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ORIGINAL ARTICLE

Characterization of fungi associated with the decline syndrome of strawberry from organic cultivation in Southern Italy

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Abstract

Severe decline symptoms (reaching 60–80% disease incidence) were recently observed on strawberry plants from organic cultivation in Southern Italy, raising a great concern among growers. Root symptomatic samples from two strawberry fields (Eboli and Caserta) were investigated, and different fungi were isolated. A total of 47 pure fungal cultures were chosen and further characterized. Using classical isolation approaches combined with multi-locus phylogeny based on sequences of two barcodes (ITS and *tub2*), a complex fungal community associated with strawberry decline was revealed. Within this community, *Neopestalotiopsis* species [63% and 55% relative abundance (RA)] and *Dactylonectria torresensis* (13% RA) were mostly isolated, indicating that these can have a critical role in the observed decline symptoms in fields. Other fungi, such as *Plectosphaerella plurivora*, *Globisporangium selvaticum*, *Chaetomium subglobosum*, *Acremonium sclerotigenum*, *Geotrichum* sp., *Neofusicoccum parvum*, and *Gnomoniopsis fragariae*, were less frequently isolated. Furthermore, some very common strawberry pathogens like *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Colletotrichum acutatum*, *Botrytis cinerea*, *Alternaria alternata*, *Biscogniauxia numularia*, *Mucor hiemalis*, and *Penicillium brevicopactum* were only sporadically isolated, suggesting that they did not play any key part in the observed strawberry decline syndrome in the Campania region. Some other non-root-pathogenic fungi, including *Clonostachys rosea*, *Aureobasidium pullulans*, *Linnemannia* sp., and *Bjerkandera adusta*, were also found and could be further evaluated for their biocontrol potential against strawberry diseases or as plant growth promoters. Results from this study about the fungal community of strawberries affected by decline provided a foundation for upcoming studies on the assessment of organically cultivated strawberries health status aiming to develop efficient disease management strategies.

Keywords *Fragaria x ananassa* · Fungal pathogens · Diversity · Molecular phylogeny · Strawberry decline (SD)

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Introduction

Berries are among the healthiest fruits, being fibre-rich and also a great source of antioxidants. Their intake can reduce cholesterol levels, provide lots of nutrients, and even help fight inflammation and cancer. Among the berries, strawberry (*Fragaria* × *ananassa* Duchesne ex Rozier), which belongs to the *Rosaceae* family, has an attractive red colour and sweet flavour and is the most relevant berry cultivated worldwide as revealed by FAOSTAT data (https://www.fao.org/faostat/en/#data/QCL accessed on 10.11.2025). The global strawberry production is massive (about 9.2 million tonnes), with China (3.4 M), the United States (1.3 M), and Turkey (728.1 K) being the top-producing countries (source: FAOSTAT data, 2023). In Europe, strawberries with a harvested area of 151,082 ha, a production of 1778614.1 t and a yield of 11772.5 kg/ha are an important crop as well, and the top producer countries are Spain, Poland, Germany, Italy and France (https://www.fao.org/faostat/en/#data/QCL accessed 10.11.2025). In Italy, strawberries are grown on about 4.350 ha located in Emilia-Romagna (a north-eastern region) and in three southern regions of Campania, Basilicata, and Calabria. In 2023, the Italian strawberry production was 119,920 tons, and the yield reached 27567.8 kg/ha (https://www.fao.org/faostat/en/#data/QCL accessed 10.11.2025).

Strawberry cultivation worldwide comprising the organic one, regardless of the progress made so far in cultivar breeding and agricultural technology development, still remains complex due to multiple factors that can affect plant health and production, especially under the global climate change scenario. Numerous fungi, bacteria, viruses, and nematodes harm strawberries, often leading to serious diseases. However, strawberry production and quality are mainly affected by the pathogenic fungi attack. The top fungal pathogens of strawberry crops, capable of causing devastating leaf, fruit, crown and root diseases, are: *Botrytis cinerea* Pers.: Fr. (Gray mould) which has a potential to cause up to 80% production reduction (Petrasch et al. 2019), *Phytophthora cactorum* (Lebert & Cohn) J. Schröter (Phytophthora crown-rot), *Colletotrichum acutatum* J.H. Simmonds (Antracnose), *Verticillium albo-atrum* Reinke & Berthold and *V. dahliae* Klebahn (Verticillium wilt), *Fusarium oxysporum* von Schlechtendal (Fusarium wilt) which are also highly limiting factors of strawberry yields (Maas 1998; Fang et al. 2023). In particular, soil-borne fungal pathogens remain the most restraining element of strawberry crops, severely affecting the plants' health, consequently leading to their death and heavy economic losses (Avilés et al. 2024). Knowledge about their diversity in field cultivation can be of great help for the development and planning of sustainable control strategies for crop health protection (Avilés et al. 2024). Monocropping easily triggers an increase in soil-borne pathogens responsible for plant diseases, thus reducing fruit yield and quality, changing soil quality, and also affecting the native microbial population (Lovaisa et al. 2017). Moreover, strawberry long-term monoculture and continuous cropping have proved to have a negative effect on plant health and fungal community diversity by increasing soil-borne pathogens like *Fusarium* and *Guehomyces* and also decreasing soil quality (Li and Liu 2019).

Among strawberry phytosanitary issues, the decline syndrome remains one of the most severe, and symptoms include a great reduction in plant development, accompanied by a damaged root system, being a multi-factor syndrome (Soppelsa et al. 2021). It was associated with soil-borne fungal pathogens, often considering more than one opportunistic soil-borne fungal agent, and the disease severity was also linked to the cultivar. The following core soil-borne common fungal pathogens responsible for crop decline in strawberry: *Pythium* spp., Cylindrocarpon-like fungi, binucleate *Rhizoctonia* sp., and *Macrophomina phaseolina* (Tassi) Goidanich were reported (Fang et al. 2023). The decline associated with the death of Spanish strawberries was attributed to soil-borne pathogens like *M. phaseolina*, *P. cactorum*, and *Fusarium* spp., and their inoculum sources and incidence were also investigated (Pastrana et al. 2017).

