TITLE: Sniffing for fungi: Use of a conservation dog uncovers high regional truffle diversity

RUNNING TITLE: Truffle diversity dog case-study

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OPEN RESEARCH STATEMENT

Genetic data are available through GenBank (individual GenBank numbers are listed in Appendix S2). Vouchers will be deposited at Southern Oregon University and Oregon State University Herbaria (SOC and OSC) and associated photographic records are available on iNaturalist (listed in Appendix S2). A complete list of truffles and the sites that they were found at will be made available on OSF.io following manuscript acceptance; however, due to the sensitive nature of some species, exact location data will not be publicly available.

KEYWORDS: fungal conservation, fungal diversity, fungal ecology, hypogeous fungi, scent dog, truffle dog, public lands, U.S. Pacific Northwest, Cascade-Siskiyou National Monument

ABSTRACT: Hypogeous aromatic fungi ('truffles') contribute significantly to overall fungal diversity but are difficult to find using traditional survey methods because they fruit underground, leading to under-documentation and a lack of understanding of truffle ecology. Truffles evolved to emit strong aromatic compounds to attract mycophagists for spore dispersal, a trait that culinary truffle harvesters exploit using trained truffle dogs. We trained a conservation dog to recognize a broad range of truffle aromas across diverse fungal lineages to increase efficiency in finding these cryptic fungi. In this case-study, we demonstrate the value of using a 'truffle diversity dog' in the floristically diverse Cascade-Siskiyou National Monument (CSNM), located on the southwestern Oregon and northwestern California border, U.S.A. The dog uncovered high biodiversity in this region, locating 103 truffle species in 34 genera across 23 families. We found that this trained dog was particularly valuable for locating species that are visually difficult to distinguish from the substrate and species that produce few fruiting bodies. Fewer than half of the unique taxa matched public sequence records, and many are likely new to science. We found high species turnover between oak and conifer habitats, suggesting high host specificity in truffles. The wide range of fungal taxonomic diversity found in this study by a conservation dog demonstrates the value of training dogs to generalize to all types of truffle aroma, providing a promising method to efficiently describe understudied fungal biodiversity.

INTRODUCTION

Fungi represent an enormous and valuable biodiversity on earth, yet scientists struggle to quantify how many are at risk of extirpation or extinction (Tedersoo et al. 2021). An increased focus on environmental sequencing to address this lack of knowledge overlooks the importance of a 'collect and sequence' approach which has long-term added values derived from preserved specimens (Truong et al. 2017). Most fungal inventories focus on fungi that produce mushrooms or other epigeous fruiting bodies, overlooking hypogeous (underground) fungi and others that are not visually obvious. Hypogeous fungi are colloquially known as ‘truffles,’ a term encompassing culinary *Tuber* species and other polyphyletic fungi with a sequestrate (closed) form that fruit belowground. Truffles can contribute significant biodiversity to a regional flora and while less researched than their epigeous counterparts, some studies have explored truffle habitat niches and seasonality (e.g. Luoma et al. (1991) in the Pacific Northwest, Claridge et al. (2000) in Australia, and Nouhra et al. (2012) in Argentina). Most truffle species are ectomycorrhizal, so these taxa may be at risk when their mycorrhizal host is disturbed (Trappe et al. 2009). Truffles are critical to ecosystem health not just as ectomycorrhizal partners, but also as key forage for small mammals (Elliott et al. 2022).

Mature truffles produce aromatic compounds to attract mammal and invertebrate mycophagists that disperse truffle spores through their digestive tract (Elliott et al. 2022). Wildlife mycophagists find truffles by scent alone, using aromas that are highly variable and range from pleasant to pungent. Traditional survey methods involve scouting the forest floor for animal truffle digs and uncovering additional fruiting bodies beneath the litter layer with a rake. This is a simple but laborious method to search for truffles that can be biased towards larger light-colored truffles easily spotted by eye. Truffle dogs take advantage of the role aromas play in the truffle life cycle, using superior olfactory abilities to locate truffles with high precision (Čejka et al. 2022). Truffle dogs have been used reliably for centuries to find culinary truffles (Rosa-Gruszecka et al. 2017) and can also be trained to find diverse truffle aromas from non-culinary species (Stobbe et al. 2012, Molia et al. 2020, Berch et al. 2023, Dawson and Dawson 2024). With training, dogs could represent the forefront of truffle diversity research. Although limits to how many unique truffle aromas a trained dog will recognize and indicate are unknown, the dog we used has found truffles from ca. 50 genera to date.

