Suillus: an emerging model for the study of ectomycorrhizal ecology and evolution

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Summary

Research on mycorrhizal symbiosis has been slowed by a lack of established study systems. To address this challenge, we have been developing *Suillus*, a widespread ecologically and economically relevant fungal genus primarily associated with the plant family Pinaceae, into a model system for studying ectomycorrhizal associations. Over the last decade, we have compiled extensive genomic resources, culture libraries, a phenotype database, and protocols for manipulating *Suillus* fungi with and without their tree partners. Our efforts have already resulted in a large number of publicly available genomes, transcriptomes, and respective annotations, as well as advances in our understanding of mycorrhizal partner specificity and host communication, fungal and plant nutrition, environmental adaptation, soil nutrient cycling, interspecific competition, and biological invasions. Here, we highlight the most significant recent findings enabled by *Suillus*, present a suite of protocols for working with the genus, and discuss how *Suillus* is emerging as an important model to elucidate the ecology and evolution of ectomycorrhizal interactions.

I. Introduction

The ectomycorrhizal (ECM) symbiosis formed between plant roots and soil fungi is characterized by the obligate exchange of plant derived carbohydrates for fungal scavenged nutrients (Smith & Read, 2010). These cross-kingdom mutualisms provide both direct and indirect impacts on plant growth, ranging from increased mineral nutrient access to improved abiotic and biotic stress tolerance (Smith & Read, 2010; Branco *et al.*, 2022). These benefits have long been recognized as critical to the health of both natural and managed forest systems, with ECM fungal inoculation regularly employed by the nursery and forestry industries to enhance and protect seedling growth (Torres & Honrubia, 1994; Cripps & R., 2011; Mateos *et al.*, 2017). ECM fungi also offer a multitude of ecosystem services, playing fundamental roles in the functioning and maintenance of community composition, water relations, and global carbon and nutrient cycling (Smith & Read, 2010; Branco *et al.*, 2022). In recent years, these attributes have attracted increased interest from diverse fields including ecosystem ecology, evolutionary biology, biogeochemistry, global change biology, and sustainability studies.

Despite the recognition of their importance and the growing interest in ECM fungi, there are still significant gaps in our understanding of how ECM fungi function and interact with their plant partners, microbial communities, and environments. The cryptic belowground nature of ECM fungi, coupled to high species diversity, variable tractability under laboratory conditions, lack of standardized protocols, as well as technical challenges associated with the isolation, sequencing, and manipulation of host-associated dikaryotic species, have hindered the advancement of the field. Many questions remain unanswered, including how ECM fungi evolve at different biological scales, the mechanisms facilitating interactions between ECM fungi and their hosts, and how ECM communities will be affected by environmental change (Plett *et al.*, 2024; Dauphin & Peter, 2024).

While recent technological advances, such as the ability to generate and analyze extensive genomic datasets, promise to improve our ability to tackle some of the aforementioned questions, our understanding of ECM mutualism remains limited due a scarcity of well-developed model systems. Establishing ECM models that are diverse, ecologically relevant, and amenable to experimentation and *in vitro* manipulation is critical to advancing mycorrhizal research. The establishment of models allows the scientific community to build capacity around common systems, increasing the breadth and utility of core methodologies, improving reproducibility by using a common set of strains and procedures, and creating discourse and momentum around novel findings and approaches.

Model systems are often defined at the species level, with each system offering unique advantages and limitations. Mycologists have built capacity around the development of systems such as *Coprinopsis cinerea* for studying fruit body development (Kamada *et al.*, 2010; Plaza *et al.*, 2014), *Schizosaccharomyces pombe* for probing fundamental molecular and cellular biology (Hoffman *et al.*, 2015), *Candida albicans* for investigating human fungal pathogenesis (Kabir *et al.*, 2012), *Neurospora crassa* for understanding gene silencing and circadian rhythms (Selker *et al.*, 1987; Roche *et al.*, 2014), and *Saccharomyces cerevisiae* for elucidating biological processes from neurodegenerative disorders to biological aging (Miller-Fleming *et al.*, 2008; Murakami & Kaeberlein, 2009; Karathia *et al.*, 2011). The development of these systems has resulted in the creation of numerous tools, and invaluable insights into eukaryotic biology. The concerted effort towards developing ECM model systems for fungal ecological and evolutionary research will foster rapid tool development and the critical mass necessary to cultivate effective collaborations and propel the field of mycorrhizal research into the future.

Several ECM models are in development including *Laccaria bicolor*, *Hebeloma cylindrosporum*, and *Pisolithus tinctorius*. These species have produced fundamental insights into the ecological, physiological, and molecular mechanisms of ECM-host interactions (Cairney & Chambers, 1997; Marmeisse *et al.*, 2004; Martin *et al.*, 2008). To date, these taxa have been primarily used as species-level models, but are rapidly accumulating genomic resources at the genus level. As of October 2023, MycoCosm web portal (https://mycocosm.jgi.doe.gov/) now includes two species of *Laccaria* (including 15 genomes of *L. bicolor*), two species of *Hebeloma*, and 10 species of *Pisolithus*. Other ECM fungal genera quickly accumulating sequenced genomes include *Tuber* (10 species) and *Lactarius* (12 species). The choice of which ECM lineages should be targeted for model development should take into consideration diversity and tractability, trait variation, reproducibility, accessibility and availability, ecological relevance, conservation/invasion status, applicability and extensibility to other systems, as well as community interest and momentum. Fungi in the genus *Suillus* fulfill of many of these considerations, including laboratory and field tractability, high ecological relevance, species and trait diversity, accessibility and abundance, and an engaged and rapidly growing research community. To this end, we have been developing *Suillus* into a genus-level system to characterize the ecology and evolution of ECM fungal symbioses.

Suillus is a speciose genus with most species displaying strong partner specificity with members of the plant family Pinaceae (Kretzer *et al.*, 1996; Nguyen *et al.*, 2016b). *Suillus* produces abundant fruitbodies, and are some of the most frequently encountered ECM mushrooms in northern temperate and boreal forest systems. The genus includes pioneer species that are instrumental for forest establishment across both natural and managed systems, where they have facilitated both the success of forestry plantations, and extensive ecological damage as invasive host-symbiont pairs encroach into introduced ranges and disrupt local communities (Policelli *et al.*, 2019). In addition, the genus includes both stress-tolerant and stress-sensitive species, enabling investigation into environmental adaptation of both ECM fungi and their associated plant partners (Colpaert *et al.*, 2011; Bazzicalupo *et al.*, 2020; Zhang *et al.*, 2021). Finally, relative to other ECM fungi, *Suillus* is highly tractable to laboratory manipulation, including but not limited to, the ability to be isolated axenically and grown apart from their hosts with specific nutritional supplementation, long term *in vitro* culture viability, and the ability to colonize host plants via both long-lived reactive spores or mycelial extension (Nguyen *et al.*, 2012).

Here, we present *Suillus* as an emerging model genus for ECM fungal ecology and evolution. Our goals are 1) to provide a primer on *Suillus* that synthesizes our current understanding of the biology and eco-evolutionary theory relevant to this unique genus, and 2) to standardize and disseminate the protocols developed to study *Suillus* both in the field and the laboratory. This primer is not intended to be systematic literature review, but rather a broad introduction to the genus and its potential to serve as a model system. Specifically, we introduce the vision and goals of the International *Suillus* Consortium (a working group of over 20 independent laboratories collectively dedicated to the development of the *Suillus* system, http://www2.hawaii.edu/~nn33/suillus), consolidate the latest findings in *Suillus* biology, ecology, and evolution, and compile molecular and experimental protocols. Sections II-VII cover the development of *Suillus* as a model genus, its taxonomy and evolution, significant ecological interactions, abiotic environmental adaptations, the biology and implications of introductions and invasions, and a look forward at outstanding questions, current challenges, and concentrations areas for system development. Section VIII outlines a series of protocols for working with the genus which we have included as a series of standalone supplementary documents.

II. *Suillus* as a model genus

II.i: The power of genus-level model systems

Unlike model systems that are based on individual species, the strength of the *Suillus* system lies at the genus level. There are multiple benefits to leveraging genus-level systems. First, they provide the biological and ecological diversity needed to ask questions and test hypotheses across broad scales, from the individual to the ecosystem level. Second, they offer a comparative framework that allows researchers to investigate eco-evolutionary questions about speciation, adaptation, and how closely related taxa respond to environmental perturbation. Third, they allow the generalization of research findings beyond individual species to identify commonalities, trends, and general biological principles that apply to close relatives as well as other organisms occupying the same guild, lifestyle or ecological niche. Finally, there are experimental and research community advantages, such as the development of shared tools that can be used by multiple research groups with minimal protocol optimization. Having shared genomic and computational resources also allows different research teams to tackle diverse questions and minimize experimental overlap while increasing collaborative opportunities. The benefits of using model clades (at the genus level or above) for ecological and evolutionary research are well recognized in other systems, driving the development of model genera such as the bivalve *Mytilus* for ecotoxicology and climate change research (Leopold *et al.*, 2019; Ribeiro *et al.*, 2019), *Mimulus* plants for studying adaptation and speciation (Wu *et al.*, 2008; Twyford *et al.*, 2015) and *Anolis* lizards for studying adaptive radiation and ecomorphology (Sanger & Kircher, 2017).

II.ii: *Suillus* is an excellent model for ECM ecology and evolution

The genus *Suillus* is diverse (Fig. 1) and displays a range of host associations and a gradient of partner specificity responses, possesses unique traits with high ecological relevance, and is both accessible and tractable, making it an ideal model for studying ECM ecology and evolution. Generally, members of the genus have strong partner specificity with the plants in the family Pinaceae (Kretzer *et al.*, 1996), making it a favorite target of fungal partner specificity research. In natural environments *Suillus* typically associates with three of the four subfamilies of the Pinaceae (Pinoideae, Laricoieae, and Piceoideae), allowing researchers to study host-symbiont associations across different taxonomic levels, including among host genera, subgenera, and species. *Suillus* also facilitates the study of host switching (Lofgren *et al.*, 2021; Zhang *et al.*, 2022), both between typical hosts and outside of these host groups, thanks to several species that defy the canonical partner specificity patterns demonstrated by most species (Lofgren *et al.*, 2018; Pérez-Pazos *et al.*, 2021).

Suillus contains species that demonstrate a variety of unique ecological attributes: they occur as pioneers (Peay *et al.*, 2012) and as introduced species (Thompson *et al.*, 2022), demonstrate variable stress tolerance (Branco *et al.*, 2022; Erlandson *et al.*, 2022), and associate with hosts across different life stages, ranging from seedlings to mature forests (Rineau *et al.*, 2016). *Suillus* serve as effective symbionts that assist in host seedling establishment and development (Jenkins *et al.*, 2018). Further, *Suillus* can act as hosts themselves for a diversity of fungi and bacteria, including related fungi in the genera *Gomphidius* and *Chroogomphus* (Olsson *et al.*, 2000) (Fig. 1 O-P) and bacteria that both associate with living hyphae and mycorrhizas (Izumi *et al.*, 2006; Timonen & Hurek, 2011), and actively decompose dead mycelia (Maillard *et al.*, 2022, 2023). Together, these traits offer exciting opportunities to help solve a broad range of ecological and evolutionary questions.

Suillus is also an accessible and tractable genus that is amenable for study by a diverse array of research specialties. Species in the genus tend to fruit abundantly, are commonly encountered, and are relatively easy to identify in the field. While many ECM taxa are difficult or impossible to culture independently from their hosts (e.g., *Cortinarius*, *Russula*, *Lactarius*, *Tomentella*) (Palmer, 1971; Brundrett *et al.*, 1996; Nygren *et al.*, 2007), obtaining *in vitro* axenic cultures of *Suillus* is a straightforward process across the genus. Cultures of *Suillus* can be grown on solid media, in liquid culture, or somewhere in between (for example, on glass beads partially submerged in liquid media) and are amenable to long-term storage. *Suillus* mushrooms produce ample long-lived spores, and mycorrhization can be carried out rapidly (in as little as one month) using either spore-based or mycelial-based inoculation, providing flexibility for either high-throughput colonization or the ability to control the provenance of specific strains. The ability to generate mycelial biomass quickly in culture allows for manipulative studies (such as nutrient use and stress tolerance) either alone, with a plant partner, or with third-party community members such as other fungi or bacteria. *Suillus* species have relatively small genomes (~50 Mbp) that are comparatively easy to sequence and assemble. Through collaborations with the Joint Genome Institute, the International *Suillus* Consortium research teams have sequenced and assembled 46 draft genomes to date, spaning the taxonomic diversity of the genus. Sequencing efforts have also resulted in over 300 hundred shallow-depth genomes for particular species of interest such as *S. luteus*, *S. brevipes*, and *S. salmonicolor* (*cothurnatus*). The depth and breadth of sequencing across the genus provides genomic tools for the community to ask questions that can span the spectrum of systematics, ecology, and evolution. These traits have enabled the development of many *Suillus* optimized protocols (outlined in section VIII) and make the genus ideal for the development of manipulative and -omics based approaches, such as functional-omics (both *in vitro* and *in situ*), and gene editing (discussed in section VII).

