in the second round, according to the protocol of Scibetta et al. (2012). Paired-end 250 bp libraries were sequenced using the Illumina NovaSeq 6000 platform.

The sequence data from each sample was processed and clustered into amplicon sequence variants (ASVs) and frequency tables using the UPARSE 10.0 pipeline (Edgar, 2013). Sequences containing incorrect bases in either primer region or less than 250 bases in either pair were discarded. Remaining read pairs had primer and barcode regions removed and were then merged using different settings for ASV generation and frequency table generation.

For ASV generation, merged reads were discarded if they contained any base differences in the merge region or had a merge length of less than 185 bases. Remaining merged reads were filtered for quality with a maximum expected error threshold of 0.05 per sequence (Edgar & Flyvbjerg, 2015). Unique sequences were identified, and sequences with fewer than four reads were discarded. Unique sequences were ordered by decreasing read frequency and unique ASVs generated by denoising.

For frequency table generation, reads were merged with a very relaxed criterion; only reads with more than 100 differences in the merged region were discarded. All remaining merged reads were mapped to the ASV representative sequences, and an ASV frequency count table was produced for statistical analysis. Taxonomy was assigned to each ASV using the SINTAX algorithm (Edgar, 2016). Oomycete ASVs were compared to the nearest matching sequences in the GenBank nucleotide database using the BLASTN+ program for identification (Altschul et al., 1990).

Raw reads were normalized for library size using the median-of-ratios method implemented in the DESeq2 v. 1.38.3 package (Love et al., 2014) in R v. 4.1.1 (R Core Team, 2021).

The alpha (α) diversity of the normalized counts was subsequently analysed using the Chao1, Simpson and Shannon indices using the Phyloseq package in R (McMurdie & Holmes, 2013). The LmPerm v. 2.1.0 (Wheeler et al., 2016) package was used to conduct a permutation analysis of variance and significance analysis of the α-diversity indices to assess the interaction between farm location, variety and substrate type.

Beta (β) diversity between farm location, variety and substrate type was subjected to principal components analysis (PCA) using variance-stabilized reads and non-metric multidimensional scaling (NMDS) using Bray–Curtis distances in the Vegan package v. 2.6–4 (Dixon, 2003). A permutation multivariate analysis of variance was conducted on the β-diversity indices using the Adonis2 function in the Vegan package to assess the effects of farm location, variety and substrate type on *Phytophthora* diversity. PCA was used to reduce the dimensionality of the dataset, allowing the data to be more easily interpreted. An analysis of variance (ANOVA) was conducted on the first four principal components to determine the contribution of each experimental factor: location, variety, and substrate type to the total variability of each principal component. Differential analysis was conducted to determine which ASVs differed between the experimental factors, that is, the effect each factor has on individual taxa, using DESeq2. The *p*-value threshold for differential analysis was adjusted to $p = 0.1$. For reporting of ASV taxonomy, a SINTAX confidence score of ≥0.65 at the lowest assignable taxonomic rank was used.

## 2.4 | Pathogenicity testing

Pathogenicity testing was performed to assess the virulence of selected isolates on raspberry leaf tissue. The panel of species for

**TABLE 1** Identities of isolates recovered from diseased raspberry root and cane tissue.

| Species | No. of isolates | Variety | Farm | Tissue type | Country |
|---|---|---|---|---|---|
| *Phytophthora pini* | 1 | A | 7 | Root | Scotland |
| *Phytophthora citrophthora* | 2 | B | 1 | Root | England |
| *Phytophthora cryptogea* | 1 | C | 7 | Root | Scotland |
| *Phytophthora pseudocryptogea* | 1 | B | 5 | Root | Scotland |
| *Phytophthora erythroseptica* | 1 | A | 6 | Root | Scotland |
| *Phytopythium litorale* | 6 | A, C, E | 5, 6, 7 | Cane, root | Scotland |
| *Phytopythium vexans* | 1 | D | 6 | Cane | Scotland |

*Note*: Sanger sequencing was performed on the *Yph1* gene of the isolate DNA.

pathogenicity testing was chosen based upon their detection in amplicon sequencing; as such, *P. erythroseptica* and *P. pseudocryptogea* were excluded from the panel. An isolate of *P. rubi* (SCRP 339) and an isolate of *P. pini* (ICO45) were obtained for pathogenicity testing from the James Hutton Institute and Berry Gardens Growers Ltd., respectively.

A detached leaf assay was modified from Loyd et al. (2014). The isolates and a plain agar control were grown on V8 agar (17 g agar, 100 mL V8 juice, 900 mL deionized water pH adjusted to 7.0 with 9 mL of 1 M KOH) in the dark at 20°C for 14 days. Raspberry leaflets from five varieties—Latham, Valentina, Tulameen and two proprietary Malling varieties—were collected from primocanes of plants actively growing in pots, with compost as a substrate, under Spanish tunnels. Briefly, the raspberry leaves were surface sterilized in sodium hypochlorite (0.25% available chlorine) for 3 min, momentarily submerged in 70% (vol/vol) ethanol, rinsed three times in sterile distilled water, and dried on blotting paper in a laminar air flow cabinet.

Six agar plugs were cut from the leading edge of 10-day-old *Phytophthora* cultures using a sterilized 7 mm cork borer and were submerged into a 500 mL plastic container with 200 mL of a 10% (vol/vol) sterile soil extract (Pérez-Sierra et al., 2010). One raspberry leaflet of each of the five raspberry varieties was sterilized as described above and then floated on top of the submerged plugs. Four replicate leaves per isolate were tested. The containers were sealed and incubated at 22°C in the dark and observed daily for signs of infection. The leaves were incubated for 7 days. The experiment was set up according to a randomized complete block design and repeated three times.

Lesion area and disease percentage for each leaf were calculated using APS Assess v. 2.0 software (Lamari, 2002). The percentage disease of each leaf was analysed with a quasibinomial model followed by ANOVA. Post hoc comparisons were performed using a Dunnett's test on the log odds ratio scale with the R statistics software. Seven days after inoculation, leaves exhibiting lesions were removed from the soil extract, surface sterilized and placed on *Phytophthora*-specific media. Reisolation from the diseased leaf lesions was performed according to the method outlined in Stewart et al. (2014). Diseased leaves, that is, those that had significant browning or apparent lesions, were cut into 10 $mm^2$ sections, soaked in sodium hypochlorite (1.2% available chlorine) for 2 min, and rinsed in sterile distilled water three times. Two pieces of leaf per sample were carefully submerged in 9 cm Petri dishes containing V8 amended with pimaricin (0.2 mL of a 5 mg/L solution), ampicillin-Na (250 mg/L), rifampicin (0.4 μL of 2.5% aqueous solution), and PCNB (5 mg/L) (V8-PARP). The plates were sealed with Parafilm and incubated in the dark at 18°C until mycelial growth was observed (5–7 days after isolation). The hyphal tips of growing colonies were transferred onto fresh V8-PARP. A rapid fungal DNA extraction and PCR amplification were performed using ITS4 and ITS5 primers (White et al., 1990) to determine the identity of the resultant cultures, and samples were sent for identification via Sanger sequencing using the same method outlined previously.