We used this trained dog to document the truffle diversity and ecology of the Cascade-Siskiyou National Monument (CSNM), a region recognized for its high biodiversity (https://www.blm.gov/programs/national-conservation-lands/national-monuments/oregon-washington/cascade-siskiyou). This biodiversity is attributed to the intersection of the southernmost U.S. Pacific Northwest flora and the northernmost California flora (see Brooks 2021). Because most species of truffles are ectomycorrhizal with specific plants, the biodiversity of overlapping florae makes this region ideal for truffle exploration. Previous truffle research in the area has led to the description of at least 5 novel species (e.g., Frank et al. 2006b, Frank 2014, Southworth et al. 2018). Twenty-one truffle species are known from the local oak habitat, twelve of which were found in fecal pellets, indicating their role in cross-kingdom species interactions (Frank et al. 2006a). However, this represents only a fraction of the known species of the region, suggesting that more extensive and thorough surveys could yield further diversity.

METHODS

Sites

Elevation ranged from 821 m to 1939 m (Appendix 1: Table S1) and to a large degree elevation controlled the tree species present in each locality. The higher elevation conifer sites primarily consisted of ponderosa pine (*Pinus ponderosa*), white fir (hybrid; *Abies grandis* x *concolor*), sugar pine (*Pinus lambertiana*), and Douglas-fir (*Pseudotsuga menziesii*), and occasionally Shasta red fir (*Abies magnifica* var. *shastensis*). Mixed conifer and true fir sites were located at higher elevations centrally and in the northeast corner of CSNM, and oak savanna and woodland sites were located at lower elevations on the western and southern sides of CSNM (Figure 1). Oak sites were dominated by Oregon white oak (*Quercus garryana*) with scattered California black oak (*Quercus kelloggii*) and ponderosa pine. The mixed oak-conifer sites had patches dominated by Douglas-fir, ponderosa pine, Oregon white oak, and California black oak, with some mature madrone trees (*Arbutus menziesii*).

We visited 33 sites on 25 days between 31 October 2022 and 26 July 2024. Of these sites, 8 were visited multiple times at different seasons, with 7 foray days in autumn (after rain began but before multiple hard frosts), 6 in winter (after multiple hard frosts), 8 in spring (after thawing and leaf regrowth), and 4 in summer (after rains ceased) (Figure 1a, Table S1). Site regions were chosen by consulting mycology experts in the area who had experience surveying fungi in CSNM. As many sites as possible were visited over the feasible length of this project, based on availability of resources and funding.

Truffle hunting

All truffles were located by Rye, a conservation dog trained to detect truffle diversity, also called a "truffle diversity dog" (Figure 1b). While most truffle dogs focus on culinary truffles, Rye is trained to indicate any aroma from hypogeous fungi. At each site, he was cued to search and allowed to roam free to sniff for truffles. He indicated targets by digging until the truffle was excavated. During our first survey, we tested Rye's thoroughness at each site by performing a separate truffle search with a hand rake. However, we discontinued raking because in the initial trial we only found one immature sporocarp of a species found many times by Rye.

We collected each truffle that we presumed to be a unique species and documented its fresh characteristics. We marked a GPS point (Garmin eTrex or 67i) for every truffle with the highest level of identification that we could confidently make in the field. This level was typically to genus, except for some taxa with consistent presentation (Table 1). During our forays, we continued each search until the dog expressed no further interest, an indication that he found most of the truffles. We dried collected truffles at 35ºC. Each collected truffle has an associated iNaturalist observation with color images of fresh characteristics (Appendix S2).