II.iii: The SuilluScope database

While genus-level models are powerful systems for addressing questions that span ecological gradients, linking response variables back to genomic variability remains a grand challenge for most biological systems. Conducting genotype-to-phenotype analysis would be greatly aided by coupling genomic resources to the generation of diverse, reproducible, phenotypic data, and the broad availability of this data to the community. The *Suillus* Genome Strain Culture Collection constitutes a living library of fungal isolates used for whole-genome sequencing projects. To help the community take full advantage of both the genomic resources available for *Suillus*, and the phenotypic and genotypic diversity across the genus, we present a new interactive *Suillus* database, SuilluScope (v1.0Beta) available at www.SuilluScope.com. The initial release of the database contains phenotype information on growth rate, optimal growth temperatures, and culture images on multiple media types for isolates in the *Suillus* Genome Strain Culture Collection. The goal of the database is to act as a centralized and accessible community repository and analysis platform for phenotype assays conducted on the same set of genome-sequenced strains. This resource will allow the community to identify optimal growth conditions for specific strains, and predict and interpret response variables across the genus for downstream analysis. Subsequent releases will include additional phenotypic variables, and new genome strains will be added as they become available. The *Suillus* Genome Strain Culture Collection has been integrated into the USDA ARS NRRL culture collection and all strains are available to the community without restriction. Strain accession numbers and detailed isolate information are available as a living document in the SuilluScope database, and will be updated as new assays are conducted, and new genome strains are integrated into the collection.

One example currently offered by the database is the selection of optimal growth conditions for specific species and strains. Many species of *Suillus* exhibit symptoms of stress on rich media including slower growth and increased pigment production (Fig. 2 A-P). This is a particularly important consideration for preparing material for sequencing and molecular work, as pigmentation is often associated with decreased extraction efficiency. For this reason, rich media preparations such as Modified Melin-Norkrans (MMN) and Hagem's (see details in section VIII) are often prepared with a 50% reduction in carbon (glucose, malt extract, or yeast extract depending on the formulation, Supporting Information S3). However, total carbon content is not the only determinant of pigment production, and individual species (or strains) often display unique requirements for pigment reduction, such varying the availability of organic and inorganic nitrogen, media pH, or temperature. Media type has additional wide-ranging effects on culture phenotype, including growth rate, topology, and the production of aerial hypha (Fig. 2 A-H). Similarly, while most species of *Suillus* will sustain growth at temperatures between 10-30 °C, growth rates vary among *Suillus* species, and optimal growth temperatures for a given species can be identified using the database. For example, maximum growth rates peak between 19 and 33 days after media transfer and vary between 29mm^2/day (*S. spraguei* EM44) to 137mm^2/day (*S. quiescens* FC197) when grown at room temperature (Fig. 3).

III. Taxonomy and evolution

III.i: Taxonomy and species divergence

In 1821 S.F. Gray first proposed splitting *Suillus* from the genus *Boletus*, which Linnaeus had originally defined to comprise all pored fungi (Murrill, 1909). The genus *Suillus* was formally sanctioned by Elias Magnus Fries (1821-[1832]), but was not widely accepted until the 1950s (Dahlberg & Finlay, 1999). Perhaps due to the distinct morphological characters typically associated with *Suillus* mushrooms, the genus *Suillus* Gray has undergone surprisingly little revision over the years outside of new species additions and occasional species splitting, often associated with the recognition of taxa occurring in distinct geographic regions. To date, there are 112 species of *Suillus* recognized as valid in the Catalog of Life Database (https://www.catalogueoflife.org/), a number we expect to increase over the coming decade, particularly as new taxa are characterized from understudied areas such as Asia and the Indian subcontinent (Nguyen *et al.*, 2016b). The *Suillus* phylogeny is broadly divided into 3-5 major phylogenetic sections, and grouped into corresponding subgenera, including *Boletinus*, *Spectabilis*, *Larigini*, *Douglasii*, and *Suillus* (Zhang *et al.*, 2022). However, this intrageneric classification has not been formally recognized and the exact number of subgenera remains controversial.

Current classification schemes place *Suillus* in the order Boletales, in the suborder Suillineae, which contains the genera *Truncocollumella*, *Rhizopogon*, *Gomphidius*, *Chroogomphus*, and the monotypic genus *Psiloboletinus* (Besl & Bresinsky, 1997; Binder & Hibbett, 2006; Wu *et al.*, 2020). The Suillineae are colloquially referred to as suilloid fungi, but represent multiple families including the Suillaceae (*Suillus*, *Truncocolumella,* and *Psiloboletinus*), Rhizopogonaceae (*Rhizopogon*), and the Gomphidiaceae (*Gomphidius* and *Chroogomphus*). The Suillineae are thought to be the first clade in the Boletales to have independently evolved the ECM lifestyle (Sato & Toju, 2019), a trait consistent across all families in the Suillineae, except the Gomphidiaceae which contains at least some mycotrophic species (Fig. 1 O-P).

Suillus diverged from other suilloid fungi between 40.2 and 71.1 MYA (Lepage *et al.*, 1997; Zhang *et al.*, 2022) (Fig. 4). Within *Suillus*, the earliest diverging clades are associated with the hosts *Larix* and *Pseudotsuga*. The most significant diversification in the genus occurred during the switch onto *Pinus* and it is within this *Pinus*-associated lineage that we find evidence for the active divergence of species complexes. However, the pattern of host switching within *Pinus* (especially between the host subgenera *Pinus* and *Strobus*) is convoluted and not yet fully resolved (Zhang *et al.*, 2022). We find notable cases of ongoing evolution associated with host-switching across the genus, both in clades that are actively diverging and in those that appear relatively stable. For example, *S. brunnescens* diverged from within the apparently stable *S. luteus* clade, concurrently switching from subgenus *Pinus* to subgenus *Strobus*.

The three most notable actively diverging clades in *Suillus* are the /albivelatus, /flavidus and /placidus species complexes (Nguyen *et al.*, 2016b). Within these clades, similar morphological forms are often difficult to distinguish among the members within each complex. For example, the /albivelus clade contains a group of Western North American species (*S. albivelatus*, *S. pseudobrevipes*, *S. volcanalis*, and *S. watsatchicus*) that are difficult to tell apart morphologically. Likewise, the members of the large-pored species within the /flavidus clade (*S. umbonatus, S. megaporinus, S. flavidus,* and *S. helenae*) are morphologically similar. Conversely, the /placidus clade contains a group of species (*S. placidus, S. subalpinus, S. anomalus* and *S. punctatipes*) which, although morphologically distinct, are not (yet) supported as monophyletic group (Nguyen *et al.*, 2016b). Among these clades are species such as *S. punctatipes*, which has the ability to associate with alternative hosts such as *Picea* and *Abies* (Pérez-Pazos *et al.*, 2021), although it should be noted that species outside of these clades such as *S. glandulosus* and *S. subaureus* also have the ability to associate with alternative hosts. The active evolution within (and outside of) these species complexes provide novel information that can be leveraged to understand the molecular and evolutionary mechanisms of host association and host-switching among plant-associated fungi.

III.ii: Atypical morphological features

Secotioid and gasteroid fruit bodies of *Suillus*-like fungi have occasionally been observed and were once classified as belonging to the genus *Gastrosuillus*. However, *Gastrosuillus* was dissolved after molecular evidence showed that these fungi were polyphyletic in *Suillus* (Baura *et al.*, 1992; Kretzer & Bruns, 1997). Transitions in fruit body form can happen rapidly and are likely controlled by a small number of genes (Bruns *et al.*, 1989). It is currently unknown whether the examples of secotioid/gasteroid *Suillus* found to date represent ongoing speciation or variants of known species. Such variants could be the result of spontaneous genetic mutations, malformations resulting from environmental stress during development, or ecological plasticity in fruit body form in response to environmental conditions. These scenarios are not mutually exclusive, and it may well be the case that while some secotioid/gasteroid fruit bodies are aberrations (as is likely the case for the secotioid examples of *S. grevillei* (Kretzer & Bruns, 1997)), others have undergone speciation and are yet to be typified, or have previously been misclassified. This is likely the case for *Rhopalogaster* cf. *transversarius*, a morphologically distinct secotioid species currently considered a member of the *Rhizopogonaceae*, but likely to be reclassified in *Suillus*, after molecular studies have found it to be nested deeply in the genus (Smith *et al.*, 2018). As the rapid evolution of truffle-like basidiocarps is thought to be adaptive in low moisture availability (as seen in the sister genus *Rhizopogon*) (Bruns *et al.*, 1989), secotioid/gasteroid *Suillus* represent an intriguing system for future work given the ongoing interest into *Suillus* drought responses (Wang *et al.*, 2021; Erlandson *et al.*, 2022; Castaño *et al.*, 2023).

Another interesting morphological feature demonstrated by some species of *Suillus* and *Rhizopogon* is the formation of tubercles. These structures are characterized by dense clusters of mycorrhized root tips surrounded in a hyphal rind (Randall & Grand, 1985). The biological and ecological function of tubercles is poorly understood, but as they bear resemblance to the bacterial nodules formed by *Rhizobium* and *Bradyrhizobium* on legumes or *Frankia* on *Alnus*, it has long been thought that they may play a role in nitrogen fixation. Paul *et al.* (2007) used an acetylene reduction assay to assess potential N-fixation in *S. tomentosus* tubercles and found evidence of nitrogenase activity up to 25098·8 nmol C_2H_4 g^{-1} per tubercle over a 24 hour period, suggesting that the ability to form tubercles could play a significant role in N-fixation. Follow up work from the same group later identified multiple species of diazotrophic bacteria in association with the interior tissue of *S. tomentosus* tubercles (Paul *et al.*, 2012), as well as isotopic evidence for N-transfer to the host tree (Chapman & Paul, 2012). Future work is needed to assess the diversity and frequency of tuberculate forming suilloid fungi, better characterize the bacterial communities associated with these structures, and quantify the contribution of tubercle-associated nitrogenase activity to host nitrogen budgets.

III.iii: *Suillus* vs. *Rhizopogon*

The genus *Rhizopogon* is closely related to *Suillus* and shares many of the traits that make *Suillus* an attractive model for studying ecology and evolution. Like *Suillus*, *Rhizopogon* species are obligately ECM, easily isolated, and speciose, currently including 225 recognized species which are divided into approximately five subgenera (Grubisha *et al.*, 2002). *Rhizopogon* species exhibit high to moderate partner specificity and typically associate with host trees in the Pinaceae, primarily *Pinus* and *Pseudotsuga* (Molina *et al.*, 1997). Rarely, primary host associations have also been observed with *Larix* (Miyamoto *et al.*, 2019), and secondary colonization is occasionally reported on non-Pinaceae hosts such as *Arbutus* and *Arctostaphylos* (Massicotte *et al.*, 1994; Kennedy *et al.*, 2012). Unlike the complex history of host-switching and reversion observed in *Suillus*, partner specificity in *Rhizopogon* is primarily reflected by fungal subgeneric classification (e.g., the subgenus *Villosuli* associates exclusively with *Pseudotsuga*, whereas the subgenus *Amylopogon* has a more generalist host range (Grubisha *et al.*, 2002)).

Unlike *Suillus*, *Rhizopogon* exclusively forms truffle-like hypogeous fruitbodies, an adaptation which likely evolved from pileate-stipitate (mushroom) ancestors (Bruns *et al.*, 1989; Sánchez-García *et al.*, 2020). Like most hypogeus fungi, *Rhizopogon* spp. largely depend on animals excavate their fruit bodies and distribute their spores (Bradshaw *et al.*, 2022). The clear differentiation in fruit body forms between *Rhizopogon* and *Suillus* has been cited as evidence of strong selection against intermediate forms, putatively driven by differences in dispersal mechanisms (Bruns *et al.*, 1989). Like *Suillus*, *Rhizopogon* species form long-lived spore-banks in the soil (Shemesh *et al.*, 2023) and are often associated with early successional colonization, including seedlings and invasion fronts (Policelli *et al.*, 2019), but often demonstrate poor competitive ability outside of these scenarios (Bruns *et al.*, 2002).