Given the severe decline observed in strawberries from Eboli and Caserta organic cultivations, the considerable economic losses triggered by it and the limited understanding about the pathogens involved in the complex phenomena noticed , this study goals were to: (i) identify the fungal species associated with strawberry decline through morphological, molecular and phylogenetic approaches, (ii) discuss the possible role played by the identified taxa in the strawberry decline syndrome.

Materials and methods

Field symptoms, sampling, and fungal isolation

In June 2024, disease symptoms resembling a general decline were observed on strawberry plants of variety (var.) Marimbella and Sabrosa Candonga® cultivar (cv.), organically cultivated in the fields of Eboli and Caserta (Southern Italy), respectively (Fig. 1).

Root samples (of 100 plants randomly selected/geographic origin) were individually taken from symptomatic plants in plastic bags. They were brought into the laboratory and washed with tap water to eliminate any soil residues. Subsequently, they were surface disinfected with 70% ethanol and 1% sodium hypochlorite and

Fig. 1 Fields with strawberry plants affected by decline syndrome, along with dead plants in Eboli (**a**) and Caserta (**b**) locations. Closer view of a decline symptomatic strawberry plant showing a necrotic crown associated with discoloured, necrotic roots (**c**)

processed shortly thereafter as fully described in previous studies (Mang et al. 2010; Forlano et al. 2022). Briefly, surface sterilized roots were cut into pieces (2–4 mm), aseptically placed in Petri dishes (five/plate) onto potato dextrose agar (PDA) media (Oxoid™ Ltd., Hampshire, UK) amended with 40 ml l^{-1} streptomycin and 50 ml l^{-1} ampicillin (Merck KGaA, Darmstadt, Germany) and incubated for about 7 days at 24 °C till colonies growth became visible. Data from fungal isolation investigation were analysed with Minitab 21 statistical software (Minitab LCC 2021), and one-way ANOVA followed by Tukey's test was performed. The dependent variable was the relative abundance (RA%), while the factor was represented by the fungal species. The analyses were conducted separately for each site to evaluate the differences between the RA% for fungal species discovered. When the ANOVA evidenced significant differences ($p \leq 0{,}05$), comparison between means was made by the post hoc Tukey (HSD) test. Results were presented as mean ± standard deviation.

Morphological analysis

Pure fungal cultures, obtained by subculturing single hyphal tips, were observed visually and under a microscope model Axioscope (Zeiss, Jena, Germany) equipped with an Olympus Camedia model C-7070 wide zoom digital camera. Plates were photographed, and fungi were preliminarily identified based on morphological features using appropriate literature resources. Specific bibliography was consulted for each fungal taxon. However, due to an extremely long list, determined by the high number of morphologically different taxa found, we only mention here: Crous et al. 2021; Maharachikumbura et al. 2012, 2014; Carlucci et al. 2012; Damm et al. 2012; Edel-Hermann and Lecomte 2019. The relative abundance (RA in %) for specific taxa present in the obtained fungal community was calculated (Ulrich and Ollik 2005) with the formula: RA% = Number of individual taxa identified / Total number of taxa discovered × 100. One to five pure fungal cultures from each group (depending on their relative abundance) were selected for species confirmation and were also long-term conserved *ex-situ* (as slants at 4 °C) in the fungal collection of the Plant Pathology Laboratory at the University of Basilicata.

DNA isolation, polymerase chain reaction (PCR), and sequencing

Total genomic DNA was extracted from fresh cultures (200 mg of mycelia) using the NucleoSpin Plant II Isolation kit (Macherey-Nagel GmbH & Co., Düren, Germany) according to protocols previously described in Mang et al. (2025). Fungal species identification was accomplished through DNA amplification followed by sequencing of a merged dataset of genes: the nuclear ribosomal internal transcribed spacer (ITS1, 5.8 S RNA, and ITS2) ITS region and partial β-tubulin (*tub2*) gene. PCRs using primers ITS1 + ITS4 (White et al. 1990) for ITS along with T1 + T22 (O'Donell & Cigelnik 1997) and Bt2a+Bt2b (Glass & Donaldson 1997) for the *tub2* gene were performed as described in Mang et al. (2025), and PCR amplicons were verified and sequenced in both directions using the same PCR primers according to Camele and Mang (2019).

Sequence analysis and phylogenetic investigation

Nucleotide sequences of the two fungal barcodes were initially visualized with Chromas v. 2.6.6 (Technelysium Pty Ltd., South Brisbane, Australia) chromatogram editor, downloaded from www.technelysium.com.au/wp/chromas, and subsequently analysed by the nucleotide Basic Local Alignment Search Tool (BLAST, Altschul et al. 1990) (https://blast.ncbi.nlm.nih.gov) with the option BLASTn in the National Centre for Biotechnology Information (NCBI) database. They were compared with reference sequences already available in GenBank and species assignment for each fungal isolate was performed for the two barcodes considering > 99% identity with ex-type cultures or isolates. Before phylogenetic analysis nucleotide sequences for each locus were aligned using the MUSCLE (Edgar 2004) algorithm in the Molecular Evolutionary Genetics Analysis (MEGA) version 12 package (Kumar et al. 2024) and manually trimmed. Afterwards, phylogenetic reconstruction was conducted separately due to a lack of sequence data for the same isolates and for both genes in the GenBank database. The ITS-based analysis comprised 47 sequences generated in this study, along with 65 closely related sequences retrieved from GenBank (Table S1, Supplementary material). In phylogenetic analysis, the ITS nucleotide sequence data were processed separately based on phyla since very diverse fungal phyla had been obtained and their highly variable sequences were unsuitable for a single analysis. The *tub2* partial gene nucleotide sequences obtained here, along with 45 *tub2* sequences downloaded from the database (Table S1, Supplementary material) were also used for phylogenetic analysis. Before phylogenetic analysis, to choose the best-fit nucleotide substitution model for each phylum, the Models tool in the MEGA12 package with the option find best DNA models (ML) was selected (Kumar et al. 2024). The Bayesian Information Criterion (BIC) scores were applied to select the optimal model, and the following models were recommended and used: T92 + G + I, T92 + I, HKY + I, and K2 + G + I (Kimura 1980; Tamura 1992; Hasegawa et al. 1985) depending on the alignments considered. Evolutionary rate variation among sites was modelled using a discrete Gamma distribution (+G) with 5 rate categories (Yang 1994), and a fraction of sites were allowed to be evolutionarily invariant (+I). For the gaps/missing data, the option partial deletion with a 60% site coverage cutoff was used, and the codon positions 1st, 2nd, 3rd, and non-coding were selected. Evolutionary history was inferred using the Maximum Likelihood (ML) statistical method (Kimura 1980), and branch support on the tree was estimated with 1000 bootstrap iterations (Felsenstein 1985). The evolutionary distances were computed using the appropriate substitution models described before depending on the dataset used to infer phylogenetic relationships, and are in units of the number of base substitutions per site. The trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The tree topology was made in MEGA12 (Kumar et al. 2024). The strain CBS 201.65 of *Mucor hiemalis*, suited for a multi-class analysis, was used as an outgroup for the taxa belonging to Ascomycota. For the taxa fitting into Basidiomycota, the strain CBS 124811 of *Schizophyllum commune* was used, while the strain DAOM 234 180 of *Rhizophagus irregularis* was used for the taxa belonging to Mucoromycota, and the strain CBS 118 360 of *Phytopythium litorale* was used as an outgroup for the taxa belonging to Oomycota phylogenetic analyses (Table S1, Supplementary material). For the phylogenetic analysis based on the *tub2* gene, the strain 4–39 of *Schizophillum commune* was used as an outgroup.