DNA analyses

We extracted DNA from fresh or dry sporocarp tissue using an alkaline extraction method following Vandepol et al. (2020) with minor modifications. The majority of the sporocarp collections (except those collected during two forays, see below) were prepared for PacBio SMRT sequencing (Pacific BioSciences). We targeted a ~1600 bp region extending from the extreme terminus of the SSU through ~1000bp of the LSU region, including the full ITS, using the primers ITS1catta (ACCWGCGGARGGATCATTA; Tedersoo and Anslan 2019) and LR5 (TCCTGAGGGAAACTTCG; Hopple and Vilgalys 1994). Primers included 16-nt dual-indexed barcodes for multiplexing and a 5-nt padding sequence. PCR amplification was performed in 25 µL reactions containing 12.5 µL GoTaq® Green Master Mix, 0.4 µL 2% BSA, 1 µL primer/barcode pairs, 0.8 µL DNA template, and 10.3 µL nuclease-free water. Reaction conditions were set to an initial 2 min denaturation at 95ºC, followed by 30 cycles of 30 s at 95ºC, 30 s at 51ºC, and 90 s at 72ºC, then a final 7 min at 72ºC. Products were visualized on a 1% agarose gel via electrophoresis and pooled according to band brightness following Runnel et al. (2022). Pools were purified using Omega Mag-Bind® TotalPure NGS Beads at 0.8X to remove small DNA fragments. Purified libraries were sequenced on a PacBio Sequel II by the University of Oregon Genomics and Cell Characterization Core facility. Circular consensus sequences with read quality scores of ≥40 were processed using a custom bioinformatics pipeline

(C. Delevich, https://github.com/cdelevich/climush) with tools from VSEARCH (Rognes et al. 2016).

DNA from truffles collected during 2023 May/June and 2024 July surveys was obtained using Sanger sequencing. The target region for these collections was only the ITS region, using primers ITS1F (CTTGGTCATTTAGAGGAAGTAA; Gardes and Bruns 1993) and ITS4 (TCCTCCGCTTATTGATATGC; White et al. 1990). Similar PCR methods were used for these samples, and 15 µL of the unpurified products were sent to MC Lab (San Francisco, CA) for purification and sequencing with ITS1 and ITS4. Contigs were edited and assembled in Geneious Prime (2024.0.5).

Final assembled (Sanger) and de-replicated (PacBio) ITS sequences were compared with similar sequences in the GenBank nucleotide database using the web BLAST search (NCBI). Species identifications were determined based on a combination of morphological characteristics and percent similarity to validated vouchered sequences in the GenBank database. We use “cf.” to denote a close species match but with a margin of uncertainty based on ITS variation or a lack of good reference sequences and “aff.” for species complexes. For sequences with no close species matches, provisional names were assigned based on the collection number (“CS-”) of our first sequenced record.

To compare all truffle sequences obtained in this study, we used the MAFFT alignment plugin (v7.490; Katoh and Standley 2013) via Geneious Prime, and Geneious Prime tree building tools (Geneious Prime, v2024.0.5). Sequences were aligned within each genus to determine which collections represented unique taxa. If multiple PacBio sequence variants were present within an individual in similar abundance, those variants were compared against each other and the number of nucleotide differences was used to estimate a typical level of variation within the taxon. The sequence variant with the greatest abundance was used as the representative sequence in the GenBank submission (see Data Availability Statement).

Data analysis

Data were analyzed and visualized in R code v4.4.1 (R Core Team 2022) using a tidyverse framework (Wickham et al. 2019). Rarefaction curves were made using iNEXT (Hsieh et al. 2024).

RESULTS

Summary of finds

Rye successfully found truffles at every site during each visit except a visit to one site during snowmelt. Most truffles were found in the upper regions of the mineral soil or in the interface between the organic layer and mineral soil, but deeper finds were up to 30 cm underground. Rye found 1186 individual truffles at 33 sites. Of 237 sporocarps collected, 202 successfully yielded DNA. Endogonaceae and Glomeraceae collections had the highest failure rate and were only identified to family in the field. We collected 103 unique truffle species from 34 genera, 23 families, 11 orders, and four phyla. Based on the large number of sequences without a match in GenBank, many of these species may be undescribed (Table 1).

Truffle ecology

Both conifers and oaks supported a wide diversity of truffle species (Figure 3); however, there was little species overlap between the two habitats. We only found three species in both: *Hymenogaster* cf. *parksii*, *Xerocomellus* cf. *macmurphyi* (Figure 3a), and *Balsamia setchellii*. Fifty-three species were found exclusively in conifer habitat, as well as four species found in both conifer and mixed stands (Table 1). Thirty-four species were found exclusively in oak habitat, as well as three species found in oak and mixed stands. Six species were only found in mixed stands so we cannot determine their likely host.