The hypogeus and cryptic nature of *Rhizopogon* fruitbodies makes them more difficult to survey and sample compared to mushroom-forming fungi like *Suillus*. Similarly, quantifying the percent of individual root tips colonized by *Rhizopogon* for field or *in-vitro* assays can be challenging due to their typically coralloid morphology (Kennedy & Peay, 2007). For fruitbodies, morphological differentiation of *Rhizopogon* is notoriously complicated by a lack of variable diagnostic characters, which confound field identification, and contribute to the instability of species concepts developed in the pre-molecular era (Bidartondo & Bruns, 2002; Bubriski & Kennedy, 2014; Koukol *et al.*, 2022; Karlsen-Ayala *et al.*, 2022). Although *Rhizopogon* was long thought to be sister to *Suillus*, multiple molecular studies have identified fungi in the family Gomphidiaceae as more closely related to *Suillus* (Grubisha *et al.*, 2002; Wu *et al.*, 2020). However, given that the Gomphidiaceae are putatively nonmycorrhizal, and the Suillaceae genera *Truncocolumella* and *Psiloboletinus* while ECM, together contain only five recognized species (Wu *et al.*, 2020), these genera have a distinct, but limited, use as comparative genus-level systems. In contrast, work in *Rhizopogon* has contributed a rich independent body of research to our understanding of ECM ecology and evolution and is the most ecologically relevant genus for comparative studies with *Suillus*. In addition to many similarities, *Rhizopogon* also demonstrates traits which are unique from *Suillus*, including a high propensity to be parasitized by mycoheterotropc plants (Bidartondo & Bruns, 2002; Dowie *et al.*, 2012; Grubisha *et al.*, 2014), and the potential for not only heat tolerance, but growth stimulation in response to fire (Bruns *et al.*, 2019). As we continue to build *Suillus* into a model genus for ecology and evolution, the concurrent development of other groups with contrasting ecological and life history strategies, including but not limited to other suilloid fungi, will complement our understanding of the mechanisms and ecological drivers at play in ECM forest systems.

III.iv: Population genetics

Because genetic divergence can often be detected before morphological divergence, deep sampling of individual species can provide a wealth of information into microevolutionary processes. To this end, member labs of International *Suillus* Consortium have focused efforts on continental-to-global sampling of *S. luteus* (the type species of *Suillus*), as well as *S. brevipes*, *S. salmonicolor (cothurnatus)*, *S. pungens*, and *S. quiescens*. The deep sampling of these species across both native and introduced ranges has facilitated our ability to define population structure, characterize diversification events, and identify genetic bottlenecks associated with global introductions, as well as other microevolutionary processes that can drive divergence (Branco *et al.*, 2017; Pildain *et al.*, 2021; Ke *et al.*, 2023).

Population genetics, the study of intraspecific variation and the forces that result in evolutionary changes in species over time (Wills, 2007), is a critical tool for understanding fungal evolution. However, the cryptic nature of fungi makes population studies challenging. Specifically, accurately counting and characterizing fungal individuals, as well as delimiting and describing fungal populations and their respective allele pools is notably more complicated in fungi than for plants or animals. *Suillus* is no exception to this generalization, and technical challenges to studying microevolutionary changes have historically complicated our understanding of how populations are structured and distributed, the patterns and processes that drive gene flow, and how environmental change affects genetic diversity.

Despite these challenges, *Suillus* fungi have been targeted for population genetic analysis since the early 1990s, capitalizing on the new availability and affordability of DNA sequencing. These studies contributed key insights about genet size (Dahlberg & Stenlid, 1994; Bonello *et al.*, 1998; Zhou *et al.*, 1999), population genetic structure (Dahlberg & Stenlid, 1990; Muller *et al.*, 2007; Burchhardt *et al.*, 2011), and gene flow (Zhou *et al.*, 2001; Burchhardt *et al.*, 2011) but were limited in scope regarding the number of individuals, the number of populations, and the number of markers used for population differentiation. Recent population-level sequencing efforts have facilitated major contributions to the understanding of fungal population genetics, including key insights relevant to both *Suillus* evolution and to ECM fungal evolution in general. Many of these advances have been facilitated by the ability to sequence whole genomes and recent efforts to assemble and annotate high quality reference strains. For example, work on *S. brevipes* showed that the species is highly outcrossing and includes several distinct populations across North America that often display restricted gene flow at the regional scale (Branco *et al.*, 2015, 2017). These studies also revealed that climate and soil chemistry play important roles in *S. brevipes* population structure. In contrast, the European species *S. luteus* displays an absence of population structure at small spatial scales, including across highly contrasting environments (Bazzicalupo *et al.*, 2020).

III.v: Mating systems

Whether fungi produce progeny by selfing, inbreeding, or outcrossing impacts the evolutionary trajectory of species by influencing genetic diversity, deviation from Hardy–Weinberg equilibrium, and the relative level of linkage disequilibrium (Nieuwenhuis *et al.*, 2013). The breeding systems of basidiomycetes are categorized based on the number of loci controlling mating type, where two functional MAT loci are present in tetrapolar breeding systems, one functional MAT locus is present in bipolar breeding systems, and a lack of any self-incompatibility loci results in homothallic (self-fertile) breeding systems (Nieuwenhuis *et al.*, 2013). Generally, basidiomycete mating compatibility and mating types are governed by two self-incompatibility loci, the HD MAT locus and P/R MAT locus (Heitman *et al.*, 2013). Two or more self-incompatible allele types at each locus dictate the mating types and the mating compatibility between gametes (Wang & Mitchell-Olds, 2017). Genomic analysis has shown that in *Suillus*, the P/R MAT locus contains three pheromone receptors and three or more pheromone precursors (Mujic *et al.*, 2017) and the HD MAT locus contains a pair of homeodomain proteins flanked by genes encoding mitochondrial intermediate peptidase (MIP), as well as a gene known as the ‘beta-flanking gene’; a syntenic arrangement that appears to be conserved across most basidiomycetes (Ke *et al.*, 2023).

In *Suillus*, breeding systems can be studied by both genomic methods and by constructing crosses. Crossing experiments require monokaryotic strains, most frequently produced via the germination of basidiospores (discussed in section VIII). Crossing experiments in *Suillus* indicate that *S. luteus*, *S. variegatus*, *S. granulatus*, and *S. bovinus* all have bipolar, multi-allelic mating systems (Fries & Neumann, 1990; Fries & Sun, 1992; Fries, 1994). In these species, incompatibility between individuals is rare, implying that there are a high number of mating types maintained at the population level. The highest diversity of mating types recorded to date are in the species *S. luteus* where eight mating types were recovered from four individuals, and all mating types were found to be unique (Fries & Neumann, 1990). Although these studies have added significantly to our understanding of ECM fungal genetics, *Suillus* presents several challenges to constructing and confirming laboratory crosses. For example, clamp connections are not a consistent trait among dikaryotic strains of *Suillus*, and therefore the presence of clamps cannot be used as a proxy for monokaryon compatibility as in other groups (Fries & Neumann, 1990). Further, the secondarily homothallic binucleate basidiospores produced by some species of *Suillus* complicate both the determination of breeding systems and crossing experiments (Jacobson & Miller, 1994). Although the incidence of binucleate spore production by *Suillus* appears to be low (Horton, 2006), the population frequency and fecundity of these binucleate spores is not yet resolved.

Insights into the evolution of breeding systems in the genus have also been supported by genome-based analyses. In agreement with studies employing laboratory crosses, comparative genomic based analysis of pheromone receptors suggests that *S. brevipes* has a bipolar breeding system (Mujic *et al.*, 2017). Allelic analysis of *Suillus* genomes suggest that many species have a long-term multi-allelic state, and a trans-specific polymorphism at the HD MAT locus (Ke *et al.*, 2023). This result is in agreement with the high diversity of mating types previously identified in crossing experiments, supporting HD MAT as the primary locus contributing to the bipolar mating system observed in *Suillus*. The closely related species *Rhizopogon rubescens*, also has a bipolar, multi-allelic breeding system (Kawai *et al.*, 2008). This fact, in combination with the recognition of the wide-spread bipolar, multi-allelic mating systems observed in multiple species of *Suillus*, suggests that a bipolar multi-allelic breeding system is likely the ancestral state for this group.

Population-level research suggests that *Suillus* generally displays high levels of outcrossing. For example, studies using molecular markers in *S. pungens* (Bonello *et al.*, 1998) and *S. spraguei* (Burchhardt *et al.*, 2011) have highlighted an excesses of heterozygosity at the population level, suggesting that outcrossing is more predominant than random mating. Similarly, population genomic analysis of *S. brevipes* has shown a sharp decay in linkage disequilibrium, which also indicates a high level of outcrossing (Branco *et al.*, 2017). The mechanisms associated with the extensive outcrossing observed in *Suillus* have not been tested experimentally but may be the result of self-incompatibility and heterosis. Crossing studies have found mixed results for the presence of a functional somatic incompatibility system, which may be present only in certain species such as *S. luteus* (Fries & Neumann, 1990). Identifying the molecular mechanisms responsible for the extremely high levels of outcrossing observed in the genus, including characterizing the identity and frequency of self-incompatibility systems, is a key future direction for understanding *Suillus* genetics.

III.vi: Genomics

While light-coverage genome sequencing of several key taxa has added important insights about population-level processes in *Suillus*, light-coverage sequencing is not adequate to capture nuanced information about gene and genome diversity. To this end, member labs of the International *Suillus* Consortium have spearheaded large-scale initiatives to sequence and annotate a phylogenetically and ecologically diverse set of *Suillus* fungi, using deep-coverage, whole genome sequencing of both DNA and RNA, with an emphasis on long-read technologies. Many of these efforts have been carried out in collaboration with the US Department of Energy's Joint Genome Institute, resulting in a genome set comprising more species from a single genus than any other ECM group to date. Currently, this genome set includes 46 *Suillus* genomes, representing approximately 40 distinct species. Many of these genomes have been released publicly on the MycoCosm Web Portal at https://mycocosm.jgi.doe.gov (Grigoriev *et al.*, 2014), facilitating community access and further work into *Suillus* biology, such as the ability to compare and contrast *Suillus* with other fungal groups (Lofgren *et al.*, 2019; Miyauchi *et al.*, 2020; Wu *et al.*, 2022).

Comparative genomic studies using these genomes have revealed the extent of genetic diversity in the genus, identifying a dynamic genomic landscape involving many gene families, including a higher number of rapid gene family expansions and a higher number of rapid gene family contractions than any other ECM group investigated to date (Lofgren *et al.*, 2021). These analyses have identified several genomic features that appear to be characteristic of the genus, including a diversity of gene clusters involved in secondary metabolism, and genes involved in the processing of reactive oxygen species (Lofgren *et al.*, 2021). The ecological roles that these genes play in *Suillus* biology is an active area of research. While secondary metabolic clusters likely function in a variety of biological processes in *Suillus*, the genus-wide expansion of terpene and non-ribosomal peptide synthetase (NRPS)-like clusters hint at roles in interspecific interactions such as microbial and host communication and are ideal targets for investigating the remarkable partner specificity displayed by the genus. Recently, LC/MS based untargeted metabolomic analysis of three genome-sequenced strains of *Suillus* identified hundreds of unique secondary metabolites, including 116 putative terpenes (Mudbhari *et al.*, 2023). The most abundant terpene classes were identified as terpene lactones, sesquiterpenes and di-terpenes, including several compounds induced exclusively when grown in co-culture with other species of *Suillus*. These results further suggest that these metabolites play a role in interactions between *Suillus* and other community members, but further work is needed to characterize these compounds and their ecological functions. Similarly, the processing of reactive oxygen species is known to be an important part of host colonization (Liao *et al.*, 2016), as well as mitigating host-mediated stress responses such as the oxidative bursts characteristic of plant drought stress (Zou *et al.*, 2021), or the unique ability of *Suillus* to withstand high levels of heavy metal exposure and to facilitate metal sequestration (Bazzicalupo *et al.*, 2020). Future work linking the genomic landscape of *Suillus* to the functions displayed by the genus will be key to developing a deeper mechanistic understanding of *Suillus* biology.

Efforts to leverage the benefits of both deep-coverage whole genome sequencing and population-level sampling are underway, with projects designed to characterize genome variation in target species such as *S. quiescens*, *S. luteus*, *S. brevipes, S. salmonicolor, S. pungens,* and *S. tomentosus*. We expect these projects to yield novel insights into intraspecific variation in *Suillus*, including genome structure, noncoding and repeat regions, and improved frameworks for linking genotypes to phenotypes. For example, comparative genomic analysis including 12 strains of *S. brevipes* found that rDNA copy number varied from 72 to 156 within the species, only slightly less variation than was estimated across the genus as a whole (44 to 198 copies) (Lofgren *et al.*, 2019). Leveraging functional-omics technologies on genome-sequenced strains will produce further insights into natural variation, and the ecology of *Suillus*-community interactions. Continuing efforts to produce more reference-quality *Suillus* genomes, the increased sampling breadth of target species for pangenomic analysis, improved pipelines for *de novo* assembly and annotation of unique strains, and an increase in the use of long read sequencing, will all propel and direct future insights into ECM fungal genomics.