Results

Field symptoms, sampling and fungal isolation

In fields, strawberry plants of var. Marimbella and Sabrosa Candonga® cv. showed typical decline syndrome symptoms, which consisted of heavily weakened and collapsed plants with necrotic crowns and discoloured necrotic roots (Fig. 1). A total of 206 fungal cultures [Eboli (98) and Caserta (108)] were isolated from decline-symptomatic roots.

Morphological analysis

A total of 47 selected representative pure cultures were identified at least at the genus level through cultural and morphological features. They were divided into groups based on similar morphological features (24 groups with Caserta origin) and (18 groups with Eboli origin). Most fungal genera were common among origins, with fewer revealed in Eboli samples. Fungal taxa associated with strawberry decline, considering features described by several authors, belonged to 25 genera listed bellow: *Neopestalotiopsis*; *Dactylonectria*; *Plectospaherella*; *Globisporangium*; *Chaetomium*; *Acremonium*; *Geotrichum*; *Neofusiccocum*; *Gnomoniopsis*; *Macrophomina*; *Rhizoctonia*; *Fusarium*; *Colletotrichum*; *Botrytis*; *Alternaria*; *Biscogniauxia*; *Mucor*; *Penicillium*; *Cylindrocladiella*; *Cadophora*; *Paraphoma*; *Clonostachys*; *Aureobasidium*; *Linnemannia* and *Bjerkandera*. Some isolates belonging to the same genus showed diverse morphological features, and it was presumed they may fit into different species, and all were molecularly analysed (Fig. S1, Supplementary material).

Molecular recognition of fungi associated with strawberry decline

Looking at all phyla detected in decline-affected strawberry roots originating from Eboli and Caserta, the Ascomycota prevailed (accounting for 78% and 85% of the taxa), while others were less represented: Basidiomycota (10%), Ooomycota and Mucoromycota (<5%), and a very high microbial diversity at genus level was revealed. However, the *Neopestalotiopsis* genus was the most frequently found, exhibiting 63% RA (Eboli) and 55% RA (Caserta), respectively. Other genera, such as *Dactylonectria* (13% RA) and *Macrophomina* (7% and 6% RA), were shown to be relatively abundant, while the *Plectosphaerella* genus (2% RA) was rarely found. Despite being less represented, both *Dactlyonectria* and *Plectopshaerella* are here fully described for the first time on strawberry in Italy. Statistical outcomes of fungal isolations showed that among all species, *N. rosae* was predominant, followed by *D. torresensis* in Marimbella var. or *M. phaseolina* in Sabrosa Candonga® cv. (Fig. 2a, b). We also found that, in both geographical places, a rich fungal community at the species level was present in decline-affected strawberry roots where *N. rosae*, *M. phaesolina*, *N. clavispora*, and *D. torresensis* were predominant, while many other species were less represented (Fig. 2a, b). In addition, in both cases, Tukey's multiple comparison analysis (p-value<0.05) highlighted statistically significant differences between the isolated fungi and the above-mentioned species, resulting significantly different from all the other fungi detected (Table S2, Supplementary material).

Phylogenetic analyses

ITS and *tub2* barcodes direct sequencing of the representative fungal isolates yielded 47 nucleotide sequences, which were identified at least at the genus level through the BLASTn web tool in the NCBI database (https://blast.ncbi.nlm.nih.gov/) at a>99% similarity threshold and deposited in the GenBank (NCBI) database, as provided in Table 1. Phylogenetic analyses were conducted separately for the two barcodes (ITS and *tub2*), as sequences of both markers from the same strain/isolate exist in the GenBank database only for some of the species identified in this study.

A total of 89 nucleotide sequences of the ITS region, including sequences of the fungal taxa isolated in this study along with reference species taken from the NCBI database (Table 1, Supplementary material), were considered in separate alignment matrices (considering four phylum taxonomy ranks Ascomycota, Basidiomycota, Mucoromycota and Oomycota) for phylogenetic analysis with the appropriate outgroups fully described earlier in the materials and methods section. The ITS alignments after manual adjustments yielded diverse datasets for each investigated phylum. In particular, Ascomycota taxa alignment contained 635 characters, of which 175 were constant, 457 were variable, 408 were parsimony-informative, and 49 singletons, while that of Basidiomycota comprised 632 characters, of which 339 were constant, 171 were variable, 98 were parsimony-informative, and 72 were singletons. In addition, the alignment of taxa belonging to Mucoromycota contained 697 characters, of which 395 were constant, 296 were variable, 211 were parsimony-informative, and 85 were singletons. Finally, ITS alignment of taxa belonging to Oomycota contained 703 characters, of which 405 were constant, 281 were variable characters, 155 were parsimony-informative, and 126 were singletons. Outcomes of the ML bootstrap analysis of the ITS region showed that all fungal taxa obtained in this study clustered closely with similar species, forming clades and subclades with very high support (mostly 100%) (Fig. 3).