## 3 | RESULTS

The 2020 and 2021 study of farms in England and Scotland resulted in the isolation of 12 isolates of five species of *Phytophthora* and seven isolates of two *Phytopythium* species from 86 raspberry root and canes samples (Table 1). No isolates were recovered from blackberry material. *Phytopythium litorale* was the most frequently isolated species, with six isolates obtained, four of which came from cane material and two were from roots, all of which came from Scottish farms. *Phytopythium* is a genus of plant-pathogenic oomycetes that share similar morphology to both oomycete genera *Phytophthora* and *Pythium* but occupies an intermediary evolutionary position (Bala et al., 2010).

### 3.1 | Sequencing data analysis

From the 134 samples sent for analysis, 64 passed the quality control parameters required to proceed with amplicon sequencing, a summary of which is presented in Table 2.

Samples from Farm I and Farm II did not produce any *Phytophthora* PCR product using the 18Ph2F/5.8S-1R and ITS6/5.8S-1R primer sets. Plants of variety B were most frequently sampled as this variety has reported *Phytophthora* resistance and is thus chosen as a primary cultivar by many UK growers. Variety E is a blackberry variety. Varieties C and G–K are experimental varieties, and 2020 was their first year of commercial trial.

TABLE 2 Summary of raw amplicon data from the 64 samples of raspberry plants sequenced in this study.

| | | No. of ASVs per sample | | No. of reads per sample | | No. of reads per ASV | |
|---|---|---|---|---|---|---|---|
| Total raw reads | Total no. of ASVs | Min. | Max. | Min. | Max. | Min. | Max. |
| 5,416,190 | 23 | 5 | 20 | 4133 | 673,749 | 2626 | 1,389,673 |

Abbreviation: ASV, amplicon sequence variant.

TABLE 3 Summary of the top 10 amplicon sequence variants (ASVs) generated using a 97% sequence similarity.

| Species | Similarity (%) | Clade | % of samples | % of total reads | Counts | Proportion of total counts |
|---|---|---|---|---|---|---|
| *Phytophthora rubi* | 100 | Subclade 7a | 100 | 32.0 | 1,734,373 | 0.211 |
| *Peronospora sparsa* | 100 | N/A | 100 | 28.9 | 1,563,729 | 0.190 |
| *Phytophthora citrophthora* | 100 | Subclade 2a | 56 | 28.8 | 1,559,085 | 0.190 |
| *Phytophthora cactorum* | 100 | Subclade 1a | 100 | 19.9 | 1,078,735 | 0.131 |
| *Phytopythium litorale* | 100 | N/A | 62 | 12.9 | 700,131 | 0.085 |
| *Phytophthora pini* | 100 | Subclade 2c | 56 | 10.2 | 549,753 | 0.067 |
| *Phytophthora cactorum* | 100 | Subclade 1a | 100 | 5.8 | 315,854 | 0.038 |
| *Phytophthora bishii* | 100 | Subclade 2c | 100 | 4.3 | 230,353 | 0.028 |
| *Phytophthora citrophthora* | 100 | Subclade 2a | 50 | 3.3 | 178,424 | 0.022 |
| *Phytopythium citrinum* | 100 | N/A | 44 | 1.8 | 97,645 | 0.012 |

*Note*: ASV sequences were obtained from high-throughput sequencing of root samples from raspberry plants showing symptoms of raspberry root rot and were compared to the GenBank nucleotide database using the BLASTN+ database for species identification.

BLAST analysis identified 41 ASVs. A summary of these ASVs and their identities is listed in Table S1. Nine *Phytophthora* (11 phylotypes), three *Phytopythium* (23 phylotypes), four *Globisporangium* (four phylotypes), two *Pythium* (two phylotypes) and one *Elongisporangium* (one phylotype) species were detected. Phylotypes of the downy mildew species *Peronospora medicaginis-minimae*, *Peronospora* cf. *fagopyri*, *Hyaloperonospora parasitica* and *Pseudoperonospora urticae* were also detected. The top 10 ASVs, that is, those with the highest counts, are outlined in Table 3. ASVs with 97% similarity to the *Phytophthora* species *P. rubi*, *P. cactorum*, *P. bishii* and the downy mildew species *Peronospora sparsa* were detected in 100% of samples. The *Phytopythium* species *Pp. litorale and Pp. citrinum* were detected in 62% and 44%, respectively. The phylotype corresponding to *P. rubi* had the highest number of counts, followed by *Peronospora sparsa* and *P. cactorum*. The most prevalent *Phytophthora* species in the samples, that is, the top 10 ASVs, came from within *Phytophthora* clades 1, 2 and 7 (Table 3). Farm II in Kent and farm 2 in the West Midlands had the highest percentage abundance of *Peronospora* ASVs, while *Phytophthora* ASVs were most abundant in farm IV in Kent and farm 6 in Scotland. *Phytopythium* ASVs were highest in farms 5 and 7 in Scotland but notably absent from farms II, IV, 8 and 9, which were all Kent farms (Figure 2).

A summary of the *Phytophthora* species detected in each farm in this study is outlined in Table S1. ASV analysis revealed *P. rubi* as the most frequently detected species across all farms; the species was detected in 100% of samples and comprised 32% of the total reads for all samples. Farm 1 in the West Midlands had a consortium of eight *Phytophthora* species present, while farms 8 and 9 in Kent had just *P. rubi*, *P. cactorum*, *P. citrophthora* and *P. bishii*. *Phytophthora crassamura*, *P. pini* and *Phytophthora* sp.1 were not detected in samples taken on farms in Kent. *P. cryptogea* and *P. pseudocryptogea* were detected in one sample on farm III and in one sample on farm IV in Kent (Table 4).

Samples from farm 7 and farm IV had the highest percentage of *P. rubi* with 61.8% and 50.5% of reads in each farm, respectively. Sequencing revealed the presence of a phylotype corresponding to *P. crassamura* in 14 samples and 0.006% of total reads; this species had the highest read in a sample taken from a soil-grown raspberry plant from farm 1. A phylotype corresponding with *P. crassamura* collected from holm oak in Spain by Català et al. (2017) in 2017 was detected in 14 samples and contributed towards 0.1% of total reads (Table S1).

Three *Phytopythium* species were detected across all samples through metabarcoding: *Pp. litorale*, *Pp. citrinum* and *Pp. vexans*. Farm 6 had the highest number of reads for all three *Phytopythium* species: 2.1%, 1.7% and 0.4% of total farm reads, respectively. Samples from Scottish farms had the highest detection rates for all three *Phytopythium* species. *Pp. citrinum* and *Pp. vexans* were not detected in Kent and were most frequently detected on samples from Scottish farms (Table 5).