IV. Ecological interactions

The unique intrinsic traits discussed above have enabled many studies on ecological interactions between *Suillus* and their surrounding communities. There has long been a focus on *Suillus* in the context of ECM partner specificity as well as many investigations of *Suillus* species as both competitors of other fungi and symbionts mediating plant responses to changing environmental conditions. Below we outline some of the major findings that have emerged from these ecologically focused studies.

IV.i: Partner specificity

Generally, co-occurring ECM host species exhibit large overlaps in ECM species associations, suggesting that partner specificity is low for most ECM taxa (Peay *et al.*, 2015; Peay, 2016; Arraiano-Castilho *et al.*, 2021). While recent work has highlighted the possibility that cryptic species may inflate estimates of host generalism by artificially combining taxa that are actually distinct, a phenomena known as cryptic specificity (Sato *et al.*, 2007; Wilson *et al.*, 2017; van der Linde *et al.*, 2018), how often this occurs in practice is unclear. Importantly, partner specificity is not a binary classification of specialists versus generalists, but rather a spectrum of interactions, ranging from fungal associations with a single host species to those spanning hundreds of host species. While it is likely that some genera such as *Laccaria, Thelephora* and *Russula*, are largely composed host generalists (Roy *et al.*, 2008; Smith *et al.*, 2009; Cho *et al.*, 2021), others such as *Lactarius* and *Strobilomyces* probably exhibit gradients of partner specificity or host preference (unequal colonization when multiple host taxa are available) that warrant more nuance then they have been historically afforded (Looney *et al.*, 2018; Tang *et al.*, 2021).

Early on, it was recognized that a small number of ECM fungal groups exhibited remarkable and conspicuously high partner specificity with certain host taxa. These specialists include *Alnicola*, *Alpova* and other basidiomycetes associated with the actinorhizal host genus *Alnus* (Tedersoo *et al.*, 2009; Rochet *et al.*, 2011), the genus *Leccinum* with various species of broad-leaf trees (Den Bakker *et al.*, 2004), and the genera *Suillus* and *Rhizopogon* with the plant family Pinaceae (Dahlberg & Finlay, 1999). Each of the major host genera (*Pseudotsuga*, *Larix*, and *Pinus*) are associated with a suite of *Suillus* species that appear to form mycorrhizas almost exclusively with that host (Table S1, Nguyen *et al.*, 2016b). While partner specificity in ECM fungi is most well recognized at the level of host genus (Molina *et al.*, 1992), *Suillus* species tend to be specific to host subgenera, particularly within the genus *Pinus*. The most detailed account of this level of specificity was presented by Liao *et al.* (Liao *et al.*, 2016), who combined seedling bioassays with metatranscriptomics to critically assess specific plant-fungus associations. The authors compared colonization and gene expression patterns across 5 species of *Suillus* and 10 species of *Pinus* and showed that several *Suillus* species, including *S. americanus*, *S. granulatus*, and *S. spraguei* only formed compatible associations with *Pinus* species in a single subgenus. This subgenus-scale specificity appeared to be associated with the expression of similar genes and metabolic pathways previously identified in pathogenic plant-fungal interactions.

While close pairings of particular *Suillus* species with specific hosts is the most common framing of this ECM symbiosis, recent studies indicate that patterns of *Suillus* partner specificity are strongly mediated by ecological context. Within the plant host family Pinaceae, multiple tree genera have long been considered non-hosts of *Suillus*, including *Picea* (a genus in the subfamily Piceoideae nested within a clade of known *Suillus* host genera; (Gernandt *et al.*, 2016) as well as *Abies* and *Tsuga* (in the subfamily Abietoideae, a clade that does not contain *Suillus* host genera) (Fig. 4). Based on sporadic field reports of *Suillus* mushrooms being collected in *Picea* or *Abies/Tsuga* forests with no known *Suillus* host genera present (Doudrick *et al.*, 2011), Pérez-Pazos et al. (Pérez-Pazos *et al.*, 2021) combined field root tip sampling and experimental seedling bioassays to assess the partner specificity of two *Suillus* species; *S. glandulosus* and *S. punctatipes*. The authors found that *Picea*, *Abies*, and *Tsuga* root tips were colonized by *Suillus* species in the field and that both *Suillus* species were capable of colonizing alternative hosts in the laboratory. Importantly, however, the colonization of alternative hosts only occurred when a known host (a species of *Pinus* or *Pseudotsuga*) was co-planted in the same container. This outcome matched prior studies of ECM partner specificity (Massicotte *et al.*, 1994), where it was shown that certain 'primary' host species are required to trigger spore germination, but that additional 'secondary' hosts could be colonized once the ECM fungus was growing as mycelium. Even greater host phylogenetic breadth was shown for *S. subaureus* by Lofgren *et al.* (2018), who demonstrated through a combination of field sampling and seedling bioassays that this species was capable of colonizing both *Pinus* and *Quercus* hosts. This unusual association with *Quercus* (an angiosperm) appeared to be associated with the local extirpation of *Pinus* hosts during intensive logging decades earlier, resulting in a unique ecological legacy effect. Differences in host range between primary and secondary colonization such as those identified for *S. glandulosus, S. punctatipes* and *S. subaureus* may seem like curious exceptions but could have significant consequences for ecosystems. Secondary colonization is likely responsible for reports of *Suillus* on other non-canonical hosts, including *Betula* (Nara, 2006), *Cupressus,* and *Ceratonia* (Avital *et al.*, 2022), and could influence the succession dynamics of plant communities, particularly if carbon transfer from secondary hosts is sufficient to produce fruit bodies or if resources are transferred via exudates or common mycelial networks. As plant host ranges shift due to a changing climate and human introductions, understanding ECM partner specificity is key to predicting the establishment of these interactions in novel ecological contexts.

IV.ii: Competition

Like all ECM fungal species, members of the genus *Suillus* compete to colonize host root tips as their main source of carbon in natural settings (Baldrian, 2009). Prior to molecular identification of ECM root tips, the relatively high annual production of sporocarps by most *Suillus* species suggested this genus was a dominant part of the ECM fungal community in many conifer forests (Dahlberg & Finlay, 1999). However, as molecular-based root tip surveys proliferated in the 1990s and 2000s, it became apparent that most *Suillus* species colonize only a limited number of root tips compared to other ECM genera such as *Tomentella* and *Russula* (Gardes & Bruns, 1996; Jonsson *et al.*, 1999; Peay *et al.*, 2007). However, in early successional forests, non-native environments, and post-fire environments *Suillus* often deviate from this pattern and dominate root tip communities (Visser, 1995; Chapela *et al.*, 2001; Hayward *et al.*, 2015; Miyamoto *et al.*, 2021; Thompson *et al.*, 2022). This pattern of root-tip dominance can also be seen in some specialized habitats such as hummocks present in *Larix* systems (accumulations of organic matter that exist directly above the waterline), where *Suillus* can occupy a significantly higher proportion of the ECM fungal community (Kennedy *et al.*, 2018). In general, however, *Suillus* is rarely the most dominant genus colonizing ECM root tips in conifer forest soils where it is endemic.

Given the aforementioned pattern, one might speculate that *Suillus* species are relatively weak competitors for host root tips or easily excluded by other ECM fungi during fungal community succession. Support for a limited competitive ability has been observed in multiple seedling bioassay studies involving *Suillus* and other ECM fungi. For example, Kennedy *et al.* (2011) found that *S. pungens* was outcompeted by two species of *Rhizopogon* when colonizing *Pinus muricata* seedlings from mycelia. Similar results were found by Moeller and Peay (2016) who demonstrated that *S. pungens* was also outcompeted by both *Thelephora terrestris* and *R. occidentalis* when colonizing *P. muricata* seedlings from spore. Collectively, these experimental findings, in conjunction with root tip abundance patterns in field studies, suggest that *Suillus* species may indeed be relatively weak competitors when faced with either spore- or mycelial-based competition from other ECM fungi. It should be noted, however, that *Rhizopogon* and *Thelephora* are aggressive colonizers of seedlings, and studies across a much wider range of *Suillus* and competitor species are needed to have greater confidence in this pattern. The pattern of low root tip abundance, increased plant health, and large and abundant fruit bodies suggests an effective carbon trading balance where only a limited number of root-tips are needed to achieve advantageous outcomes for both hosts and symbionts.

Perhaps to compensate for limited interspecific competitive ability, members of *Suillus* appear to have specialized in reproductive traits that favor rapid and abundant dispersal (Policelli *et al.*, 2019), which likely favors their ability to colonize root tips that may have few other ECM fungi present. This has been best evidenced in the quantitative estimates of ECM fungal spore dispersal at increasing distances from established forests, which typically reveal that *Suillus* represent an increasing proportion of ECM spores at the farthest distances (Peay *et al.*, 2012; Thompson *et al.*, 2022). Similar tradeoffs in competitive versus colonization ability have been routinely observed in plant communities (Tilman, 1994) and *Suillus* is arguably one of the best documented examples of this phenomenon within ECM fungi (Peay *et al.*, 2007).

Studies focused on congeneric competition among *Suillus* species have also revealed interesting ecological trends. Working with a set of species that are commonly associated with *Pinus strobus*, Kennedy *et al.* (2020) found a clear competitive hierarchy of *S. americanus* > *S. subaureus* > *S. spraguei*. The outcome of the competitive interactions appeared to be largely the result of differences in spore germination timing, with *S. americanus* being the first and most consistent to colonize *P. strobus* seedling root tips, followed by *S. subaureus*, and then *S. spraguei*. The early colonization by one species precluding the establishment of subsequent colonizers, also known as a priority effect, has been observed consistently in a variety of other ECM study systems as well (Kennedy, 2010; Bogar & Kennedy, 2013). Interestingly, *S. subaureus*, is a much rarer species than either *S. americanus* or *S. spraguei*, which suggests that other factors besides competitive ability are also likely important in shaping abundance in ECM fungal communities.

To investigate the extent to which ECM host plants may influence ECM fungal competitive outcomes, Bogar et al. (2019) conducted a pair of seedling bioassays using the *Larix*-associated species; *S. clintonianus*, *S. grisellus*, and *S. spectabilis*. The authors used a split root design in which competing *Suillus* species were spatially isolated when colonizing a shared host (eliminating the role of direct fungal-fungal interactions). They found that colonization by two of the three species was not changed by a second species being present on a different portion of the root system, but that the colonization by *S. spectabilis* was significantly lower if a second *Suillus* species was present. This suggested that plants can differentiate amongst different *Suillus* species and can potentially mediate root tip colonization to privilege preferred species. Further evidence for differential plant investment was found in the second bioassay, in which seedlings allocated greater amounts of carbon to the portion of the split-root system that provided the greatest amount of nitrogen. Intriguingly, seedlings independently colonized by *S. spectabilis* were not significantly lower in needle nitrogen content than those colonized by *S. grisellus* or *S. clintonianus*, so exactly why the *Larix* host plants were preferentially colonized by the latter two species was not clear. In natural habitats, *S. spectabilis* can only be found with mature host plants, whereas the other two species can be found with younger hosts, suggesting a host age-dependent context of interactions. This study, along with additional studies manipulating soil nitrogen levels (Bogar *et al.*, 2022), highlight that competitive outcomes among ECM fungi may be influenced by host plant tendencies toward specific taxa.

IV.iii: Soil nutrient cycling

Similar to other ECM fungi, *Suillus* are thought to have a limited capacity to decompose complex plant cell wall polymers (Kohler *et al.*, 2015). As the vast majority of ECM fungi evolved from saprotrophic ancestors (Tedersoo *et al.*, 2010) many have retained the ability to decompose less complex polymers such as protein and chitin, which could account for observed patterns of organic matter turnover (up to 30%) in introduced *Pinus* plantations (Chapela *et al.*, 2001). Protein decomposition appears to be dependent on forest age, with *Suillus* species in younger forests having lower protein decomposition activity (Rineau *et al.*, 2016), which could be advantageous given that more mature forests contain higher levels of organic nitrogen.

Genomic and functional assays have demonstrated that *S. luteus* also uses endochitinases to decompose complex chitin polymers (Maillard *et al.*, 2023). Initial work suggests that in low carbon soils, *S. salmonicolor (cothurnatus)*, like some other ECM species, can prime decomposition by saprotrophic fungi (Bhatnagar *et al.*, 2021). These pieces of evidence indicate that although limited in their capabilities to decompose complex plant cell wall polymers, *Suillus* can substantially contribute to decomposition of soil organic matter, and thus nutrient cycling, especially nitrogen cycling in soil.