It is well known that using ITS barcode alone is insufficient for precise fungal species recognition. Thus, the more informative barcode tubulin gene (*tub2*) was employed, following the same methodology as indicated for the ITS region. The *tub2* nucleotide sequences obtained in this study, along with those of reference species downloaded from the NCBI database (Table 1, Supplementary material), were used to construct the alignment matrix (comprising 78 nucleotide sequences) for phylogenetic investigation. The *tub2* alignments consisted of 504 characters, of which 119 were constant, 383 were variable characters, 353 were parsimony-informative characters, and 28 were singletons.Based on data from the *tub2* secondary barcode, all taxa obtained in this study from strawberry decline symptomatic roots clustered together with similar species, and their grouping was supported by high (>70% and mostly 100%) bootstrap values, adding more precision to the initial ITS barcode and morphological identifications (Fig. 4).

Discussion

The present study explored for the first time the fungi associated with the decline syndrome of strawberry (SDS) from organic cultivation in Southern Italy (Campania). Campania remains one of the largest Italian regions for greenhouse strawberry cultivation, which covers around 1,100 ha, producing, along with Basilicata, 50% of the national production (ISTAT, 2025; Pergola et al. 2023). Recently, a severe decline was observed in strawberry crops worldwide, including those from Italy and from the Campania region, which severely affected the plants and consequently reduced their production. Strawberry decline is a complex syndrome determined by fungal pathogens in association with other

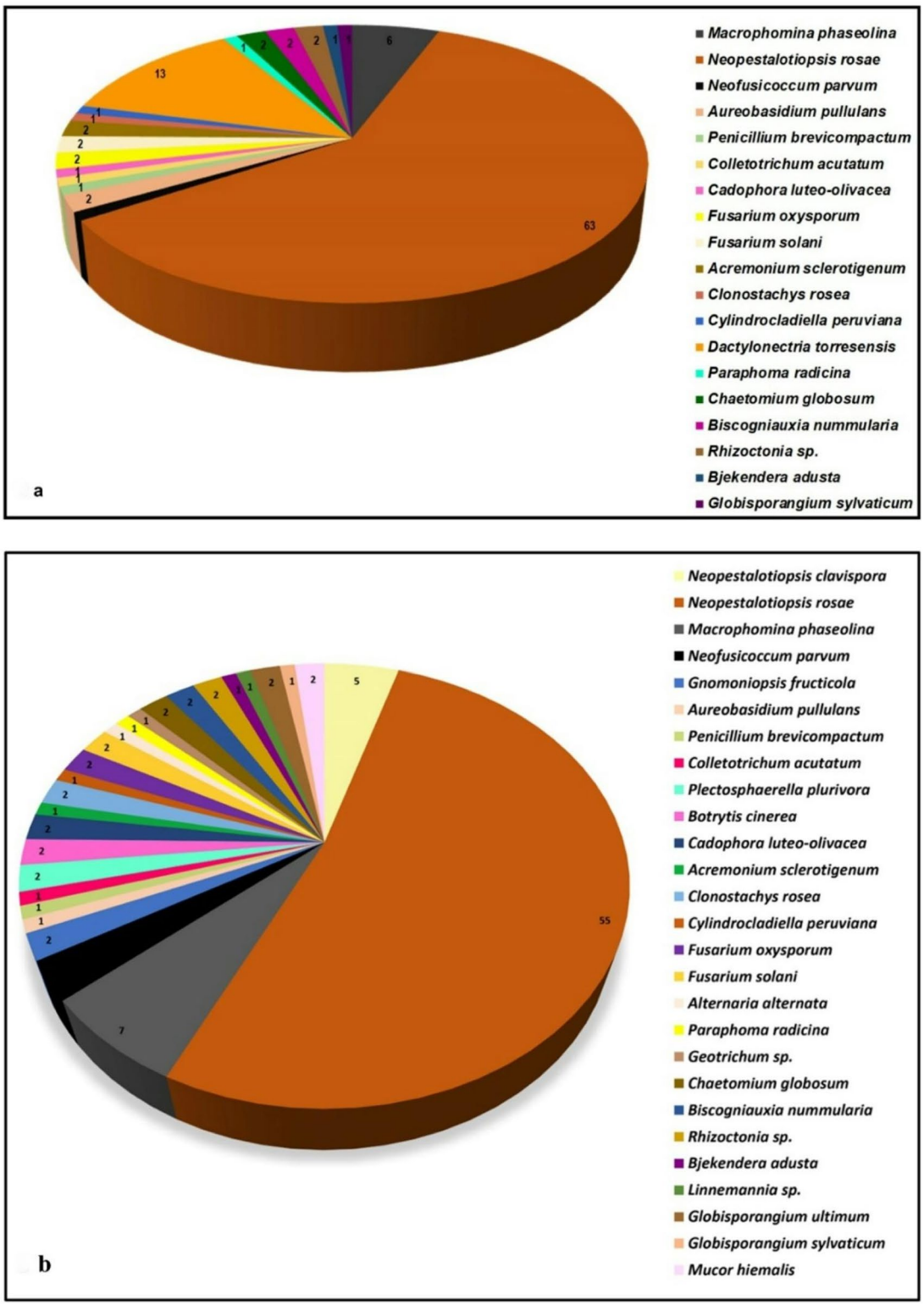

Fig. 2 Relative abundance (RA %) of fungal species isolated from decline-affected strawberry roots of Marimbella var. . (**a**) and Sabrosa Candonga® cv. (**b**)

Table 1 Details about the fungal taxa associated with strawberry decline identified in this study