## 3.2 | α-diversity

Chao1 and Simpson α-diversity indices were used to assess the effects of location and variety on *Phytophthora* diversity within

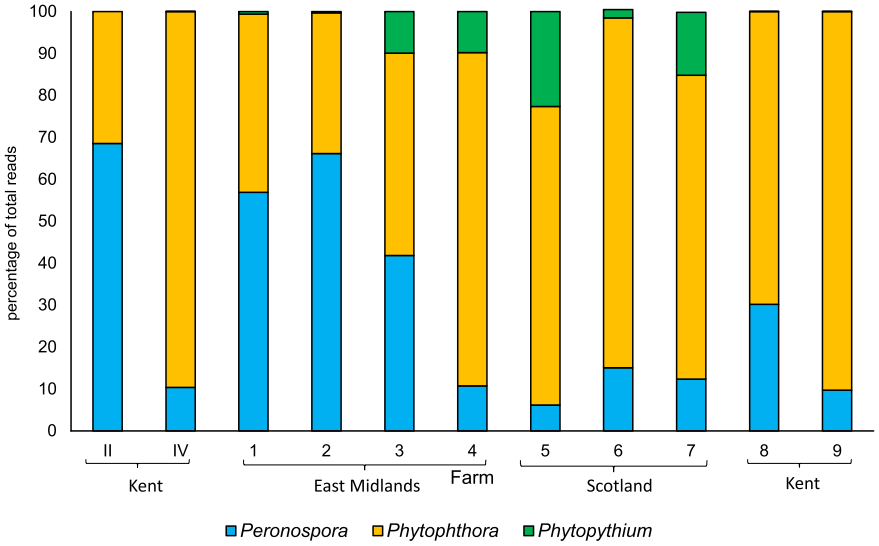


**FIGURE 2** Percentage of total reads from high-throughput sequencing aligning to *Peronospora*, *Phytophthora* and *Phytopythium* species present on raspberry plants showing symptoms of raspberry root rot collected from 11 commercial raspberry farms in 2020 and 2021. [Colour figure can be viewed at wileyonlinelibrary.com]

samples. The Chao1 index accounts for species of low abundance in a sample, whereas the Simpson index accounts for the number of taxa and their abundance and typically gives more weight to dominant taxa. The farm and plant variety had the largest effect on $\alpha$-diversity on the Chao1 index, $p<0.002$ and $p<2\times10^{-16}$, respectively, indicating that the species richness and evenness of *Phytophthora* communities differ between site and plant variety (Table 6). Farms based in Kent had lower α-diversity on the Chao index than those in Scotland and the West Midlands (Figure 3). The α-diversity of samples on the Simpson index had lower variability amongst farms.

## 3.3 | β-diversity

Principal differences in *Peronosporales* populations between samples were calculated using PCA and ADONIS analysis using the Bray–Curtis distance index. β-diversity analysis of samples can determine if factors such as location, variety and substrate affect species composition. A higher β-diversity indicates each location has a unique community structure while a lower β-diversity indicates a more homogeneous community composition. PCA showed the significant effects of farm location and variety effect on PC1, with $p=3.6\times10^{-11}$ and $p=0.003$, respectively (Figure 4). This farm effect was also detected in PC3 ($p=0.0006$) and PC4 ($p=0.0005$). Farm location had the highest effect on β-diversity through PCA, accounting for 47% of total variance. Variety contributed to 11% of the variance observed. This can be seen in the clustering of points by colour gradient in Figure 4. The percentage variation in the first four PCs was 38.1%, 14.1%, 10.4% and 9.5%, respectively. This analysis was performed on samples from 2021 and not 2020 as there was more farm and varietal variation in the 2021 dataset.

ADONIS analysis of the Bray–Curtis index also highlighted the significant effect of farm location on β-diversity ($p=0.001$; Table 7). This farm effect is further supported by the ADONIS analysis of the β-diversity using the Bray–Curtis distance similarity index showed farm location had a significant effect on the β-diversity of samples (Table 7).

In NMDS ordination (Figure 5) while there was some trend, as per the PCA, for farm-level samples to cluster together (e.g., farm_9, upper left), much more striking, however, was visible clustering of samples in the West Midlands and Kent. This suggests samples from within each of these regions have a similar β-diversity on the Bray–Curtis index. Samples from farms in Scotland had similar β-diversity to the other samples, with no apparent clustering.

## 3.4 | Differential analysis

There were a number of confounding effects between farm, variety and substrate type. Samples from variety J and K were unreplicated, and four varieties were completely confounded with farm effect. The DeSeq2 model used in the differential analysis of these samples is not suited to confounded data; thus, the effect of these factors on the abundance of taxa in the samples cannot be reliably concluded. The DeSeq2 model ran successfully on the effect of plant health status, that is, symptomatic vs. asymptomatic of root rot (Table 8). Two ASVs corresponding to *P. citrophthora*, one ASV corresponding to *P. pini* and one to *P. cactorum* were significantly more abundant in symptomatic samples, indicating these species were more prevalent in these samples. One ASV corresponding to *P. cactorum* was of higher abundance in the asymptomatic samples.

## 3.5 | Pathogenicity assay

Visual analysis of raspberry leaves after 7 days' incubation indicated *P. citrophthora*, *P. pini*, *Pp. litorale* and *Pp. vexans* had the highest lesion areas compared to the control treatment. Leaves of Valentina and variety 4 floated in soil extract infected with *P. rubi* had large lesion areas, whereas the lesion areas of the other varieties were low, and some leaves had no lesions and appeared healthy (Figure 6). No

**TABLE 4** *Phytophthora* species detected on 11 commercial raspberry farms, identified via metabarcoding of the rRNA internal transcribed region.

| Farm | Region | Total no. of samples | Number of samples with reads | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *P. rubi* | *P. cactorum* | *P. citrophthora* | *P. pini* | *P. bishii* | *P. cryptogea* | *P. pseudocryptogea* | *P. crassamura* | *Phytophthora* sp. 1 |
| III | Kent | 3 | 3 | 3 | 2 | 0 | 3 | 0 | 1 | 0 | 0 |
| IV | Kent | 4 | 4 | 4 | 1 | 0 | 4 | 1 | 0 | 0 | 0 |
| 1 | W. Midlands | 10 | 10 | 10 | 7 | 7 | 10 | 6 | 4 | 7 | 6 |
| 2 | W. Midlands | 4 | 4 | 4 | 3 | 3 | 4 | 3 | 2 | 2 | 1 |
| 3 | W. Midlands | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 4 | W. Midlands | 9 | 9 | 9 | 8 | 8 | 9 | 4 | 6 | 2 | 0 |
| 5 | Scotland | 7 | 7 | 7 | 7 | 7 | 7 | 4 | 3 | 1 | 1 |
| 6 | Scotland | 13 | 13 | 13 | 13 | 8 | 13 | 9 | 9 | 2 | 0 |
| 7 | Scotland | 5 | 5 | 5 | 1 | 0 | 5 | 5 | 4 | 0 | 0 |
| 8 | Kent | 2 | 2 | 2 | 1 | 0 | 2 | 0 | 0 | 0 | 0 |
| 9 | Kent | 4 | 4 | 4 | 1 | 0 | 4 | 0 | 0 | 0 | 0 |
| Total no. of samples | | 62 | 62 | 62 | 45 | 34 | 62 | 33 | 30 | 14 | 8 |