Genea, Tuber, and *Balsamia* were the most abundant truffle genera, accounting for approximately half of our finds (Figure 2). Other genera were rarer, and some were limited to single or only a handful of finds, frequently only from one site. Based on the species accumulation curve, there are at least 152 ± 21.5 truffle species to be found in CSNM (95CI, sample coverage 81.9%; Appendix S1: Figure S1), of which we found two-thirds of in 25 foray days spread across all seasons.

Notable finds

A full treatment of all the truffles we collected from CSNM is beyond the scope of this paper, but certain truffle species and genera were of noteworthy conservation value (Appendix S3). *Balsamia* and *Genea* were among the most abundant and speciose, suggesting that CSNM may harbor unusual diversity for these genera (Boxes 1 and 4). We documented an IUCN Red List data deficient species (*Lepiota viridigleba*, Box 3) and unusual truffles closely related to crust fungi, one of which is under IUCN assessment (Box 2). Other taxa of conservation interest include *Elaphomyces, Choiromyces, Tuber, Sarcosphaera,* and *Xerocomellus*, and four instances of truffle parasitism detailed in Appendix S4.

ascomata.

DISCUSSION

Truffle diversity, ecology, and conservation

We found truffles at all sites throughout the year, indicating how widespread truffles are in CSNM. The 103 truffle species found represent 29% of the estimated truffle species diversity and 62% of the truffle genera known from the Pacific Northwest (Trappe et al. 2009). However, our two-year effort covered just two-thirds of the modeled diversity (Appendix S1: Figure S1). Our truffle collections and occurrence data offer important insight into the distribution and fruiting patterns of these cryptic and vulnerable organisms during a time of rapid climate change.

Of the 103 unique species identified, we could only match 40 to species names or other close identity through the GenBank BLAST database. GenBank sequence data provide incomplete coverage for North American hypogeous fungal taxa and it is uncertain how many of our collections may match described species without sequence data. For many truffle genera, confident identification to the species level without expert assistance is difficult, historically leaving many collections either unnamed or incorrectly named. Without better sequencing coverage of type specimens, many species that exist as only single or few collections will continue to be identified only to genus. DNA sequence data greatly improve the value of herbarium specimens and the taxonomic resolution of hypogeous fungi diversity estimates.

Ten genera we identified were found fruiting year-round, although they were not always present in all habitats (for example, in the summer *Balsamia* was only found with conifers and in the spring *Tuber* was only found with oaks; Fig. 2). This follows the known trend that truffles in southern Oregon are highly seasonal (Frank et al. 2006a). Only 3% of the truffle species identified in CSNM grew in both oak and conifer habitats, highlighting the importance of conserving both habitats for fungal diversity. Ectomycorrhizal fungi can have high host specificity (Bruns 2002) and most of the truffles documented in this survey are likely to participate in these symbiotic relationships. Out of the 34 genera that we identified, 26 are confirmed as mycorrhizal with oak and/or conifer hosts (Tedersoo and Smith 2013, Põlme et al. 2020). While most truffles are ectomycorrhizal (Trappe et al. 2009), we also found truffles from several saprotrophic genera: *Agaricus* and *Lepiota* (Agaricaceae), *Coniophora* and *Sedecula*

(Coniophoraceae), *Geastrum* and *Schenella* (Geastraceae), *Phallogaster* (Phallogastraceae), and *Trappea* (Trappeaceae) (Põlme et al. 2020, Davoodian et al. 2021). Many saprotrophic fungi are specialists and only found in specific habitats and ecological niches, despite not relying on living plant hosts (Zhou and Hyde 2001). Saprotrophic truffles were among some of our least commonly encountered taxa, indicating rarity in CSNM. These taxa were predominantly seen in the conifer habitats, except the novel *Coniophora* truffle found only with oaks and *Schenella pityophila,* which was found in conifer and mixed forests.

Despite the ubiquity and importance of fungi in ecosystems worldwide, conservation efforts for fungal diversity have been lacking, especially compared to plants and animals (Tedersoo et al. 2021). Many fungi listed on the IUCN Red List either remain under assessment due to a lack of data or are given the official designation 'Data Deficient' (Dahlberg and Mueller 2011). Five of the truffle species we identified from CSNM have been recommended for assessment through the IUCN (Appendix S1: Table S2). We found greater representation of the truffle species we collected in CSNM in the Oregon Biodiversity Information Center (ORBIC) rare species list with 13 of the species we recorded from CSNM list included in the January 2023 list ([ORBIC] Oregon Biodiversity Information Center 2023).