Suillus themselves can be consumed, decomposed, and returned to the soil as labile sources of carbon and nutrients. Members of this this genus often produce large aggregations of fruit bodies, estimated to be upward of 63.9kg ha^{-1} year^{-1} in dry weight for *S. variegatus* (Ohenoja & Koistinen, 1984), to one metric ton ha^{-1} year^{-1} dry weight for *S. luteus* (Hedger, 1986; Chapela *et al.*, 2001). Notably, these estimates did not include hyphae and rhizomorphs, which likely represent a substantial additional pool of tissue available for decomposition. These high nutrient resources are rapidly consumed by insects, in particular phorid and mycetophilid flies (Bruns, 1984). The fruit bodies (Gohar *et al.*, 2022) and mycelium (Brabcová *et al.*, 2016) can also be decomposed by bacteria and fungi that appear to have specificity towards these fungal-derived resources (Nguyen, 2023). Enabled by the ready abundance of fruit bodies and the easy culture of *Suillus* mycelium Fernandez *et al.* (2019) and Maillard *et al.* (2022) used *S. grisellus* to conduct manipulative experiments characterizing the contributions of *Suillus* necromass to soil nutrient cycling as well as identifying the microbial members involved in this process. This area of research is currently experiencing a renaissance as modern tools and techniques provide exciting opportunities to dissect the molecular mechanisms and community interactions that facilitate the degradation of fungal necromass (Fernandez & Kennedy, 2018; Kennedy & Maillard, 2023) and will be influential in elucidating the contributions of *Suillus* (and other high-biomass producing fungal species) to soil nutrient cycling.

V. Environmental (abiotic) adaptation

Studies in the genus *Suillus* have enabled significant advances in our knowledge of fungal abiotic adaptation. In this section, we outline recent progress in our understanding of how the genus impacts, and is impacted, by environmental variation. Specifically, we highlight studies assessing climate change, ionic radiation, metal pollution, and enzymatic activity and degradation. The availability of public protocols and genomic resources are expected to catalyze further research in these areas and assist in the development of new strategies to remediate and recover degraded habitats.

V.i: Climate change

Climate change, characterized by elevated temperatures and shifts in patterns of precipitation, is increasingly impacting forest ecosystems, and influencing symbiotic relationships between fungi and their host plants (Baldrian *et al.*, 2023). Recent research has identified *Suillus* as a key player in enhancing host plant resilience to environmental stress, and advanced our understanding of how ECM fungi respond and adapt to changing environmental conditions (Malcolm *et al.*, 2008; Branco *et al.*, 2017; Li *et al.*, 2021; Qi & Yin, 2022; Hou *et al.*, 2022; Erlandson *et al.*, 2022).

As a result of shifting climatic conditions, there has been a global increase in the frequency and severity of drought in recent years (Dai, 2012), posing a significant threat to the resilience of forest ecosystems (Brodribb *et al.*, 2020). *Suillus* exhibit considerable drought tolerance, which potentially sustain symbiotic relationships and enhance plant stress tolerance under drought conditions. The long-distance rhizomorphs of *Suillus* are well known to aid water transport (Duddridge *et al.*, 1980), which may help host plants to maintain positive water relations during times of abiotic stress. Support for this hypothesis was recently demonstrated by Castaño et al. (2023), who found that *Pinus pinaster* seedlings exposed to experimental drought in Spain were enriched in ECM fungal taxa with long-distance rhizomorphs. In particular, they found that *S. variegatus* was an indicator species of drought (i.e. was more relatively abundant under the drought treatment), suggesting that some species of *Suillus* may be better able to tolerate low water availability than other ECM taxa. At the same time, this study also reported that the abundance of *S. bovinus* declined significantly in the drought treatment, indicating that not all *Suillus* species are drought resistant. This result is consistent with the findings of Erlandson et al. (2022), who showed that long-term water reduction dramatically decreased root tip colonization by *S. pungens* on *Pinus muricata* seedlings. This study found that while short-term water reduction led to major changes in the expression of functional genes associated with water acquisition, gene expression under long-term water stress was very similar to that of well-watered controls. This suggests that ECM fungi may be capable of significant acclimation under prolonged stress, which may aid in maintaining symbiotic functioning under challenging environmental conditions (Palumbi *et al.*, 2014). Erlandson *et al.* (2022) further found that drought-exposed *S. pungens* was able to maintain cellular integrity by up-regulating genes related to fungal cell wall synthesis.

Significantly, *Suillus* may pass drought tolerance benefits onto their host trees. Several studies have found evidence for this effect and have proposed several potential mechanisms including osmoregulation, oxidative protection, and the preferential relocation of plant resources. For example, osmoprotective compounds such as proline and trehalose have been shown to contribute to plant osmoregulation by providing protection against drought-induced damage, and appear to be upregulated in *Suillus* during drought stress (Li *et al.*, 2021; Erlandson *et al.*, 2022). Osmoregulation may also occur by increasing host water uptake via the induction of water-channel proteins such as aquaporins (Lee *et al.*, 2010; Xu & Zwiazek, 2020). While multiple studies have indicated that *S. tomentosus* can improve host tolerance to salt exposure via aquaporin-mediated water transport (Lee *et al.*, 2010; Calvo-Polanco & Zwiazek, 2011), researchers have been unable to provide direct evidence that *Suillus* regulates host-water movement under drought conditions. As such, future research is needed to confirm the induction of water-channel proteins as a drought tolerance mechanism in the genus.

A second proposed mechanism for *Suillus* mediated drought tolerance is via oxidative defense. Li et al. (2021) found that roots colonized by *S. placidus* appeared to trigger antioxidant defense against drought-induced oxidative stress through the activation of antioxidant enzymes. These results suggest that some species of *Suillus* may be capable of counteracting the negative impacts of drought-induced oxidative damage, thereby promoting plant health and survival.

Finally, *Suillus* may mediate drought responses by altering the physiology of their hosts. For example, Qi and Yin (2023) found that under drought conditions *S. luteus* altered host root traits including diameter, length, surface area, and volume, improving water and nutrient uptake efficiency. A similar pattern was detected in *S. pungens*-inoculated mycorrhizal roots which responded to drought by up-regulating genes involved in energy production, signal transduction, metabolism, and the transport of amino acids, lipids, and carbohydrates (Erlandson *et al.*, 2022). Despite the inhibitory effects of drought stress on photosynthesis, plant development, and growth, *Suillus* appears to assist in mitigating these effects by enhancing photosynthetic performance and increasing stomatal area and density (Li *et al.*, 2021; Qi & Yin, 2022). Taken together, these results suggest that *Suillus* may confer drought tolerance to host plants by increasing the production of photosynthetically-fixed carbon that is then preferentially reallocated to belowground structures, which in turn support mycorrhizal root growth and hyphal water uptake.

V.ii: Ionizing Radiation

High levels of radiation are a major concern for human health and ecosystem function. *Suillus* has provided important insights into how ionizing radiation affects the fungal kingdom. Irradiation results from radionuclides, unstable isotopes that release radiation when they break down to more stable elements. While these elements occur naturally, they are primarily concerning when produced artificially and subsequently enter the environment in high concentrations, typically via industrial waste or the detonation of nuclear weapons (Hu *et al.*, 2010). Exposure to high levels of ionizing radiation has wide ranging negative effects, including genetic and epigenetic changes, decreased reproductive ability, abnormal morphology, and mortality (Hinton *et al.*, 2007).

The devastating accidents that occurred in Chernobyl (Ukraine) in 1986 and in Fukushima (Japan) in 2011 heavily impacted vast areas with radio-active fallout. These events have been widely studied to determine how chronic irradiation affects species and ecosystems. The genus *Pinus* is extremely sensitive to irradiation (Geras'kin *et al.*, 2008), and the nuclear accidents that occurred in Japan and Ukraine affected large swaths of pine-dominated forest (Vinichuk & Johanson, 2003; Pumpanen *et al.*, 2016; Bondarenko *et al.*, 2023). Studies from these areas suggest that some fungi, including *Suillus*, are relatively tolerant towards ionizing radiation (Dighton *et al.*, 2008). These fungi act as ^{137}Cs radionuclide sinks, effectively removing them from the soil. This process may influence the cycling of radionucleotides across soil compartments, as both *S. luteus* and *S. variegatus* accumulate high levels of ^{137}Cs in fruit body tissue (Nikolova *et al.*, 1997; Ronda *et al.*, 2022). Similarly, Vinichuk and Johanson (2003) found that an astounding 19% of total soil ^{137}Cs were transported into *S. variegatus* mycelium. Importantly, these effects appeared to be species-specific, as not all *Suillus* species accumulated ^{137}Cs to the same extent. The ability of some *Suillus* species to act in the bioaccumulation of ^{137}Cs raises the question of whether *Suillus* impacts ^{137}Cs uptake in host trees. In plants, ^{137}Cs uptake is mediated by K transporters and K channels (Zhu & Smolders, 2000). In *Suillus* however, the concentration of ^{137}Cs is not correlated with K concentration (Dighton *et al.*, 2008; Vinichuk *et al.*, 2011), suggesting that in fungi, ^{137}Cs uptake may be independent of K pathways. Interestingly, there seem to be differences in irradiation effects and molecular responses among fungal species that differently impact growth, melanin production, and ROS scavenging enzymes. For example, Kothamasi (2019) found that while multiple *S. luteus* isolates displayed reduced biomass upon irradiation exposure, *S. bovinus* was unaffected. Similarly, while all *Suillus* isolates showed melanin accumulation in response to irradiation, the response of ROS decomposing enzymes varied both between and within species.

Precisely how irradiation affects ECM fungi, if ECM fungi are involved in plant tolerance to ionizing radiation, and if so, by what mechanisms, are active areas of research (Ladeyn *et al.*, 2008). Given the extensive areas affected by irradiation and the abundance and relevance of *Suillus* species in these ecosystems, *Suillus* is an ideal model to investigate the impacts of radiation, and a prime candidate for the development of bioremediation systems. To this end, members of the International *Suillus* Consortium are currently building a *Suillus* culture collection from Fukushima radionuclide-contaminated soil to investigate both the effects of irradiation in ECM systems and develop urgently needed bioremediation strategies.

V.iii: Metal tolerance

Soil metal contamination through anthropogenic activity can be extremely detrimental to ecosystems, negatively impacting metabolism, growth and reproduction (Branco *et al.*, 2022). Metal tolerance in *Suillus* is a widely documented phenomenon and several species are routinely found in metal contaminated soils, including sites near mining and metal manufacturing facilities, decommissioned smelters and mills, and sites with heavy ore enrichment (Colpaert & van Assche, 1987; Leski *et al.*, 1995; Blaudez *et al.*, 2000; Adriaensen *et al.*, 2005). This finding has driven multiple studies focused on characterizing metal tolerant isolates with the goal of assessing whether the presence of metal contamination has selected for metal tolerant traits and led to local adaptation (Colpaert & van Assche, 1987; Bazzicalupo *et al.*, 2020).

Early studies demonstrated that metal tolerance in *Suillus* not only varies across species, but also across isolates within the same species. For example, *S. luteus*, *S. variegatus* and *S. granulatus* display interspecific variation in tolerance to Cd, Pb, Sb and Zn (Hartley *et al.*, 1997), while both *S. luteus* and *S. bovinus* display intraspecific variation in tolerance to Cd, Cu, Ni and Zn (Colpaert & Van Assche, 1992; Blaudez *et al.*, 2000; Adriaensen *et al.*, 2005). Interestingly, although *Suillus* isolated from metal contaminated sites generally display higher levels of metal tolerance, metal sensitive isolates have also been collected from metal contaminated soils (Blaudez *et al.*, 2000; Fomina *et al.*, 2005; Adriaensen *et al.*, 2005). Fomina *et al.* (2005) proposed that this observation could be explained by the heterogeneous nature of soil, which could lead to uneven metal concentrations across a single soil patch, allowing metal sensitive isolates to persist. However, metal tolerant *Suillus* have also been discovered in non-contaminated areas (Leski *et al.*, 1995; Hartley *et al.*, 1997; Colpaert *et al.*, 2004), suggesting that the fitness costs to metal tolerance may be inconsequential, or that there may be benefits to retaining metal tolerance traits apart from metal exposure. While Colpaert (2004) suggested that gene flow could occasionally bring tolerant genes into sensitive populations, genetic analysis of *S. luteus* from contaminated and non-contaminated sites did not support the presence of separate populations (Bazzicalupo *et al.*, 2020).