**TABLE 5** *Phytopythium* species detected on 11 commercial raspberry farms, identified via metabarcoding of the rRNA internal transcribed region.

| Farm | Region | Number of samples with reads | | | |
|---|---|---|---|---|---|
| | | Total | *P. litorale* | *P. citrinum* | *P. vexans* |
| II | Kent | 3 | 0 | 0 | 0 |
| IV | Kent | 4 | 1 | 0 | 0 |
| 1 | West Midlands | 10 | 4 | 5 | 2 |
| 2 | West Midlands | 4 | 1 | 1 | 0 |
| 3 | West Midlands | 1 | 1 | 1 | 1 |
| 4 | West Midlands | 9 | 7 | 7 | 2 |
| 5 | Scotland | 7 | 6 | 5 | 5 |
| 6 | Scotland | 13 | 12 | 8 | 9 |
| 7 | Scotland | 8 | 8 | 0 | 0 |
| 8 | Kent | 2 | 1 | 0 | 0 |
| 9 | Kent | 4 | 1 | 0 | 0 |
| Total number of samples | | 64 | 39 | 27 | 19 |

*Note*: Total values represent the overall number of samples with reads from each site, and the number of which attributed to each *Phytopythium* species is given.

lesion development was detected in control leaves that were floated in soil extract with uncolonized agar plugs.

A one-way ANOVA and Dunnett's post hoc test were performed on the mean disease percentage area of detached raspberry leaves floated in soil extract infected with *Phytophthora* and *Phytopythium* isolates and revealed significant differences in the disease percentage of leaves induced by isolate ($p < 10^{-9}$) and plant variety ($p < 10^{-14}$), but their interaction was not significant ($p > 0.813$).

**TABLE 6** Permutation-based analysis of variance on the α-diversity index variability accounted for by farm location, plant variety, and the interaction between location and variety.

| Index | *p*-Value | | | |
|---|---|---|---|---|
| | Farm location | Variety | Interaction | Residuals |
| Chao1 | **0.002** | $<2\times10^{-16}$ | 0.073 | 672 |
| Simpson | 0.8824 | 0.7562 | 0.8824 | 13757.2 |

*Note*: Values in bold indicate $p < 0.05$.

Leaves of the variety Valentina showed the highest mean disease percentage area across all isolates, while leaves from cv. Latham had the lowest (Figure 7).

Leaves floated on soil extract containing *Pp. litorale* and *P. pini* showed significantly higher disease percentage than the control leaves in all varieties. No significant difference in the disease percentage of leaves infected with *P. rubi* compared to the control was observed on all varieties tested (Figure 8). All the isolates tested in the detached leaf assay were successfully reisolated from the diseased leaf tissue onto V8-PARP, and the resultant isolates' identity was reconfirmed via PCR and Sanger sequencing.

## 4 | DISCUSSION

This study highlights the community composition and diversity of *Peronosporales* species present across commercial raspberry farms in the UK. The results presented in this study add to our understanding of the exceptional diversity of the raspberry pathosphere. Traditional methods of pathogen identification through direct isolation and Sanger sequencing were complemented with characterization of the *Peronosporales* community via HTS. Direct isolation from roots and canes of raspberry plants exhibiting symptoms of root rot resulted in the recovery of two species of *Phytopythium*—*Pp. litorale*

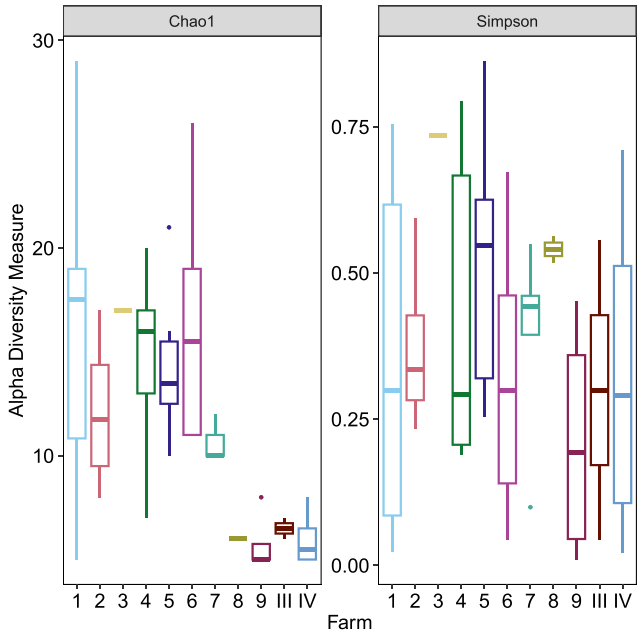


**FIGURE 3** Boxplots representing the interquartile range of the α-diversity measures, Chao1 and Simpson index for *Peronosporales* species present in the roots of red raspberry in UK farms. The *x*-axis indicates the farm number, which are differentiated by colour of box. The bold line intersecting the boxes indicates the median α-diversity measure. The lines on the top and bottom of each box represent the upper and lower quartiles, which indicates the range within which 50% of the diversity values fall. The bold points indicate outliers. The boxplot was made using R Studio. v. 1.4.05.5. [Colour figure can be viewed at wileyonlinelibrary.com]

and *Pp. vexans*—from symptomatic raspberry roots. The cane and root tissue from which they were isolated exhibited near-identical symptoms to those from which *Phytophthora* was isolated. The site in which *Phytopythium* isolates were recovered in this study notably used water from a local river to irrigate their plants. Furthermore, *Pp. vexans* and *Pp. litorale* were isolated from sites at a low elevation, and residual pooling of drip irrigation around the pots was observed. Open water systems such as lakes and rivers can be a significant source of oomycete pathogens such as *Phytopythium*, with many representatives of this genus detected in freshwater and flooded environments (Nam & Choi, 2019; Redekar et al., 2019). A recent study in apple has shown that *Phytopythium* species such as *Pp. irregulare*, *Pp. ultimum* and *Pp. sylvaticum* exhibit higher disease severity on apple seedlings under higher irrigation regimes (Moein et al., 2019). Benfradj et al. (2017) and Jabiri et al. (2021) noted a higher prevalence of *Pp. vexans* in drip irrigation systems versus submersion irrigation in citrus trees and in apple and pear orchards. Furthermore, over 30 species of *Phytophthora* have been described in water systems around the world (Redekar et al., 2019; Scibetta et al., 2012). Thus, the management of irrigation systems to prevent overflooding and regular disinfection of irrigation lines may reduce the spread of these pathogens in raspberry crops.

*Phytophthora* spores can transfer to plants at any stage of the transport process to grower sites, making it challenging to identify a source of inoculum. The production of oospores in suboptimal conditions that can persist for up to 10 years further compounds the need for rigorous sterilization of pots between plantings (Parke et al., 2014; Sapkota et al., 2022).