No.	Species	Isolate	Geographical origin	GenBank accession numbers ITS	tub2
1	*Fusarium oxysporum*	Fus-ox1	Italy (Caserta)	PV594412	PZ602942
2	*Fusarium oxysporum*	Fus-ox2	Italy (Caserta)	PV594413	PZ602943
3	*Fusarium oxysporum*	Fus-ox3	Italy (Eboli)	PV594414	PZ602944
4	*Fusarium solani*	Fus-sol4	Italy (Caserta)	PV594415	PZ602945
5	*Fusarium solani*	Fus-sol5	Italy (Eboli)	PV594416	PZ602946
6	*Biscogniauxia nummularia*	Biscg-num6	Italy (Caserta)	PV594417	-
7	*Biscogniauxia nummularia*	Biscg-num7	Italy (Caserta)	PV594418	-
8	*Biscogniauxia nummularia*	Biscg-num8	Italy (Eboli)	PV594419	-
9	*Cylindrocarpon peruvianum*	Cylnd-peruv9	Italy (Eboli)	PV594420	PZ602947
10	*Chaetomium globosum*	Cht-glob10	Italy (Caserta)	PV594421	PZ602948
11	*Chaetomium globosum*	Cht-glob11	Italy (Caserta)	PV594422	PZ602949
12	*Chaetomium globosum*	Cht-glob12	Italy (Eboli)	PV594423	PZ662943
13	*Cadophora luteo-olivacea*	Cadph-lut-olv13	Italy (Eboli)	PV594424	-
14	*Macrophomina phaseolina*	Macr-phas14	Italy (Caserta)	PV594425	PZ602950
15	*Macrophomina phaseolina*	Macr-phas15	Italy (Caserta)	PV594426	PZ602951
16	*Macrophomina phaseolina*	Macr-phas16	Italy (Eboli)	PV594427	PZ602952
17	*Macrophomina phaseolina*	Macr-phas17	Italy (Eboli)	PV594428	PZ602953
18	*Neopestalotiopsis rosae*	Neop-ros18	Italy (Caserta)	PV594429	PZ622911
19	*Neopestalotiopsis rosae*	Neop-ros19	Italy (Caserta)	PV594430	PZ622912
20	*Neopestalotiopsis rosae*	Neop-ros20	Italy (Eboli)	PV594431	PZ622913
21	*Neopestalotiopsis rosae*	Neop-ros21	Italy (Eboli)	PV594432	PZ622914
22	*Neopestalotiopsis rosae*	Neop-ros22	Italy (Eboli)	PV594433	PZ622915
23	*Neopestalotiopsis clavispora*	Neop-clavsp23	Italy (Caserta)	PV594434	PZ622916
24	*Neopestalotiopsis clavispora*	Neop-clavsp24	Italy (Caserta)	PV594435	PZ622917
25	*Neofusiccocum parvum*	Neofus-parv25	Italy (Caserta)	PV594436	PZ622918
26	*Neofusicoccum parvum*	Neofus-parv26	Italy (Eboli)	PV594437	PZ622919
27	*Rhizoctonia* sp.	Rhiz27	Italy (Caserta)	PV594438	-
28	*Rhizoctonia* sp.	Rhiz28	Italy (Eboli)	PV594439	-
29	*Alternaria alternata*	Alt29	Italy (Caserta)	PV594440	PZ554485
30	*Alternaria alternata*	Alt30	Italy (Caserta)	PV594441	PZ554486
31	*Clonostachys rosea*	Clonst-ros31	Italy (Eboli)	PV594442	-
32	*Paraphoma radicina*	Parphom32	Italy (Caserta)	PV594443	-
33	*Colletotrichum acutatum*	Colle33	Italy (Eboli)	PV594444	PZ662937
34	*Aureobasidium pullulans*	Aurb-pulns34	Italy (Caserta)	PV594445	PZ662938
35	*Acremonium* sp.	Acrem35	Italy (Caserta)	PV594446	PZ662939
36	*Acremonium* sp.	Acrem36	Italy (Eboli)	PV594447	PZ662940
37	*Plectosphaerella plurivora*	Plect-plurv37_1	Italy (Caserta)	PV594448	PZ638812
38	*Botrytis cinerea*	Botr-cin38	Italy (Caserta)	PV594449	PZ662941
39	*Penicillium brevicompactum*	Penic39	Italy (Eboli)	PV594450	PZ528908
40	*Bjekendera adusta*	Bjerk-adst40	Italy (Eboli)	PV594451	-
41	*Linnemania* sp.	Linnem41	Italy (Caserta)	PV594452	-
42	*Mucor hiemalis*	Mucor42	Italy (Caserta)	PV594453	-
43	*Geotrichum* sp.	Geotric43	Italy (Caserta)	PV594454	-
44	*Globisporangium ultimum*	Glob-ult44	Italy (Caserta)	PV594455	-
45	*Globisporangium sylvaticum*	Glob-sylv45	Italy (Eboli)	PV594456	-
46	*Dactylonectria torresensis*	Dactyl-torr46	Italy (Eboli)	PV594457	PZ662942
47	*Gnomoniopsis fragariae*	Gnom-fragr47	Italy (Caserta)	PV594458	-

environmental or cultivation factors. In the present study, two strawberry varieties affected by decline and organically grown in two locations in Campania (southern Italy) were examined, aiming to investigate the fungi involved in the decline syndrome in order to make a precise diagnosis and uncover possible ways to prevent or control it. Field investigations performed in Eboli and Caserta organic strawberry cultivations allowed the collection of decline-symptomatic root samples from both varieties. Different pure fungal isolates were obtained and mainly grouped as similar to