Metal tolerance in *Suillus* appears to result from the ability to maintain metal homeostasis through a combination of metal immobilization, exclusion, and detoxification. These mechanisms rely on a large number of genes, including transmembrane transporters (which move metal into, out of and around the cell), chelators (that bind and immobilize ions both within and outside the cell), and antioxidants that counteract the effects of metal toxicity (Bazzicalupo *et al.*, 2020; Branco *et al.*, 2022). Analysis of *S. luteus* demonstrated that metal tolerance is likely achieved through genetic variants arising from standing genetic variation (Bazzicalupo *et al.*, 2020). In a recent study, Smith *et al.* (2023) conducted a transcriptomic analysis of Zn-tolerant and Zn-sensitive *S. luteus* isolates exposed to high Zn concentrations and confirmed that Zn tolerance in this species is polygenic. This study showed that different isolates achieve tolerance through distinct mechanisms and that Zn-tolerant isolates are largely constitutively tolerant regardless of metal exposure. However, the authors also identified two Zn related genes for which expression was affected by Zn concentration, suggesting that Zn tolerance in *S. luteus* also includes an environmental component.

Studies investigating the genetics and physiology of *Suillus* metal tolerance have demonstrated that metal accumulation in cells is a limiting factor for tolerance (Colpaert & van Assche, 1987). For example, Colpaert *et al.* (2005) found that Zn uptake is negatively correlated with tolerance in *S. luteus*, and that the exclusion of Zn from cells allows tolerant isolates to persist when exposed to high Zn concentrations. Cadmium tolerance in *S. luteus* may similarly rely on a metal exclusion mechanism, as tolerant isolates have been shown to take up less metal into the mycelium than sensitive isolates (Krznaric *et al.*, 2009). Cadmium tolerant *S. luteus* isolates have also been shown to express antioxidant transcripts at much lower levels than Cd sensitive isolates, suggesting Cd tolerance is grounded in exclusion, and that Cd tolerant *Suillus* likely avoid cellular damage by preventing Cd uptake in the first place (Ruytinx *et al.*, 2011).

There has been substantial progress in illuminating the mechanisms of metal exclusion and metal binding in *Suillus*. Multiple transmembrane transporters have been identified including Cation Diffusion Facilitator (CDF) family transporters and Zrt-, Irt-Like Protein (ZIP) transporters. Whereas CDF transporters are membrane bound proteins that transport a variety of metals (Kolaj-Robin *et al.*, 2015), ZIP transporters are Zn pumping proteins that are typically localized to the plasma membrane. These transporters act to bring external Zn into the cells and to draw Zn from vacuolar stores to prevent toxicity (Coninx *et al.*, 2017), and have been characterized in *S. bovinus* (Ruytinx *et al.*, 2013). In *S. luteus* there are four CDF proteins predicted to be Zn transporters. Two of these (SIZnT1 and SIZnT2), have been functionally characterized, and S1ZnT1 has been shown to confer Zn tolerance using heterologous expression in yeast (Ruytinx *et al.*, 2017). *S. luteus* also has four predicted ZIP transporters, two of which have been described. SIZRT1 is a plasma membrane transporter heavily involved in maintaining cellular Zn homeostasis (Coninx *et al.*, 2017) and SIZRT2 is localized to the plasma membrane and the perinuclear region and also appears to play a role in Zn uptake (Coninx *et al.*, 2019).

Metal binding is also an important mechanism of metal tolerance in *Suillus*. In *S. luteus*, there are two known metallothioneins (small proteins that can bind and immobilize metals) involved in Cu homeostasis (SlMTa and SlMTb). These genes are upregulated in excess Cu, but not in excess Zn or Cd. Heterologous expression of these genes in a Cu sensitive yeast mutant has been shown to restore tolerance (Nguyen *et al.*, 2017). Metallothionein homologues with a role in Cu homeostasis have also been identified in *S. himalayensis*. These genes were shown to be induced by excess Cu, but not by Cd and heterologous expression in yeast confirmed a role in Cu tolerance, along with possible roles in Cd and Zn homeostasis (Kalsotra *et al.*, 2018).

Notably, *Suillus* metal tolerance appears to have significant downstream effects on host plant fitness (Adriaensen *et al.*, 2005). When associated with plant roots exposed to high metal concentrations, some *Suillus* isolates can reduce metal transfer and accumulation in host plant tissues while still efficiently transferring nutrients to the host (Adriaensen *et al.*, 2006; Colpaert, 2008; Krznaric *et al.*, 2010; Colpaert *et al.*, 2011; Branco *et al.*, 2022). While metal-tolerant and metal-sensitive *Suillus* seem to be equally effective mycorrhizal partners regarding nutrient uptake and transfer, there are clear differences in plant nutrient uptake and nutrient status when exposed to high metal concentrations (Adriaensen *et al.*, 2004; Krznaric *et al.*, 2010). For example, Andriaensen *et al.* (2005) demonstrated that inoculation with Cu-tolerant isolates of *S. luteus* significantly improved plant phosphorus and nitrogen uptake in Cu-contaminated soil, while Cu-sensitive isolates reduced nutrient uptake and transfer. *Suillus* colonization can also facilitate metal tolerance indirectly, for example, Liu *et al.* (2020, 2021) demonstrated that *S. luteus* activates multiple plant stress pathways, enhancing plant fitness during metal exposure. Similarly, metal exclusion mechanisms in tolerant *Suillus* isolates have been shown to reduce metal transfer to the host (Krupa & Kozdrój, 2004; Zhang *et al.*, 2021). For example, when associated with *Pinus* roots under high external Zn, *S. luteus* accumulates Zn in the hyphal mantle, preventing Zn accumulation in host tissues (Zhang *et al.*, 2021).

VI. Biological introductions and invasions

The strong ecological linkages between *Suillus* and the Pinaceae help explain the key role of the genus in the spread and establishment of Pinaceae into novel habitats. While this ability has long been beneficial for the forestry industry, enabling the wide-spread establishment and success of tree plantations, it has also facilitated the invasion of introduced host taxa into native plant communities around the world, displacing species and disrupting ecosystems (Read, 1998; Policelli *et al.*, 2019). While many *Suillus* host species have been introduced across the world, not all introductions have resulted in biological invasions. The variables determining invasion capacity, along with the ecological impacts of *Suillus*-associated host-tree establishment in non-native environments are important areas of research.

VI.i: Introduction via forestry

The genus *Pinus* is native to the northern hemisphere, but has been planted globally for shade, shelter and the establishment of softwood plantations. Exotic *Pinus* plantation forests currently cover over five million hectares, and their global effects on primary productivity, biogeochemistry, and biodiversity represent some of the most widespread and impactful introductions on Earth (Simberloff & Von Holle, 1999; Hoeksema *et al.*, 2020).

Most early attempts to introduce conifers into the southern hemisphere failed due to the lack of availability (and knowledge) of suitable ECM fungi (Mikola, 1970). As foresters became aware of ECM and their role in forest nutrition, inoculating seedlings with ECM fungi became common practice. Historically, the most commonly used inoculum source was transplanted soil from established *Pinus* plantations (Marx, 1992; Marx *et al.*, 2002). This practice led to the successful establishment of conifer plantations across both the northern and southern hemispheres, along with the widespread dissemination of conifer-associated ECM fungi. Early on, it was recognized that the ECM species that established themselves in exotic conifer plantations represented only a fraction of native ECM biodiversity, and overwhelmingly consisted of early colonizers such as *Suillus* and *Rhizopogon* (Theodorou, 1967; Theodorou & Bowen, 1973). In areas where conifers have been introduced, this process of ecological filtering has resulted in as few as 1-5 species of ECM fungi in New Zealand *Pinus* plantations (Chu-Chou & Grace, 1988), and as few as one species (*S. luteus*) in *Pinus* plantations in Chile (Hayward *et al.*, 2015). This reduction in diversity is also evident for other introduced members of the Pinaceae. For example, *Larix* and *Pseudotsuga* have been widely planted in the southern hemisphere, where they exhibit strong partner specificity with *Suillus*, including *S. cavipes* with introduced *Larix* and *S. lakei* with *Pseudotsuga* (McNabb, 1968; Moeller *et al.*, 2015; Pietras *et al.*, 2018).

VI.ii: *Suillus-Pinaceae* co-invasions

Successful afforestation and introduction of Pineaceae across the southern hemisphere has inevitably resulted in the escape of introduced trees that interact with and sometimes displace native plant communities (Richardson *et al.*, 1994; Simberloff & Von Holle, 1999; Nuñez *et al.*, 2009). Burdon and Chilvers (1977) were the first to report the process of escape and invasion by wilding conifers. The study detailed *P. radiata* invasion from plantations into adjacent *Eucalyptus* forests near Canberra, Australia, resulting in the formation of a new type of hybrid forest community. Follow up studies monitoring these forests over the subsequent 14 years, found that the growth of *P. radiata* was strongly affected by the presence of the other plant species (Chilvers & Burdon, 1983; Burdon & Chilvers, 1994), leading to the eventual coexistence between *Pinus* and native vegetation. Within Australia, the impact of *Pinus* invasions seems to be relatively small with most wilding tree escapes occurring after periodic disturbance or fire (Williams & Wardle, 2007; van Etten *et al.*, 2020). This is in contrast to *Pinus* introductions on other continents, which have resulted in active invasion fronts, tremendous loss of native biodiversity, and habitat alteration. The negative impacts of these invasions have been greatest in regions where host trees are able to grow and shade out other plant species, such as in the Cape Region of South Africa where *P. radiata* now threatens native Fynbos vegetation (Richardson *et al.*, 1994), and in Brazil, Argentina, and Chile, where invasive *Pinus* have escaped into native grasslands (Nuñez *et al.*, 2009). To date, efforts to eradicate invasive Pineaceae hosts and restore these ecosystems have achieved only limited success.

The concept that ECM fungi had a role in facilitating host tree invasions was first suggested by Richardson *et al.* (1994) and Read *et al.* (1998), and later confirmed using molecular approaches (Nuñez *et al.*, 2009; Dickie *et al.*, 2010; Hynson *et al.*, 2013; Moeller *et al.*, 2015; Horton, 2017). This pattern of invasion has been termed "Linked Plant-Fungal Co-invasion" (Dickie *et al.*, 2017). Today, plantation trees are inoculated with ECM fungi as a matter of course, typically using soil-free spore-based methods or mycelial slurries where strain origin can be controlled (Marx *et al.*, 2002). Contemporary commercial inoculum is produced using many

ECM taxa, including *Pisolithus tinctorius*, *Laccaria spp.*, *Rhizopogon* spp. *Hebeloma* spp., and *Suillus* spp. (Marx *et al.*, 2002; Charya & Garg, 2019). Whether due to innate trait-based effects, or legacy effects from soil-based inoculation, *Suillus* seem to have a disproportionate invasion potential: in almost every study conducted to date on linked plant-fungal co-invasions, *Suillus* species have been identified as keystone community members. While tree plantations are the best studied source of *Suillus* introductions into non-native ranges, it is important to note that ECM fungi can be translocated along with any compatible host tree or introduced intentionally or inadvertently with the movement of soil. This underscores the importance of knowing mycorrhizal status and the identity of the ECM fungi associated with introduced trees for applications such as forest restoration, shelterbelts (windbreaks), and ornamental plantings.

To generate an up to date assessment of *Suillus* introductions, we used the Global Database of Alien Macrofungi (Monteiro *et al.*, 2020). This database includes 770 ECM distribution records originally assembled by Vellinga *et al.* (Vellinga *et al.*, 2009), but is extended to include multiple guilds, and studies completed since 2009, for a total of 1,966 observations. We pruned this dataset to exclude taxa with uncertain species-level assignments, and those annotated as having established from uncertain geographic origins. To identify ECM species, guild was assigned across the database using FUNGuild (Nguyen *et al.*, 2016a), retaining only species that could be confidently identified as ECM and excluding mixed-guild types, resulting in 946 observations, spanning 55 genera and 241 species. Out of these 55 ECM genera, *Suillus* made up the vast majority of observed introductions, with a total of 193 records, followed by *Scleroderma* at 91, and *Rhizopogon* at 72 (Fig. 5A). While the dominance of *Suillus* is striking, as noted by Vellinga et al. (Vellinga *et al.*, 2009), occurrence records of species such as *Scleroderma* and *Rhizopogon* are likely underestimated due to the relative difficulty of locating their fruitbodies. These results broaden our understanding of genus-level ECM invasion frequency, and emphasize the ongoing prevalence of *Suillus* in ECM introductions as reported 15 years ago in Vellinga *et al.* (Vellinga *et al.*, 2009). In total, there were 24 species of *Suillus* in introduced ranges (Fig. 5B). Largely in agreement with previous studies (Read, 1998; Dunstan *et al.*, 1998; Policelli *et al.*, 2019), the most common species were the European *Pinus* associated taxa *S. luteus* (44 observations) and *S. granulatus* (34 observations), followed by the larch associate *S. grevillei* (18) and Douglas Fir associate *S. lakei* (15). It should be noted that the western north American species *S. quiescens* (typified in 2010) is also an important introduced species (Policelli *et al.*, 2019) but was absent from the database (likely combined with the morphologically similar *S. brevipes*).