Graham et al. (2011) posited the spread of RRR through north-western Europe indicates pathogen proliferation through propagator networks. The samples analysed in this study came from a diverse panel of growers; however, it was not possible to obtain propagator information on the plants sampled, thus a potential route of infection is missing. The growers involved in this study maintained potted coir or compost-grown plants for 2–4 years, whereas soil-grown plants were kept for up to 6 years, which may increase the potential pathogen load in the soil and allow for more widespread disease transmission. Novel substrates are continuing to be developed for soilless raspberry production, such as coconut coir produced from coconut husk. Coir has a higher porosity than compost, which can reduce waterlogging, a contributing factor towards *Phytophthora* root rot development (Wilcox, 1989). The oomycete composition of coir has recently been investigated by Frederickson-Matika et al. (2024). Their study identified no *Phytophthora* species via baiting; however, DNA metabarcoding revealed seven species of *Phytophthora*, *Phytopythium*, *Elongisporangium* and *Peronosclerospora*.

The results of our study and that of Frederickson-Matika et al. (2024) suggest that the move from soil to substrate-based production has not been entirely successful in the elimination of RRR in the UK. This work highlights the need for further studies on the *Phytophthora* composition of plants at each stage of the propagator–grower network. Such work is critical to ensure species such as those detected in this study are appropriately identified at an early stage. Random lateral flow testing of plants, prior to container planting, with lateral flow devices (LFDs) capable of detecting a wide range of *Phytophthora* species, such as those used by Wedgwood et al. (2020), combined with regular testing of irrigation sources may help to reduce the spread of root rot. An LFD capable of detecting *Phytopythium* species is yet to be reported; however, a rapid loop-mediated isothermal amplification (LAMP) method has been developed for detection of *Pp. vexans* by Wang et al. (2021), which can be used for screening plant material from propagation stock and by plant pathologists surveying for this species. HTS of symptomatic raspberry roots in this study revealed 11 ASVs corresponding to *Pp. citrinum*, four to *Pp. vexans* and two to *Pp. litorale*. *P. rubi* was the *Phytophthora* species with the highest read number across all farms in our study, although no isolates were recovered. This finding corresponds with previous reports of the dominance of *P. rubi* in the RRR complex (Duncan et al., 1987; Gigot et al., 2013; Stewart et al., 2014; Wilcox et al., 1993). The findings of our study are in agreement with Wedgwood et al. (2020) who identified the *Phytophthora* species present in 89 raspberry root tissue, 79 cane tissue samples and 12 raspberry leaf samples exhibiting symptoms of RRR from England, UK, through nested PCR with the same primers used in the current study. In the Wedgwood et al. (2020) study, *P. rubi* was detected in 43% of samples and was the most detected *Phytophthora* species in all tissue types.

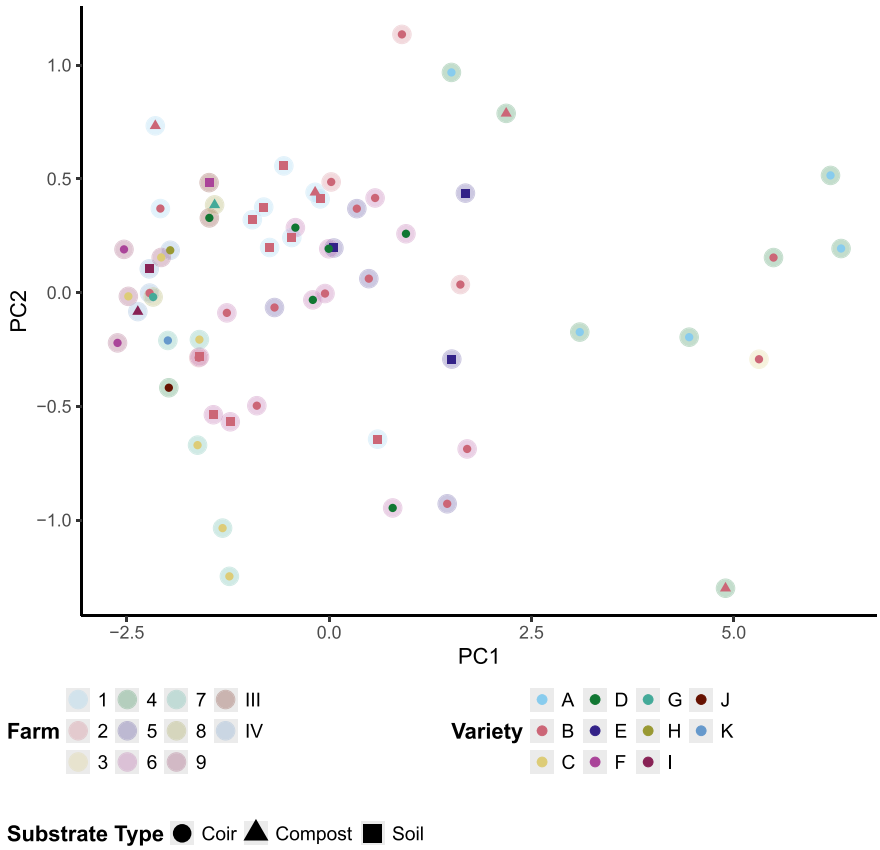


**FIGURE 4** Principal component analysis plot of PC1 versus PC2 showing the effect of factors farm location, variety, and substrate type on the β-diversity of *Peronosporales* species in each sample of raspberry plants showing symptoms of raspberry root rot, represented by a single point. Farm and variety are separated by colour, substrate type is separated by shape. [Colour figure can be viewed at wileyonlinelibrary.com]

**TABLE 7** ADONIS analysis of the effect of farm location, plant variety and their interaction with substrate type on the Bray–Curtis index.

| | *p*-Value | | | | |
|---|---|---|---|---|---|
| Index | Farm location | Variety | Variety:Substrate | Farm:Variety:Substrate | Residuals |
| Bray–Curtis | **0.001** | 0.213 | 0.640 | 0.969 | 10.808 |

*Note*: Value in bold indicates $p < 0.05$.

*Phytophthora rubi*, *P. cactorum*, *P. bishii*, *P. citrophthora* and *P. pini* were the most frequently detected *Phytophthora* species on the farms sampled in our study. Wedgwood et al. (2020) reported a similar species composition in English raspberry samples where *P. citrophthora*, *P. bishii*, *P. pini* and *P. cactorum* were detected in 8.9%, 6.1%, 2.8% and 1.7% of samples, respectively. *P. citrophthora* was first reported as a highly virulent pathogen of raspberry in Chile by Latorre (1993). Wilcox (1989) reported *P. pini* as an extremely virulent pathogen to whole plants of raspberry in the United States, while *P. cactorum* was mildly virulent. Prior to Wedgwood et al. (2020), *P. bishii* (syn. *P. bisheria*) had only been noted in raspberry in Australia, and the virulence of this species is unknown (Abad et al., 2008).