Fig. 3 Maximum Likelihood (ML) phylogenetic trees of fungi isolated from strawberry associated with decline based on ITS region: **A)** Ascomycota taxa; **B)** Basidiomycota taxa; **C)** Mucormycota taxa; **D)** Oomycota taxa. Trees with the highest log likelihoods (-6490.07; -1420.09; -1260.58; -1790.30) are shown, and drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Meaningful branch support values (bootstrap) > 70% are indicated above branches. Lack of support indicates the branch was not supported by values higher than 70%. The evolutionary distances were computed using the Maximum Likelihood method and are in units of the number of base substitutions per site. Fungal isolates obtained in this study are marked with red dots, while the outgroups are marked with blue squares. The following type strains were used as outgroups: *Mucor hiemalis* CBS 201.65 (for Ascomycota taxa); *Schizophyllum commune* CBS 124811 (for *Basidiomycota*); *Rhizophagus irregularis* DAOM 234180 (for Mucormycota taxa); *Phytopythium littorale* CBS 118360 (for Oomycota taxa)

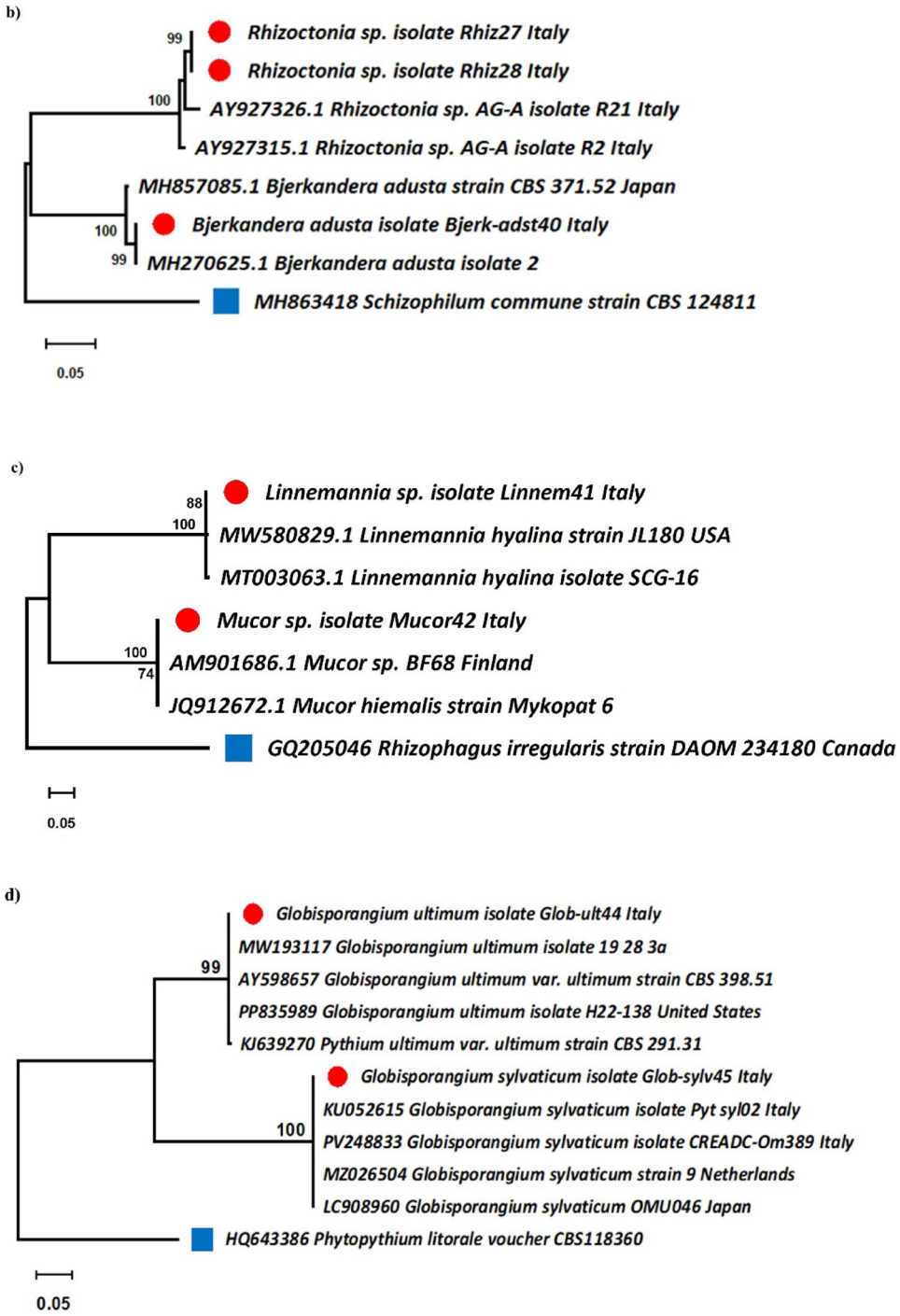

Fig. 3 (continued)

Neopestalotiopsis, *Dactylonectria*, and *Macrophomina* genera based on morphological features provided by Maharachikumbura et al. 2012, 2014; Lombard et al. 2014; and Viejobueno et al. 2022. The other 23 less abundant fungal genera were also morphologically identified following appropriate keys (among the authors, we cite Crous et al. 2021; Carlucci et al. 2012; and Damm et al. 2012). Since morphology only provided limited information for a precise fungal species identification, molecular investigations based on two markers (ITS and *tub2*) sequencing and phylogenies were used to identify the selected isolates at the species level. Thirty fungal species were identified in association with the decline in organic cultivations of strawberries in the Campania region . The emergent pathogens *N. rosae* and