The dominance of *Suillus* in ECM introductions is likely the result of both the historical frequency of using soil-based inoculations, and the confluence of several traits exhibited by the genus. Policelli *et al.* (2019) identified several ecological traits which help to make *Suillus* strong facilitators of host tree invasions. These include 1) long-distance dispersal capacity, 2) the ability to generate a long-lived resistant spore bank, 3) the establishment of positive biotic interactions with mammals, 4) the rapid colonization of host roots, and 5) the long-distance exploration type of *Suillus* extraradical mycelium.

Within their exotic range, and in the absence of competition from other fungi, *Suillus* species often fruit especially prolifically, further contributing to their proliferation and spread in these habitats. Hedger (1986) and Chapela (2001) estimated that *S. luteus* fruit body production in introduced Ecuadoran *P. radiata* forests, could amount to over one metric ton of fungal tissue per hectare (dry weight): an impressive yield by any measure. These fruit bodies produce enormous numbers of basidiospores which are widely dispersed well beyond the forest boundary. Once dispersed, *Suillus* spores become part of a long-lived drought and fire-resistant soil spore-bank, and are able to wait many years for a wilding *Pinus* seed to land beside them (Bruns *et al.*, 2010).

In addition to wind dispersal, mycophagy is recognized as an important dispersal mechanism and likely contributes to the establishment of *Suillus* in invasive ranges (Wood *et al.*, 2015;

Caiafa *et al.*, 2021; Elliott *et al.*, 2022). Numerous studies have documented fruit body consumption by both native and non-native animals including rodents, marsupials, deer, pigs, rabbits and potentially birds, which can translocate spores over great distances and disperse them through their excrement (Ashkannejhad & Horton, 2006; Nuñez *et al.*, 2013; Wood *et al.*, 2015; Horton, 2017; Aguirre *et al.*, 2021; Caiafa *et al.*, 2021; Policelli *et al.*, 2022).

Perhaps the strongest factor linking *Suillus* to host tree invasions is the fact that *Suillus* species colonize young host seedlings so readily. Once established, *Suillus* can rapidly spread to nearby root systems by the production of the abundant rhizomorphs that are a hallmark of this clade. This type of extramatrical mycelium, which Agerer (2001) termed the "long-distance exploration-type", not only enables mycelial-based colonization of neighboring host trees, but likely helps to facilitate the success of *Pinus* across exotic ranges by enabling better water withdrawal, increased access to resources patches, and increased acquisition of organic nitrogen (Hobbie & Agerer, 2009; Koide *et al.*, 2014). Although *Suillus* are often thought of as weak competitors, new plantation environments are typically depauperate in ECM symbionts capable of colonizing the introduced host trees, resulting in reduced competition (Hedger, 1986; Chapela *et al.*, 2001). In addition, novel environments offer a high likelihood of 'enemy escape' from co-evolved pathogens, which may also contribute to establishment across introduced ranges (Dickie *et al.*, 2010).

VI.iii: Downstream effects and unresolved questions

The strong obligate symbiosis between *Suillus* and the Pinaceae is an example of a positive plant-soil feedback. Plant-soil feedbacks are often associated with biological invasions, and have numerous downstream effects on ecosystems (Simberloff & Von Holle, 1999; Simberloff, 2006). *Pinus* plantations are known to greatly alter soil biogeochemistry by increasing soil acidity and litter production, and depleting soil carbon (Chapela *et al.*, 2001). This feedback is thought to be one of the primary factors preventing effective ecological restoration (Hoeksema *et al.*, 2020). Even after host removal, the enzymatic function of soil can be slow to recover (Sapsford & Dickie, 2023). As primary colonizers of introduced Pineaceae forests, *Suillus* spp. likely contribute to the biogeochemical changes associated with plantation forestry. The precise role that *Suillus* fungi play in altering soil chemistry and the mechanisms involved in this phenomenon are active areas of research.

The combined impacts of simultaneous co-invasion can have disastrous consequences on native biodiversity, a process which has been termed "invasional meltdown" by Simberloff and Von Holle (1999). Whether facilitated directly by the fungus, or via host-mediated mechanisms, early evidence points toward the capacity of *Suillus* (and also likely *Rhizopogon*) to displace native fungal communities at least when the density of introduced hosts is high (Sapsford *et al.*, 2022; Mujic *et al.*, 2023). In linked plant-fungal co-invasions, it is difficult to uncouple the invasive capacity of the fungus from that of the host, complicating the question of whether *Suillus* should be treated as invasive species in their own right. Generally, ECM species are primarily constrained to their introduced hosts (Vellinga *et al.*, 2009; Policelli *et al.*, 2020). While the high partner specificity exhibited by *Suillus* should reduce overall invasion potential compared to host generalist species that are more likely to establish on novel hosts after introduction, this benefit seems to be overwhelmed by the influence of the early-successional life history traits exhibited by *Suillus* (Vlk *et al.*, 2020).

Previous work has shown no phylogenetic signal for informing which species of *Suillus* are likely to be introduced (Vellinga *et al.*, 2009), and while phylogenetically diverse *Suillus* species are capable of establishing in introduced ranges, occurrence records are dominated by a small number of species. Interesting questions remain as to what traits and qualities make these species invasive, while the majority of *Suillus* species have never been associated with introduction or linked plant-fungal co-invasions.

VII. Conclusions and future directions

Here, we have shown that *Suillus* fungi play essential roles in natural and managed ecosystems, have greatly added to our understanding of fungal evolution, the ecology of fungal-host and fungal-fungal interactions, the stress responses of ECM fungi to both biotic and abiotic factors, as well as to population genetics, comparative genomics, and invasion biology. *Suillus* holds immense potential to serve as a model genus to advance our understanding of the ecology and evolution of ECM systems. Additionally, the combination of experimental tractability, the availability of experimental protocols, the flexibility of a well-defined genus-level system, a large set of publicly available genomes and respective annotations, and the cultures associated with these genomes, make *Suillus* one of the few well-established ECM fungal models for addressing questions that can scale from genes to ecosystems.

VII.i: Outstanding questions and research priorities

Research leveraging *Suillus* over the last decade has helped to define and prioritize outstanding questions in the field. First, phylogenetic analysis to define and place taxa from under sampled areas will help to fully delineate taxonomic boundaries and clarify the evolutionary history of host switching and diversification in the genus. While the strength of the *Suillus* system is based at the genus level, selecting target species for deep investigation will allow researchers to leverage both intra- and interspecific comparisons to derive the true impacts of fungal trait variation. Currently, the most well-studied species of *Suillus* is *S. luteus*, driven largely by the fact that it was the first species in the genus to undergo whole genome sequencing (Kohler *et al.*, 2015). However, current efforts to develop other species including *S. brevipes, S. quiescens, S. salmonicolor, S. pungens,* and *S. tomentosus* will soon provide more insight into trait and genome variation and provide a framework for testing specific hypotheses that have so far been difficult to address. These efforts include both population-level sampling, and chromosome-level reference assemblies, enabling a deeper understanding of genomic diversity, and the influence of genome structure on ecological traits.

Another research priority is to define the mechanisms facilitating partner specificity and partner switching in *Suillus*. This line of research will not only enhance our fundamental understanding of these ecological phenomena but also has practical implications for effective ecosystem management and conservation, particularly in the face of climate change-induced shifts in host tree distributions.

Ongoing research efforts using *Suillus* should aim to quantify key aspects of the ECM symbiosis such as realized nutrient trading dynamics. For instance, employing emerging techniques such as quantum dots and new advances in stable isotope tracing will help to address a multitude of both ecological and mechanistic questions related to nutrient cycling. In addition to quantifying carbon and nitrogen trading across species and environmental variables, defining the role and importance of non-canonically traded macro and micro-nutrients should be a priority for gaining a comprehensive understanding of metabolic interactions.

Clarifying the roles and mechanisms of non-nutritional partner benefits, such as stress tolerance to abiotic factors like drought, metal exposure, and ionizing radiation will help researchers to understand how *Suillus* and their hosts will adapt to a rapidly changing world. Understanding these variables will also help to identify the characteristics that contribute to the invasive potential of certain *Suillus* species, thereby enabling researchers to safeguard native ecosystems while supporting forestry practices.

Finally, research on the interactions between *Suillus* and other community members beyond the plant-fungal partnership is also vital for understanding the broader ecological context in which the ECM symbiosis operates. Exploring the consequences of these interactions, including those involving other fungi, bacteria, non-host plants, macro- and micro-fauna, will provide insights into the mechanisms of coexistence, competition, and facilitation.

VII.ii: Limitations

While the *Suillus* system presents numerous advantages, it is important to also acknowledge its limitations within specific research contexts. In fact, some of the very traits that make *Suillus* an ideal model for certain research objectives render it unsuitable for others. For example, the close association between *Suillus* and the Pinaceae, makes the genus valuable for studying partner specificity and diversification events associated with host switching, but also confines its applicability primarily to host associations within the Pinaceae (although associations of certain *Suillus* species with angiosperms may bring different perspectives). Similarly, because *Suillus* is naturally found only in the northern hemisphere where Pinaceae hosts are native, and in the southern hemisphere only as introduced species, it serves as an excellent system for studying reproductive bottlenecks, dispersal, and invasion biology across introduced ranges. These benefits do not negate the fact that *Suillus* is non-native on half the planet, limiting its applicability as a model for understanding many systems across the global south, such as understudied tropical ECM forests.

Finally, we emphasize that the advancement of the *Suillus* system as a model does not preclude the progress of other systems. On the contrary, we hope that formalizing the development of the *Suillus* model will catalyze researchers to establish similar collaborative working groups on other promising ECM model genera such as *Laccaria*, *Russula*, and *Pisolithus*, which may be better suited for specific research objectives. The ECM lifestyle is thought to have evolved independently at least 78 times over, and represents at least 251 distinct genera, with diverse traits, host-associations, and geographic distributions (Tedersoo & Smith, 2013). With recognition that no single set of taxa can hope to represent the entirety of ECM diversity, concentrating efforts around the development of carefully chosen groups will enable the rapid development of resources needed to address the most pressing questions in ECM ecology and evolution.

VII.iii: Technical challenges and future directions

Fully developing *Suillus* as a model genus presents some technical challenges that the community is currently working to address. Resolving these issues will soon open up a multitude of possibilities to further use *Suillus* as a means to understand ECM ecology, biology, and evolution.

First, using *Suillus* to fully address the mechanisms underlying ECM metabolism, requires the development of systems capable of tracking nutrient dynamics under field conditions, with adult trees, and in complex communities. Bolstered by the availability of a wide array of publicly available *Suillus* genomes, continual improvements in functional -omics and meta-omics approaches promise to facilitate comprehensive links between genomes and functional traits. These advancements are expected to have significant implications across both laboratory and field settings, facilitating a better understanding of ECM biodiversity across biological scales: linking genes to communities and ecosystems.

Another significant challenge is the complexity of working with heterokaryotic dikaryons; a biological consideration for most ECM basidiomycetes. Optimized protocols to dependably monokaryotize and dikaryotize *Suillus* strains in culture, along with efficient assays to confirm karyotic state, would go far to facilitate studies in ECM fungal genetics, gene regulation, and nuclear control.

An additional challenge common to most ECM fungi is the inability to dependably produce fruit bodies under laboratory conditions. In theory, this limitation is related to the size of the carbon stores necessary to support the development of reproductive structures. Seedling-based bioassays are favored in ECM studies because of the impracticality of manipulating large trees in the laboratory. This is thought to limit total photosynthetic capacity and the amount of carbon transferred to the fungus, particularly over limited time scales. Circumnavigating this limitation would require a system which increases carbon transfer from the host without disrupting the symbiosis, or a system for triggering fruit body development apart from the host entirely. The development of such a system would facilitate significant advances in our understanding of ECM genetics, enabling gene association studies, and studies in morphogenesis and histology.

Finally, the development of gene editing systems for *Suillus* will radically improve our ability to manipulatively address the genetic basis of adaptation, evolution, and the role of specific genes in important ecological and biological processes. The International *Suillus* Consortium considers the development of gene editing tools to be of the highest priority and has formed a working group to address this challenge, making use of multiple gene editing platforms and targeting multiple species in the genus.

VII.iv: Conclusion

Studies in *Suillus* have already made significant contributions to our understanding of the biology and ecology and evolution of the ECM symbiosis. The availability of tools and protocols optimized for *Suillus,* including specific approaches for *in vitro* culturing, plant partner bioassays, high quality DNA and RNA extraction included in Section VIII, along with the large number of publicly available reference genomes will catalyze future research to address long-standing questions in the field. We expect that continued community development of the *Suillus* system will solidify the genus as a model for understanding the ecology and evolution of plant-fungal mutualisms. We invite anyone interested in collaborating with The International *Suillus* Consortium to please contact the authors.