Three *Phytopythium* species (*Pp. litorale*, *Pp. citrinum* and *Pp. vexans*) were detected in this study. The genus *Phytopythium* was first described by Bala et al. (2010) with the type species *Phytopythium sidhum* isolated from declining banana trees in Pakistan. This pathogen was categorized as part of a new genus as the isolate produced ovoid, internally proliferating sporangia like *Phytophthora*, but the mechanism of zoospore discharge via cell differentiation in a vesicle outside of the sporangium was more similar to *Pythium*. A previous paper from our investigation reported the pathogenicity of *Pp. vexans* and *Pp. litorale* on raspberry (Browne et al., 2023).

*Phytopythium* species have also been reported as pathogens of strawberry (*Fragaria* × *ananassa*), another soft fruit member of the *Rosaceae* family. Pánek and Střížková (2021) isolated *Pp. vexans*, *Pp. litorale* and *Phytopythium mercuriale* from plants suffering strawberry decline in 2021. The group confirmed the pathogenicity of each isolate on detached leaflets of five strawberry varieties. None of the *Phytopythium* isolates had consistent virulence, that is, high total number of lesions on more than one variety. *Pp. litorale* was the only *Phytopythium* species reported as highly virulent in the study, contrary to reports from apple (Tewoldemedhin et al., 2011). A recent report by Ibañez et al. (2022) on the oomycete species recovered from strawberry crowns and roots in Argentina included *Pp. vexans*, *Pp. litorale* and *Pp. mercuriale*. Phylotypes corresponding to species of *Pythium* and *Globisporangium* were also detected in our study. *Pythium* and *Globisporangium* are oomycetes and members of

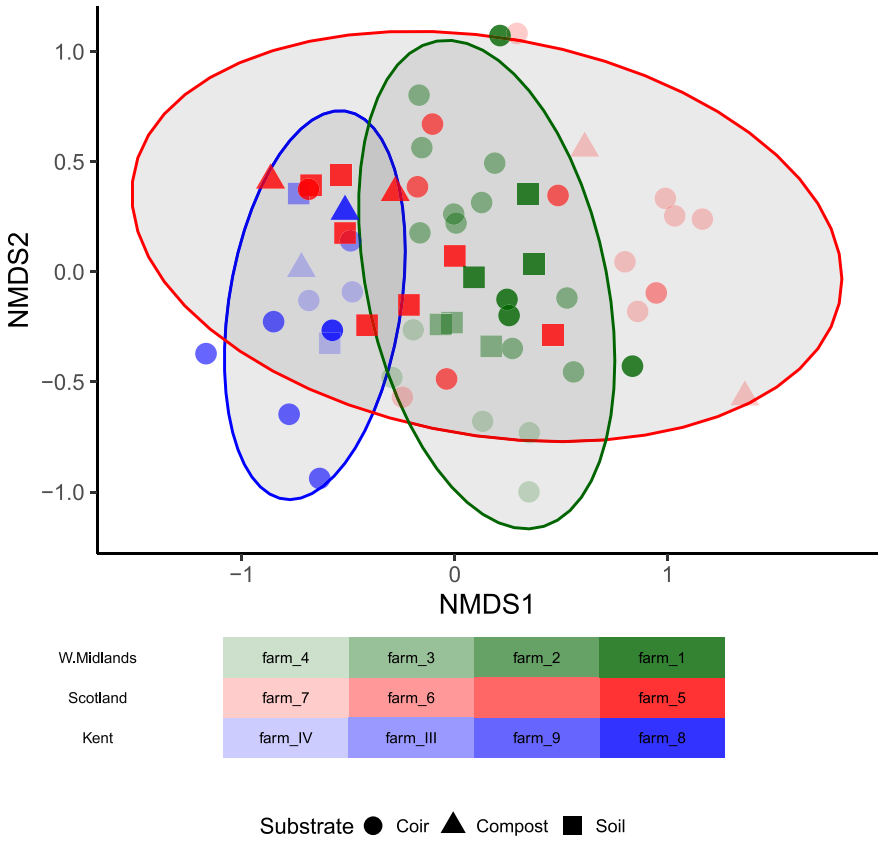


**FIGURE 5** Non-metric multidimensional scaling (NMDS, stress-value = 0.158) analysis of the Bray–Curtis similarity index showing β-diversity of *Peronosporales* species in root samples from raspberry plants exhibiting symptoms of root rot in three UK regions. Farm regions are separated by colour, different shapes indicate the three substrate types assessed. [Colour figure can be viewed at wileyonlinelibrary.com]

**TABLE 8** DESeq2 results for the differential analysis on the *Peronosporales* present in raspberry plants asymptomatic and symptomatic of root rot.

| DESeq2 model | No. of ASVs | LogFC > 0 (higher) | LogFC < 0 (lower) | Low counts |
|---|---|---|---|---|
| Symptomatic versus Asymptomatic | 22 | 4 (18%) | 1 (4.5%) | 1 (4.5%) |

*Note*: The number of amplicon sequence variants (ASVs) indicates the number remaining after ASV filtering. Log fold-change (FC) >0 indicates the ASVs are higher in the first treatment (symptomatic) than the second (asymptomatic). The reverse is true for LogFC <0.

the order *Pythiaceae*. *Pythium* has previously been implicated in replant disease in raspberry (Schilder & Gillett, 2007). *Globisporangium* has yet to be reported in raspberry but has been reported to cause root rot disease in strawberry (Pánek et al., 2022).

*Peronospora sparsa* was detected in 100% of samples in our study. *P. sparsa* (syn. *Peronospora rubi*) is the causal agent of downy mildew in rose and has been reported to infect leaves, flowers, fruits and stems of raspberry (Williamson et al., 1995). The virulence of *P. sparsa* to raspberry is unknown; therefore, the threat it poses to UK production cannot be assessed, and no isolate was recovered in this study. In the study conducted by Wedgwood et al. (2020), *P. sparsa* was detected by nested PCR in one out of 89 root samples and three out of 79 cane samples, but the species was not detected in symptomatic leaf samples tested. *P. sparsa* has been shown to overwinter in the belowground parts of arctic bramble (Lindqvist et al., 1998); this may explain why we detected *P. sparsa* so readily in our study, as the species can survive harsh cold weather such as that experienced by bare-root plants in a cold store. Additionally, bare-root raspberry plants are typically lain on top of one another in a cold store during the winter. Plants with *P. sparsa*-infected leaves may have been in close proximity to the roots of plants sampled in the present study, causing cross-contamination, which may explain its omnipresence amongst the samples analysed. Random sampling of raspberry bare-root plants in cold storage for *P. sparsa* would be needed to confirm this.

Farm location had the most significant effect on the abundance and diversity of the oomycete species we detected. The α-diversity on the Chao1 index of samples taken in farms from Kent was significantly lower than that of samples from farms in the West Midlands and Scotland. This finding is in agreement with previous reports of location being the most significant factor in *Phytophthora* abundance and diversity (Bose et al., 2018; Català et al., 2017; Gyeltshen et al., 2021; Sapp et al., 2019).