Inherent difficulties in assessing population sizes and distributions of underground-fruiting fungi are a key factor in the lack of official recognition for truffle taxa at risk for population decline or extinction. Distribution data can be more comprehensive for epigeous fungi because they are more easily spotted during both formal surveys and spontaneous observations. For hypogeous fungi, which typically require greater targeted efforts and a level of skill to reliably find, a lack of data can either mean the species is truly rare or that previous truffle surveys have been less successful in locating the hidden fruiting bodies. As a result, the more conspicuous fungi are easier to assess for population shifts, and often very little is known on the less conspicuous fungi.

Using a truffle diversity dog

Conservation dogs have been successfully used to target rare plant and animal taxa (Bennett et al. 2020) and can outperform other methods in locating species of interest (Grimm-Seyfarth et al. 2021). The label "conservation dog" is considered a parent term covering scent dogs trained for a variety of purposes, including locating plant and animal targets (Hurt and Smith 2009), but scent dogs are rarely utilized for fungal conservation (Amor et al. 2024). Using a "truffle diversity dog" in a conservation setting is a unique application of training a dog to locate target organisms by evolutionary strategy, rather than individual species. Many applications of scent-detection dogs require a high level of specialization, which requires proofing a single target odor under different conditions (DeMatteo et al. 2019). A dog's ability to detect a wide variety of aroma across different truffle lineages follows a similar type of training but demands a broader understanding from the dog of what "truffle" aroma is vs. a general "fungal" aroma.

While certain aromatic compounds have been identified as targets in culinary truffles (Epping et al. 2022), little is known about how dogs spontaneously generalize truffle aroma across a wide variety of fungal lineages. Their ability to efficiently locate truffles at a distance in the manner of wildlife mycophagists provides an advantage over traditional raking methods to locate these cryptic organisms. While the dog in our study was not trained to focus on either rare or common taxa, he routinely demonstrated the ability to switch between aromas produced by different taxa. We achieved this training by rewarding voluntary attempts by the dog to locate a truffle with a novel aroma. As a result, the dog placed importance on new aromas and experimented with alternative taxa. This reduces species-specific bias where the dog focuses solely on the most common taxon in the location and increases the chances that species with unfamiliar truffle aromas are also found. This training is generally undesirable for truffle dog handlers focused on harvesting culinary truffles, and as a result, the natural tendency of many truffle dogs to experiment with alternative aromas is frequently discouraged or ignored (e.g., Stobbe et al. 2012).

There is little understanding of how much "odor bias" determines which species may be more frequently targeted by dogs trained to find a wide range of truffle aromas. Future studies should focus on developing a metric for this bias as it likely varies between individual dogs. We theorize that dogs trained for culinary truffle species but also used for diversity surveys would show odor bias towards the species they most frequently receive a reward for finding. However, culinary truffles are often found in specific conditions and their presence would not distract the dog in other survey locations. We noticed that truffles with stronger aromas are more readily located at a distance by our dog, and those with weaker aromas have a smaller scent radius and require him to be in the right position to detect the aroma. The scent radius can also be influenced by meteorological and topographical conditions (Čejka et al. 2022). In absence of an unbiased, non-destructive, accurate large-scale survey method, the truffle dog method should be used as one of several valid techniques to assess truffle ecology and distribution.

Dogs can be particularly useful in locating aromatic but visually indistinct fungi. While some truffle taxa lack distribution data due to their rarity, other truffles are likely underrepresented because it is difficult to distinguish the fruiting body from soil and organic matter. For example, most *Genea* truffles are small, range from brown to black (Figure 7), and apt to be covered with soil. Prior to our surveys, *Genea* was not considered common in CSNM

(Scot Loring, pers. comm.). However, our truffle dog surveys revealed that *Genea* was the most abundantly fruiting truffle genus in CSNM, as well as the most speciose with 14 distinct taxa, only one of which matches a described species (*Genea arenaria*; Box 4, Figure 7A), although *G.* spp. CS45, CS46, and CS224 may fall within the *G. harknessii* species complex. Raking surveys have a visual bias towards larger sporocarps that contrast with the soil, but as truffle dogs rely on odor to locate truffles, these factors are rendered inconsequential. Some of the truffles Rye found were large and visually contrasted with the soil, but others were small and indistinguishable from the soil. Many of these finds would have likely gone unnoticed during a raking survey.