VIII. Resources and protocols

Despite the importance of ECM fungi to both natural and managed forests, manipulative work has largely lagged behind other experimental systems due in part to the technical challenges of working with host-associated, dikaryotic species. In an effort to standardize protocols and stimulate future improvements that will build off of these resources, we present a series of protocols for working with *Suillus* that will allow researchers to circumnavigate many of these challenges. These protocols are available as a series of supplemental files on topics ranging from axenic isolation and long-term storage, to mycorrhizal synthesis and the application of DNA based approaches. Here, we provide instruction for navigating these resources, outline specific use cases, limitations, and recommendations.

VIII.i: Fruit body and spore collection (Supplemental file S1)

One of the benefits of the *Suillus* system is that fruit bodies of the genus are conspicuous, frequently abundant, and relatively easy to identify. Both amateur and professional mycologists alike have long targeted the collection of charismatic *Suillus* mushrooms, with over 46,000 vouchered specimens currently digitized in the MyCoPortal collections database (https://www.mycoportal.org/) and over 62,000 observations of *Suillus* currently in iNaturalist (https://www.inaturalist.org/). Vouchered collections have facilitated phylogenetic reclassifications across the genus (Nguyen *et al.*, 2016b), illuminated the association between species diversification and host shifts (Zhang *et al.*, 2022), clarified species rarity (Kennedy *et al.*, 2020), and helped to identify the biotic and abiotic factors facilitating range shifts and invasion potential (Pietras *et al.*, 2018). The collection of *Suillus* fruit bodies is the first step to obtaining pure cultures, spores for use in bioassays, and preserved specimens for morphological studies or new species descriptions. Supplemental file S1 details the process of finding, identifying, and collecting *Suillus* fruit bodies for down-stream applications, and the processing of fruit bodies for spore collection and preservation.

VIII.ii: Isolation (Supplemental file S2)

Unlike many groups of ECM fungi, *Suillus* can be readily isolated and grown in pure culture. Cultures can be isolated fruit bodies, ECM rootlets, or basidiospores, to flexibly obtain dikaryotic or monokaryotic strains. Supplemental file S2 gives an overview of best practices and specific instructions for obtaining pure cultures of *Suillus* from all three of these sources.

VIII.iii: Culture conditions and culture storage (Supplemental file S3)

Suillus can accommodate growth on a variety of media types, but are most commonly cultured on Fries Media, Hagems Agar, Modified Melin-Norkrans (MMN) or Pachlewski's Media (Px). Recipes for all four media types, slightly modified from their original formulations for optimized *Suillus* growth, are available in Supplemental file S3, along with notes on optimized growth conditions and recommendations for culture storage and long-term culture preservation.

VIII.v: Sequencing (Supplemental files S4-S6)

Whole genome and next-generation sequencing using genomic, transcriptomic, and metatranscriptomic approaches have revolutionized our ability to identify and characterize ECM fungal communities (Bork *et al.*, 2015; Thompson *et al.*, 2017; Quince *et al.*, 2017; Lu & Salzberg, 2020), and helped to shed light on fungal ecology and evolution (Miyauchi *et al.*, 2020; Feurtey *et al.*, 2023; Ali *et al.*, 2023). In *Suillus*, these technologies have enabled the characterization of high resolution phylogenies, revealed the extent of intrageneric genome diversity, and helped to illuminate the functional mechanisms of important traits like stress tolerance and partner specificity (Liao *et al.*, 2016; Bazzicalupo *et al.*, 2020; Lofgren *et al.*, 2021; Erlandson *et al.*, 2022). However, compared to other ECM groups, *Suillus* spp. present unique technical challenges to sample preparation for -omics analysis. The genus is a notorious producer of pigments and extraction inhibitors that can complicate quality control measurements, interfere with PCR amplification, and confound DNA/cDNA library construction. DNA and RNA extraction from mixed biological samples such as mycorrhizal root tips is a critical but often difficult step in preparing material for molecular work. In general, ectomycorrhizae (which are a mix of plant and fungal tissue) contain polysaccharides, hydroxybenzenes, esters, and other secondary metabolites that can confound extraction protocols. Compared to other ECM groups, *Suillus* also has a greatly expanded secondary metabolite repertoire (Lofgren *et al.*, 2021), that likely further complicates DNA extraction. Additionally, because *Suillus* associates almost exclusively with plants in the family Pinaceae, which famously produce a wide array of secondary metabolites (Bashalkhanov & Rajora, 2008), the confounding effect of extraction inhibitors are likely compounded across the genus.

To minimize the impact of inhibitors, we have developed and optimized three methods for nucleic acid extraction that cover a broad range of applications including fresh and dried mushrooms, root-tips, cultures, and soil, and include them here as three separate Supplementary protocols. The NaOH extraction method yields good DNA for fast genotyping of cultures or root-tips (Supplementary file S4). The CTAB extraction method is generally used for whole genome sequencing and can be performed either for DNA alone, or as a DNA/RNA co-extraction (Supplementary file S5). The high-molecular weight DNA extraction method is generally good for long-read sequencing (Supplementary file S6).

VIII.vi: Host-*Suillus* bioassays (Supplemental file S7)

The tractability of *Suillus* spp. to *in vitro* mycorrhization via both spore inoculation and mycelial extension is one of the primary factors making this genus an excellent experimental system for ecological and evolutionary research. While spore-based inoculation methods are high-throughput and easily incorporate fungal genetic variation into mycorrhizal synthesis studies, mycelial-based methods provide a mechanism to control strain provenance and can be used to construct either mid-throughput pot-based assays, or low-throughput but highly flexible mesocosms that are otherwise axenic, facilitating the ability to control and manipulate the rest of the microbial community. All *Suillus* species we have examined to date are amenable to laboratory mycorrhization using spore and mycelial methods, achieving a high level of colonization (up to 100% of root tips) when paired with the appropriate host (Table S1). In Supplementary file S7, we provide an overview of these procedures and specific protocols for inoculating *Suillus* onto host trees to conduct bioassays using spore-based, and mycelial-based mycorrhizal synthesis.

VIII.vii: *Suillus*-host associations (Supplemental Table S1)

Understanding the specificity patterns between different *Suillus* species and their host trees in natural environments is fundamental to successful experimental design, interrupting high throughput sequence data, and to locating and identifying *Suillus* fruitbodies. In Table S1, we provide putative host associations for all currently recognized species of *Suillus* and as well as several additional taxa that are yet to be formally typified, but clearly represent independent species based on molecular analysis. Citations are provided for all putative host associations. Species that have one or more associated whole genome sequencing projects are noted.

Author contributions

SB, LL, and NHN developed the concept and outline for this manuscript. SB and LL coordinated all author contributions. LL synthesized and edited the initial and final drafts. SB and LL wrote section I, NHN wrote section II with contributions from LL. LL wrote section III with contributions from NHN and SB. PK and EP-P wrote section IV with contributions from NHN. SB, HW, AS, JF, JR, and KZ wrote section V. RV and LL wrote section VI. LL, SB, and NHN wrote section VII. LL wrote section VIII with contributions from H-LL, HW, HVTC, KMH, EE, and NHN.

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Author's note

Suillus fungi have long captivated the attention of the mycological community. The formalized efforts of the International *Suillus* Consortium over the last decade represent only the most recent expression of this ongoing research trajectory. The literature dedicated to *Suillus* is extensive, spanning over a century and encompassing a breadth of knowledge that far exceeds the scope of this text. Considering this context, the authors would like to acknowledge and appreciate the pioneering work of the many experts whose profound contributions paved the way for contemporary researchers and continue to inspire future advancements in the ecology and ecology of this enigmatic yet enchanting genus.

Figure Legends

Figure 1: Morphological and phenotypic variation across *Suillus*. A-H) Representative members of the major clades within the *Suillus* phylogeny: Larch associate species A) *S. ampliporus* showing the radiating pores characteristic most members of the genus. B) *S. clintonianus* displaying the copious viscus slime often produced by *Suillus* fruit bodies. C) *S. grisellus* highlighting morphological variation present just within the *Larix*-associated species. D) *Pseudotsuga*-associated *S. lakei* (Rocky Mountain form) highlighting the significant carbon allocation needed to form Suillus fruit bodies, which here exceed ~15 cm in diameter. *Pinus* associated species: E) *S. subalutaceus* representing species with longer stipes. F) *S. luteus*, the type species of the genus, displaying a prominent veil, a diagnostic feature for some species. G) *S. spraguei*, displaying distinctive red scales on the cap cuticle. H) *S. tomentosus* with robust fruiting bodies and variable bluing reactions of the trama tissue. I-P) Other diagnostic and morphologically important characteristics of *Suillus* I) A young specimen of *S. weaverae* showing milky droplets and clearly punctate stipe as a result of glandular cells. J) An old specimen of *S. caerulescens*, strongly staining blue, with insect larvae tunnels. K) Insect larvae quickly consume *Suillus* fruit bodies and can quickly ruin an attempt at making spore prints. L) A spore print of *S. lakei* on aluminum foil for spore collection. M) A portion of a *S. lakei* fairy ring with large fruit bodies which require substantial carbon allocations for the host. N) A mycorrhizal root of *S. paluster* with rhizomorphs at the base, typically found in *Suillus* mycorrhizae. O) The mycoparasitic *Chroogomphus vinicolor* (left) fruiting next to its host, a senescing *S. pungens* (right) P) Clusters of hyphae of *C. vinicolor,* stained dark violet with Melzer's reagent, within a mycorrhizal root of *S. pungens*.

Figure 2: Representatives of the *Suillus* genome-strain culture collection on four media types. Cultures were grown in 9 cm petri plates on four media types including (A, E, I, M) Modified Melin-Norkrans (MMN), (B, F, J, N) Modified Fries Media (Fries), (C, G, K, O) Modified Hagem's Agar (Hagem's), and (D, H, L, P) Pachlewski's Media (Pachlewski's). See supplemental material S3 for *Suillus* optimized media recipes. All media types were prepared at their full respective carbon concentrations and adjusted to pH 6 prior to autoclaving. Cultures were grown for 28 days, at room temperature, in the dark, prior to being photographed. Strains represented here are (A-D) *S. weaverae* FC27, (E-H) S. pungens FC27, (I-L) *S. fuscotomentosus* FC203, and (M-P) the monokaryotic strain *S. hirtellus* EM16. These four strains are part of the *Suillus* Genome Strain Culture Collection, and the *Suillus* phenotype database, SuilluScope. To access phenotype information and NRRL accession numbers for the full strain collection, please visit www.SuilluScope.com.

Figure 3: Total colony area over time for representatives of the *Suillus* genome-strain culture collection. Cultures were started by placing 3mm agar plugs on Modified Melin-Norkrans (MMN) media, adjusted to pH 6 prior to autoclaving, and grown in temperature adjustable incubators, in the dark, for a total of 33 days (at n=4 replicates per species per temperature treatment). Starting on day 8, colony area was recorded twice per week over the course of the assay by marking the colony margin on the back of each petri dish with a fine-tip sharpie. After 33 days of growth, the back of the petri plates were imaged using a flatbed scanner, and colony area was calculated for each time point using the program imageJ. The data presented here is for replicates grown at room temperature (20°C). To access growth data at other temperatures, and for the full *Suillus* Genome Strain Culture Collection please visit www.SuilluScope.com.

Figure 4: Associations between *Suillus* and their Pinaceae hosts. Time calibrated phylogenies of proposed *Suillus* subgenera and Pinaceae genera and subgenera, displaying distinct topologies and no indication of cospeciation. Molecular clock dating supports the earliest divergence of *Suillus* between 40.2 and 71.1 MYA depending on the method of fossil calibration used. Dashed lines represent host associations, and ancestral host-switching events, highlighting ancestral associations with *Larix* and the host switch of *Suillus* subgenus *Suillus* from the host subgenera *Strobus* to the subgenus *Pinus*, which is thought to have occurred at least four times independently. Figure redrawn

with permission from Zhang *et al.* (Zhang *et al.*, 2022).

Figure 5: Biological introduction records for A) all ECM fungi and B) Suillus. Records from the Global Database of Alien Macrofungi (Monteiro *et al.*, 2020) were pruned to exclude taxa with uncertain species-level assignments, and those annotated as having established from uncertain geographic origins. Guild was assigned across the database using FUNGuild retaining only species that could be confidently identified as ECM, resulting in 946 observations. A) Out of 55 genera of ECM fungi with introduction records, *Suillus* accounted for the majority of observed introductions, totaling 193 records. B) In total, 24 species of *Suillus* have introduction records, with the most common species identified as *S. luteus* (44 observations) and *S. granulatus* (34 observations), but was absent from the database (likely combined with the morphologically similar *S. brevipes*).

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