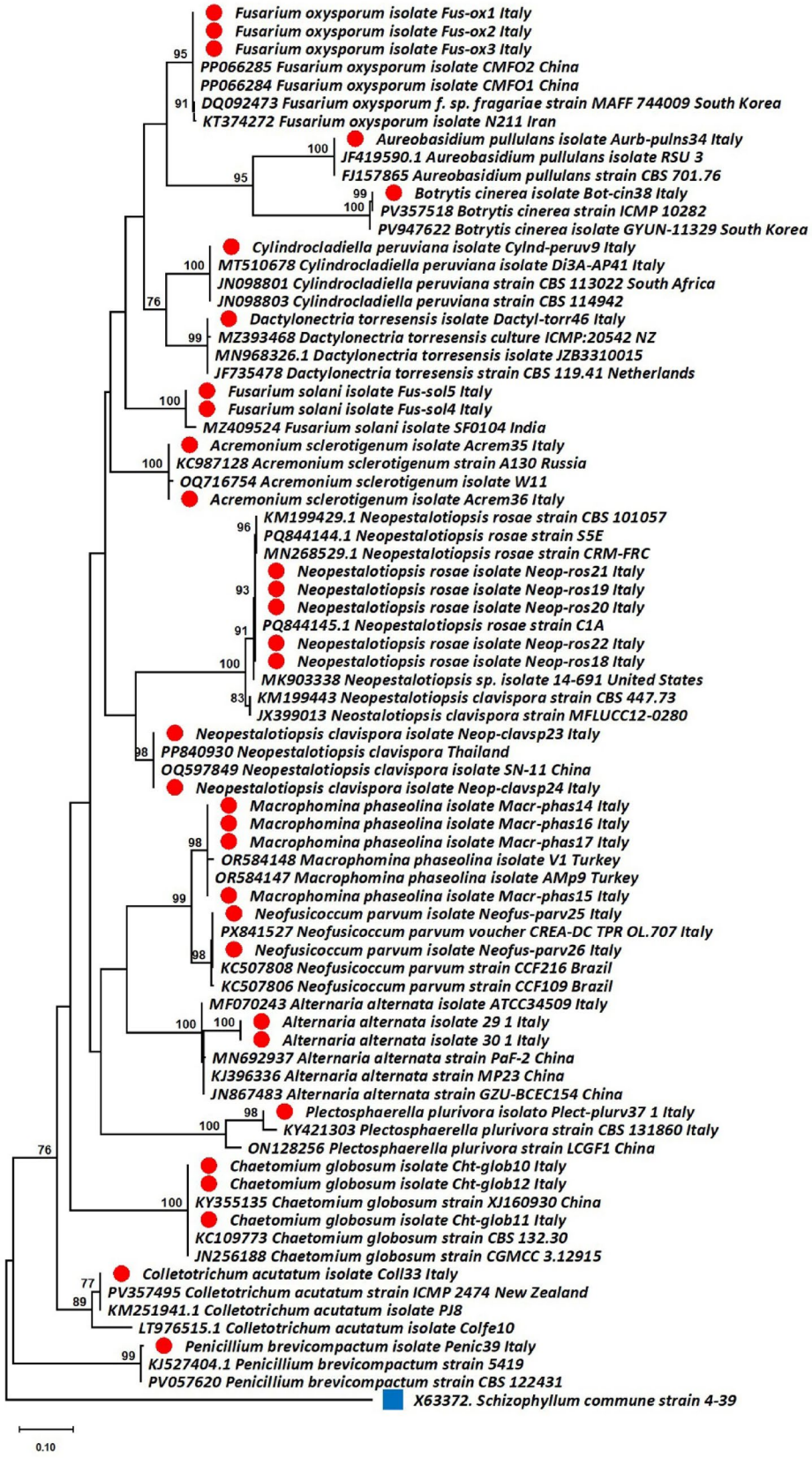

Fig. 4 Maximum likelihood (ML) phylogenetic tree of fungal taxa based on *tub2* nucleotide sequences. The tree with the highest log likelihood (-3.771.24) is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2+G+I parameter model and are in units of the number of base substitutions per site. Fungal isolates obtained in this study are marked with red dots. The strain 4–39 of *Schizophyllum commune* was used as an outgroup (blue square)

N. clavispora most frequently isolated in this study and have recently been reported to cause epidemic outbreaks on strawberries in many countries (Sigillo et al. 2020; Baggio et al. 2021; Salvas et al. 2024; Mang et al. 2025), raising concerns for the local growers from the region due to their severe impact on plant health and yield, and since their control remains difficult. In particular, *N. rosae* was abundantly found (63% and 55% RA) in both varieties investigated while *N. clavispora* was discovered with less frequency (5% RA), but it was still more frequently isolated than almost all other species from the Sabrosa Candonga® cv. . Therefore, we can assume that *Neopestalotiospis* spp. can play an essential role along with abiotic factors in the complex decline syndrome observed on plants. The observed progressive symptoms noticed in fields mostly resembling *Neopestalotiopsis* species assaults, and *N. rosae* predominant presence let us once more connect these severe attacks to the above-described pathogens, and as already reported by Ávilla-Hernández et al. (2025), Avilés et al. (2024), and Baggio et al. (2021), the source of infection can be linked to infected runner plants, contaminated nursery tools being favoured by elevated humidity and warm temperature conditions. Species belonging to *the Dactylonectria* and *Ilyonectria* genera have been generally reported as weak pathogens, but they also include major species that are described as causal agents of the black foot disease of strawberries and raspberries (Weber and Entrop 2017). Furthermore, Cylindrocarpon-like fungi are implicated in strawberry yield decline, their presence being negatively correlated with plant growth (Weber and Entrop 2017; Soppelsa et al. 2021). In our study, *Dactylonectria torresensis* , here reported for the first time in Italy on strawberry, was only found in Marimbella var. but not with a high abundance (13% RA). Nevertheless, due to its pathogenic nature, the evolution of this fungus should be observed over the years, and its exact role in the complex strawberry decline syndrome observed in strawberries in southern Italy needs further investigation. Another relevant pathogen, *M. phaseolina*, a common soil-borne fungus, was found in this study at 13% (RA), which is still relatively high compared to other fungi discovered, and many studies have linked this pathogen to charcoal rot of strawberry, triggering high economic losses worldwide (Pastrana et al. 2023). In Italy, *M. phaseoliona* was first reported in 2017 in three fields with cv. Mellissa (from Basilicata) causes crown and root rot on strawberry (Gerin et al. 2018) and generally does not cause visible symptoms on fruits, but the overall yields are still decreased, especially when optimal temperatures of about 25–30 °C and low moisture around 60% for pathogen development are recorded. Less abundant in both cultivars investigated were *G. sylvaticum* and *G. ultimum*, and thus, their primary role in SDS could be ruled out. Nevertheless, *G. ultimum* was reported to be highly virulent to strawberry cultivars (Pánek and Střížková 2021), and recently, *G. sylvaticum* caused black root rot on strawberry in the USA (Shrestha et al. 2023). Similarly, *F. solani* (von Martius) Saccardo and *F. oxysporum* registered lower frequencies in our study, but their presence could contribute to aggravating the SDS due to their severe pathogenic nature and the discovery of novel resistance-breaking strains (Henry et al. 2017). *Rhizoctonia* sp., *C. acutatum*, *(A) alternata*, *P. brevicompactum*, and *(B) cinerea*, also rarely found in our study, can cause relevant diseases in strawberry, leading to production decreases of up to 30% (Fang et al. 2023). In addition, another rarely found fungal species from the present investigation, *Chaetomium globosum*, was reported to be linked to root and crown rot on strawberry in Pakistan (Alam et al. 2017). *A. sclerotigenum*, found in the present study, is not a strawberry pathogen so far reported in Italy; still, its relative *(A) strictum* was reported to cause disease in strawberry in Argentina, inducing leaf necrosis symptoms but no crown rot even in the later stage (Racedo et al. 2013). *Plectosphaerella plurivora* , not yet reported on strawberry in Italy, but found to be associated with root and collar rot in horticultural crops (Carlucci et al. 2012), was found in our study also with a very low frequency (<2%). However, this fungal pathogen should be kept under observation as it may generate outbreaks similar to what was recently reported in strawberries in Korea, where it was demonstrated to be pathogenic on strawberry and associated with crown and root rot (Hassan and Chang 2022). Another rarely isolated species, *G. fragariae*, was reported for the first time on strawberry in Italy in 2022, causing leaf spots on cv. Elodi and the effects of this disease on yields could be very significant (Pugliese et al. 2023). The same pathogen was also recently reported in strawberries in the United States and Australia (Farr and Rossman 2023). Some other fungal species, like *Penicillium brevicompactum*, *Acremonium sclerotigenum*, and *F. oxysporum*, found in this study, may have a neutral behaviour in the decline syndrome found in organically cultivated strawberries in the Campania region. Instead, a few interesting taxa, *Bjerkandera adusta*, *Clonostachys rosea*, and *Aureobasidium pullulans*, have been reported as having beneficial effects on strawberry plants (Sun et al. 2020) and could be further investigated and used as biocontrol agents against the detected phytopathogens. Organic strawberry consumption is in high demand worldwide. However, as for other crops, a restricted use of agrochemicals for plant health control is required in organic strawberry farming. In the search for low-risk solutions for sustainable plant health protection in organic strawberry farming, studies on microbial diversity, like those focusing on the detection of taxa associated with root diseases, can furnish relevant information serving to protect crop health and assure yield security. In addition,