Traditional truffle survey methods have yielded the wealth of knowledge we have on truffles today, but these methods take a keen eye and patience. A handful of recognized experts have done a majority of the work on truffles in western North America such as early work by Harkness (Harkness 1899) and Helen Gilkey (Gilkey 1939) and modern work by Trappe, Trappe, Luoma, Castellano, and Cazares (Trappe et al. 2009). Our foray into truffle ecology is atypical as we [H.A.D. and H.R.D.] are relative newcomers to the field with only two years of experience in truffle diversity at the start of this study. Using a truffle diversity dog allowed us to thoroughly cover each site we visited with a minimal amount of time and effort, reduce unnecessary ground disturbance, and collect mature specimens, allowing for a better description of truffle characteristics and the preservation of valuable material in a fungarium for future study (Truong et al. 2017).

Conclusions

In this case study we demonstrate how a truffle dog trained to detect a wide range of truffle aromas can efficiently document ‘hidden’ diversity in the form of underground-fruiting fungi. In 25 days surveying the 46,134 ha Monument in 21 months from October 2022 to July 2024, we documented 103 truffle species just with one dog. Conservation dogs are well-established as a reliable method to detect nuisance fungi and rare animals and plants, so this case-study demonstrates that dogs can also be used to locate hard-to-find fungi that have evolved to be found via scent rather than visual cues.

We found truffle diversity varied throughout the Monument and throughout the year. Only nine genera were found year-round; and species varied seasonally within these genera. CSNM had 15 truffle species that are under evaluation by the IUCN Red List or listed as species of concern in Oregon, and many species without genetic matches in GenBank, including potential undescribed endemics. Both oak woodlands and conifer forests supported a diversity of truffle fungi with very little overlap in the species composition of the two habitats. This differentiation in community composition emphasizes the importance of continued management in CSNM to support both oak woodlands and conifer forests. It also demonstrates the importance of maintaining forests with botanical diversity rather than monotypic timber plantations of uniformly aged trees.

ACKNOWLEDGEMENTS

We thank Scot Loring for advice on where to find truffle diversity hotspots at CSNM, Tina Arredondo for assistance with PacBio sequencing methods, Matthew Smith for advice on this manuscript, and Lucas C.R. Silva for guidance and supervision of H.R.D. This study was funded by two the Friends of CSNM student research grants and NSF DEB-2106130 Macrosystems Biology with supplementary funding provided by Lucas C.R. Silva. Work was also supported by the NSF GRFP under Grant No. 2236419-DGE. Locating truffle diversity in CSNM would been impossible without Rye's superb nose–good boy, Rye.

AUTHOR CONTRIBUTIONS

Conceptualisation: HAD, HRD; Data curation: HRD, HAD, CD; Formal analysis: HAD, HRD, CD; Funding: HRD, HAD, BR; Investigation: HAD, HRD, JLF; Methodology: all authors; Resources: JLF, BR; Supervision: JLF, BR, HRD; Writing-original draft: HAD, HRD; Writing-review and editing: all authors.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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BOXES

Box 1: Balsamia

The ectomycorrhizal truffle genus *Balsamia* (Helvellaceae) is widespread across western North America, with 12 species currently described from this region (Southworth et al. 2018). We found *Balsamia* truffles fruiting in high abundance in CSNM. At least five of 12 described western North American *Balsamia* species are found within CSNM: *B. alba*, *B. filamentosa*, *B. latispora*, *B. quercicola* (Figure 4), and *B. setchellii*. Three of these species are listed as species of concern through ORBIC (Table S2; 2023). Additionally, we sequenced two potentially novel taxa. *B.* sp. CS101, related to *B. quercicola* and *B.* sp. CS166, related to *B. cascadensis*.