Growers typically chose to grow a variety as a primary crop that had noted resistance to root rot and good quality fruit. Variety B was the primary crop on seven out of 11 farms sampled in this study. This variety is reported to have field resistance to *Phytophthora*. However, resistance of raspberry varieties to *Phytophthora* is usually tested

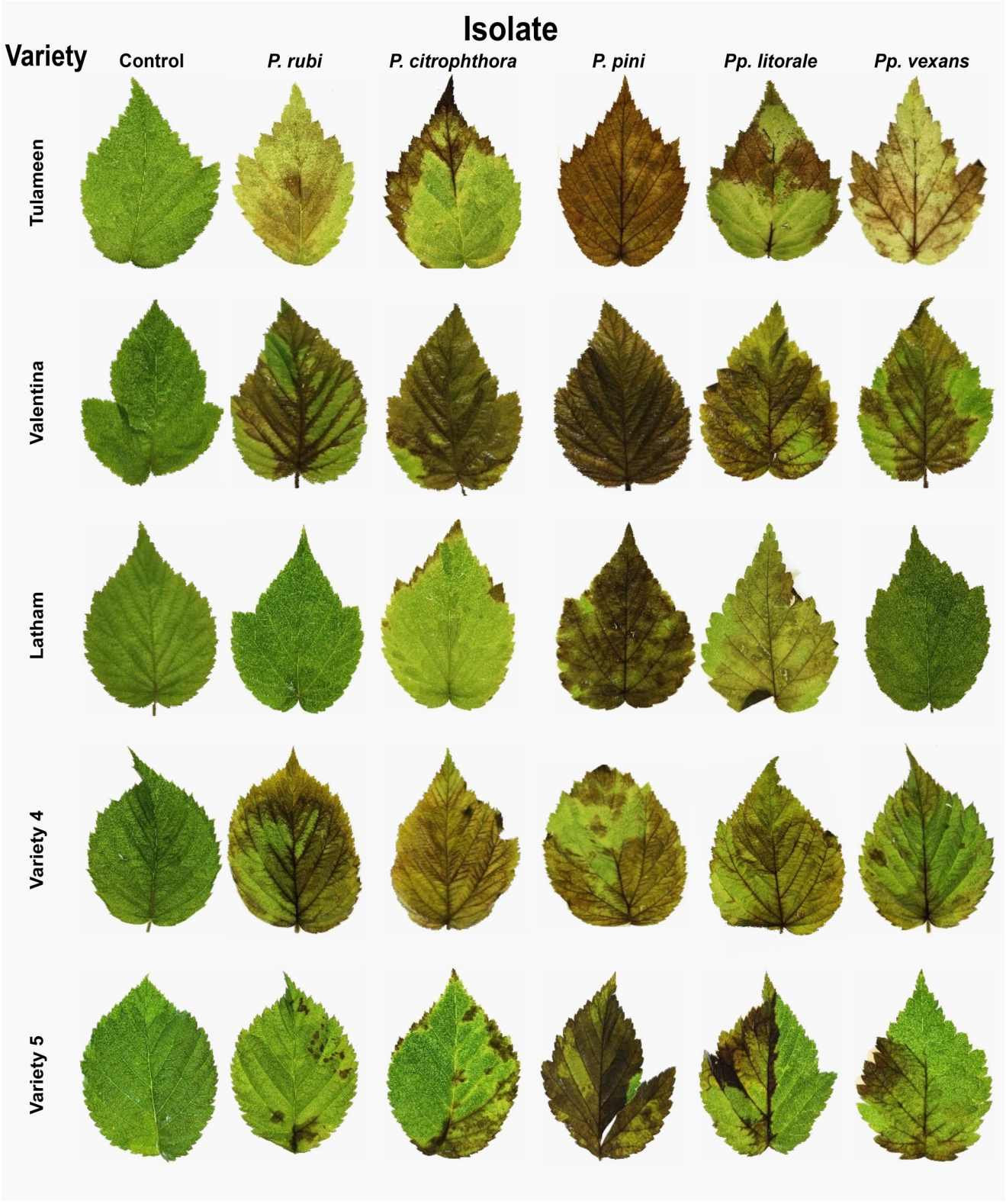


FIGURE 6 Example raspberry leaves of five varieties floated in soil extract infested with *Phytophthora* and *Phytopythium* isolates after 7 days' incubation at 20°C. Control leaves were floated in soil extract with uncolonized agar plugs. [Colour figure can be viewed at wileyonlinelibrary.com]

against, or reported in fields containing, *P. rubi* (Graham et al., 2011; Kempler et al., 2012; Nestby & Heiberg, 1995). Plants from variety B had high read numbers of *P. rubi*, *P. cactorum* and *P. bishii* (100% of samples). The perceived resistance of raspberry varieties to *P. rubi* is reportedly linked to a larger root density and diameter (Graham et al., 2011). Thus, the more vigorous root growth associated with *Phytophthora*-resistant varieties may reduce the ability of pathogens to encyst on the root.

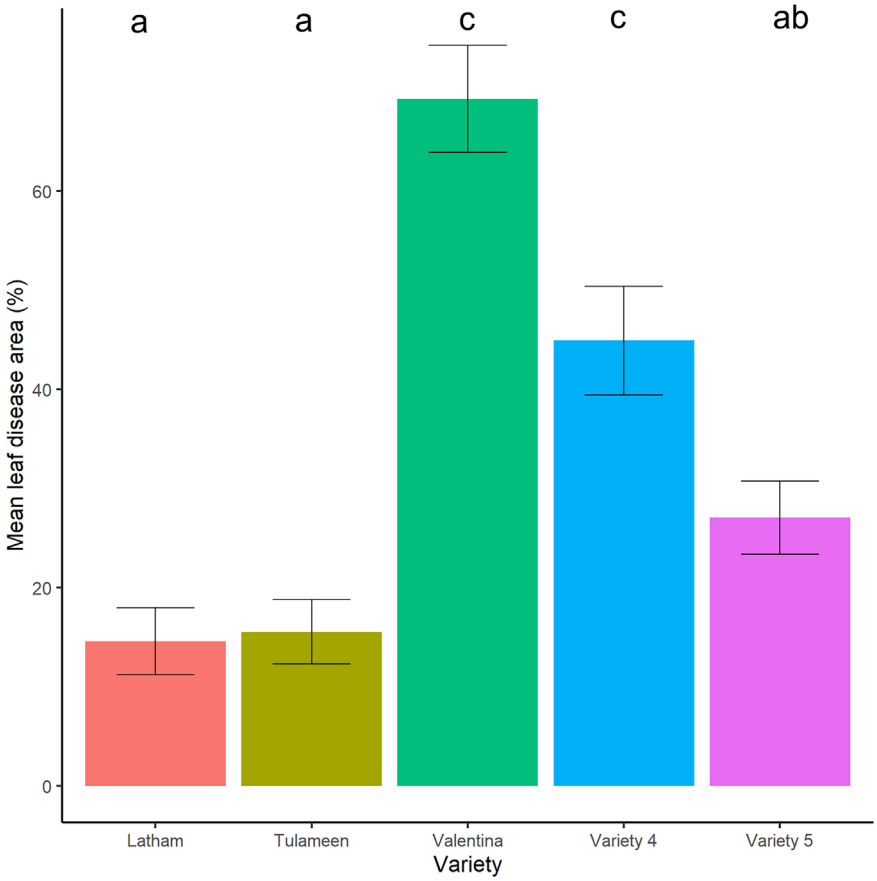


FIGURE 7 Mean percentage leaf disease area of raspberry (*Rubus idaeus*) leaves of five raspberry varieties infected with isolates of *Phytophthora* and *Phytopythium*. Statistically significant differences based on analysis of variance and Dunnett's test are given as labelled letters. [Colour figure can be viewed at wileyonlinelibrary.com]