future studies focusing on pathogenicity assays conducted on more strawberry cultivars (including those grown locally) will be planned. In particular, the resistance of different strawberry cultivars against the relevant fungal pathogens described in this study, along with others, will be evaluated. Furthermore, recent studies conducted in our laboratory in order to find green alternatives to synthetic fungicides for the phytopathogens control allowed us to isolate and characterize diverse *Bacillus* species from cultivated soil. Notably, a few *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus velezensis* isolates were selected through *in vitro* screening for their strong antifungal activity against several key phytopathogens (comprising some of the fungal pathogens reported in the present work) of some agricultural and forestry plants (unpublished data). Therefore, they seem like promising candidates for future biocontrol studies of strawberry fungal pathogens.

Conclusion

The present study investigated for the first time the fungi associated with strawberry decline on Marimbella var. and Sabrosa Candonga® cv. of strawberry from organic cultivation in southern Italy. We found that Sabrosa Candonga® cv. had a higher fungal taxa diversity in the decline syndrome-affected roots compared to Marimbella var. Furthermore, the relative abundances of fungal groups associated with decline symptoms were significantly higher in Marimbella var. compared with those found in the Sabrosa Candonga® cv. samples. Furthermore, this study evidenced that many potential fungal pathogens, along with beneficial fungi, were more abundant in Marimbella var. than in Sabrosa Candonga® cv. of strawberry. All fungal isolates obtained here and long-term conserved are available for future bioactive molecules identification, and a few can be potential candidates for biocontrol assays against the strawberry phytopathogens. Detection of destructive pathogens such as *Neopestalotiopsis* spp., *M. phaseolina*, *D. torresensis* and *P. plurivora* confirms the complex nature of the SDS in strawberry involving multiple biotic factors, often acting synergistically, which stress the necessity for more integrated and updated defence management strategies.

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Author contributions Stefania Mirela Mang, Ippolito Camele and Carmine Marcone were involved in the conceptualization of the manuscript; Stefania Mirela Mang, Mihnea Ioan Cezar Ciocîrlan, and Carmine Palmieri carried out the pathogen isolation and morphological analysis work; Stefania Mirela Mang performed the molecular biological laboratory work and evaluated molecular biological information; Carmine Palmieri and Alberto Sellitto assisted in pathogens identification; Stefania Mirela Mang, Mihnea Ioan Cezar Ciorcîlan and Carmine Palmieri contributed to manuscript compilation; Stefania Mirela Mang, Carmine Palmieri, Mihnea Ioan Cezar Ciorcîlan, Alberto Sellitto, Ippolito Camele and Carmine Marcone were involved in the writing and editing the manuscript; Stefania Mirela Mang, Ippolito Camele and Carmine Marcone revised the manuscript; Carmine Marcone provided funds for the present study. All authors read and approved the final version of the manuscript.

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Data availability All nucleotide sequences obtained in this study have been deposited in GenBank under the following accession numbers: PV594412-PV594458 for ITS and PZ528908; PZ554485-86; PZ602942-PZ602953; PZ622911-PZ622919; PZ662937-PZ662943 for *tub2*.

Declarations

Conflict of interest Stefania Mirela Mang, Carmine Palmieri, Ippolito Camele, Alberto Sellitto, Mihnea Ioan Cezar Ciorcirlan and Carmine Marcone declare here that they have no competing interests.

Ethical approval No experiments have been conducted on animals or humans in this study.

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