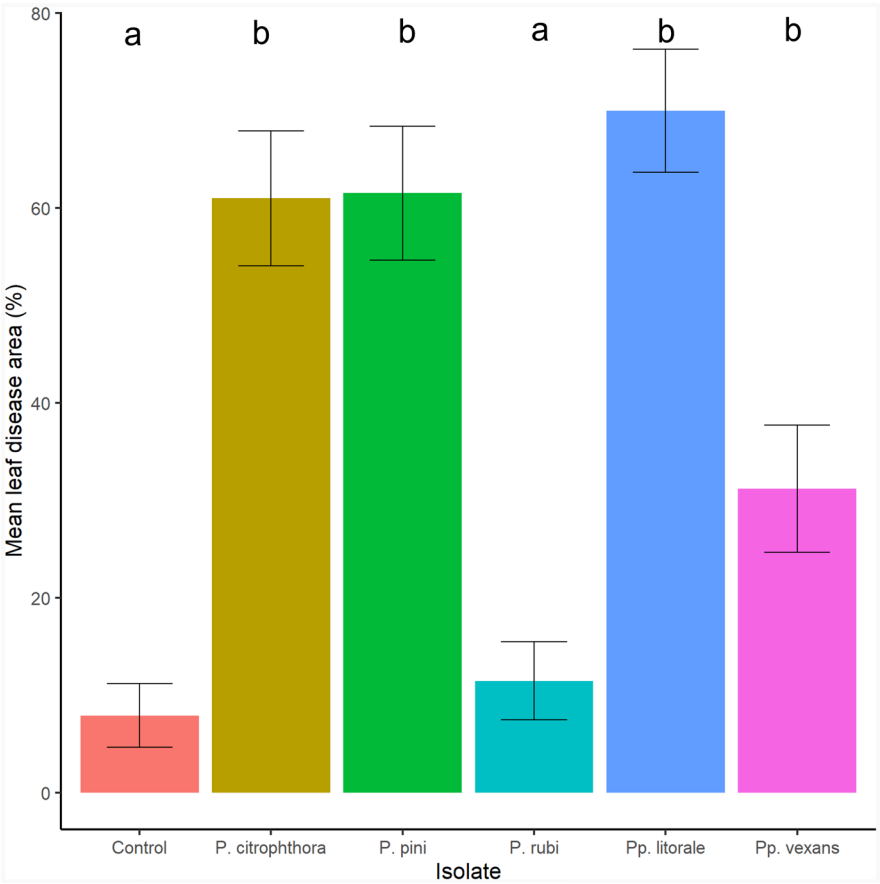


FIGURE 8 Mean percentage disease area of raspberry (*Rubus idaeus*) leaves of five raspberry varieties floated in soil extract infected with one of three *Phytophthora* and two *Phytopythium* isolates. Statistically significant differences based on analysis of variance and Dunnett's test are given as labelled letters. $n = 74$. [Colour figure can be viewed at wileyonlinelibrary.com]

Samples were not evenly collected from each variety due to growers trialling a few plants of newer varieties C and G–K, and just three samples were taken from the blackberry variety BB; therefore, a robust statistical analysis of the effect of plant genotype on *Phytophthora* community diversity and abundance was not possible. The α-diversity estimated using the Chao1 index revealed plant genotype has a significant effect on the species richness and evenness of *Phytophthora* species in the samples. However, β-diversity analysis using the Bray–Curtis index indicated variety does not have a significant effect on the diversity of *Phytophthora* species in raspberry roots. Therefore, the impact of genotype cannot be fully inferred from these conflicting results. β-diversity analysis relies more on the abundance of the *Phytophthora* groups, while α-diversity is a measure of species richness and evenness; thus, plant genotype affects the richness of *Peronosporales* species but not their overall abundance. Future work should analyse an even number of samples from multiple genotypes to assess their effect on the diversity and abundance of *Peronosporales* species associated with RRR.

Pathogenicity assays in this study were conducted using detached leaves. Leaf assays provide preliminary information on the aetiological relationship between a pathogen and its host. While all isolates tested in this study are soilborne, the purpose of this experiment was to assess their ability to infect plant material. Although leaves are not a major route of natural infection, leaf assays are nondestructive and thus more cost-effective method for screening large breeding populations for host susceptibility and the pathogenicity of numerous isolates. To assess accurately the pathogenicity of these isolates on raspberry, an additional trial on detached roots, such as that outlined in Browne et al. (2023), and/or a whole-plant pathogenicity trial should be conducted. It is worth noting that cv. Valentina, whose leaves were easily colonized by *P. rubi* and other species in this study, was found to be highly resistant to *P. rubi* in whole-plant glasshouse tests carried out in 2003 and 2004. In those experiments, potted plants were inoculated with sporulating mycelium disks of FVR11-race 3 and FVR67-race 1 isolates (JHI, Scotland) according to Knight and Fernández-Fernández (2008). This discrepancy could be caused by race-specific responses or by differences in the mechanism of infection of leaves versus roots, and further investigations using the same pathogen isolates are needed for validation. The work presented in this study represents a preliminary step in assessing the virulence of *Phytopythium* species in raspberry. We detected a more diverse oomycete community in red raspberry than previously thought; indeed, *Phytopythium* may be a much more important genus in the RRR complex than some *Phytophthora* species. The focus on screening for *P. rubi*-specific genotype resistance may not be as effective with changing patterns in the diversity of RRR species, as reported in this study. We have also demonstrated the potential of a detached leaf assay as a preliminary method in the assessment of host resistance to *Phytophthora* and *Phytopythium* and pathogenicity analysis of these species.

The research outlined in this study offers more insight into the understudied field of the raspberry *Phytophthora* complex, presenting a more diverse oomycete community in the crop than previously thought. The prevalence and abundance of *Phytopythium* species is of note as this genus has only recently been reported in raspberry and in the UK (Browne et al., 2023). Furthermore, this study adds to the growing body of work on HTS as a diagnostic tool for *Phytophthora* and as a method to identify potential targets for pathogenicity studies and resistance screening of emerging raspberry breeding lines, although further validation might be needed to correlate leaf and whole plant response.

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## CONFLICT OF INTEREST STATEMENT

We declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Sampling was performed on raspberry grower sites in Scotland and England; locations remain confidential on grower's request. The amplicon sequencing data that support the findings of this study are openly available in the ENA database at https://www.ebi.ac.uk/ena/browser/home under the accession PRJEB76314.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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