We found distinct seasonality and habitat patterns for *Balsamia* in CSNM. *Balsamia* was the most abundantly fruiting truffle genus during winter, with over half our *Balsamia* finds in that season. Mature *Balsamia* truffles were consistently found near buckbrush (*Ceanothus cuneatus*, Rhamnaceae) in oak habitat, occasionally at great distance from oaks. *C. cuneatus* is a nitrogen-fixing shrub not known to form ectomycorrhizal relationships (Spears et al. 2001), but we suspect some form of symbiotic relationship between *Balsamia* and *Ceanothus*. We highlight the need for further study on this potential relationship between *Ceanothus* presence and *Balsamia*

Box 2: Sededcula *and* Coniophora

Two species of truffles we found from the wood decay family Coniophoraceae (Boletales), *Sedecula pulvinata* (Figure 5a) under conifers and a novel *Coniophora* sp. (Figure 5b) under oaks**,** represent an unusual transition from a resupinate form to a sequestrate form (Trappe et al. 2015) with only one other known transition in Stephanosporaceae (Lebel et al. 2015). *Sedecula pulvinata* is currently the only recognized truffle-forming species in Coniophoraceae, and is under IUCN assessment (https://redlist.info/iucn/species_view/290852/). We found this species in dry conifer forest in autumn and recorded it from three locations. While the fruiting bodies are inconspicuous, they are typically large and found near the surface.

In November 2023, we found two collections of a large, amorphous truffle under *Quercus garryana.* The gleba of this truffle was dense and marbled, with soil particles embedded between the tightly packed veins and producing a chemical, nutty odor. Sequence data show this truffle is closely related to crust fungus *Coniophora marmorata,* and we plan to describe this novel species in a separate publication.

Box 3: Lepiota viridigleba

We found four occurrences of the little-known truffle species *Lepiota viridigleba* localized under mixed conifers at 1645 m in June 2023 (Figure 6). These sporocarps were all uniquely blue-green and aromatic, smelling sweet and almond-like. We did not see this species during any other surveys and consider it rare in CSNM. It is otherwise known from two locations in California and was designated Data Deficient on the IUCN Red List based on the paucity of records (https://redlist.info/iucn/species_view/541256/). The habitat of our records in CSNM is very similar to the record from Siskiyou County, California, with shared tree species *Abies concolor* and *Abies magnifica,* as well as a similar elevation (~1525m). Further surveys in this habitat could help determine the risk level of this species.

Box 4: Genea

The ectomycorrhizal truffle genus *Genea* (Pyronemataceae) is widespread across Europe and North America, producing warty, lobed ascomata with open to convoluted interior chambers. Around 60 species of *Genea* are currently listed in the MycoBank database (Crous et al. 2004), with only 5 from western North America: *G. harknessii* (species complex), *G. compacta*, *G. bihymeniata*, *G. arenaria*, and *G. gardneri* (Smith et al. 2006). *Genea* truffles are notoriously difficult to distinguish in the field. Our results suggest there is greater undescribed diversity in this genus than previously expected. Truffle dogs may be the best method to expand our currently limited knowledge on North American *Genea* diversity and ecology as they show an affinity for the highly aromatic sporocarps (Berch et al. 2023, Dawson and Dawson 2024).

Genea was the most abundant truffle genus we recorded in CSNM (n = 229 sporocarps, Figure 2a). Most were found in the spring (n = 158, Figure 2d) and most from oak habitat (n = 165). We identified a single species with a brown peridium as *G. arenaria*, consistently found under oak (Figure 7a). The number of unnamed *Genea* we found suggests that the CSNM is a hotspot for *Genea* diversity.

TABLES

Table 1: Presence/absence list of truffle species found in CSNM by taxonomic group including which habitat(s) and season(s) they were recorded.

	Habitat			Season			
Taxon	**Conifer**	**Oak**	**Mixed**	**Winter**	**Spring**	**Summer**	**Autumn**
Ascomycota							
Eurotiales							
Elaphomycetaceae							
Elaphomyces	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Elaphomyces decipiens	X	--	--	--	--	X	--
Elaphomyces sp. CS2	--	--	X	X	--	--	X
Elaphomyces sp. CS50	--	X	--	--	X	--	--
Elaphomyces sp. CS64	X	--	--	--	X	X	--
Elaphomyces sp. CS72	--	X	--	--	--	X	--
Ascomycota							
Pezizales							
Discinaceae							
Hydnotrya	**X**	**--**	**--**	**--**	**--**	**X**	**--**
Hydnotrya sp. CS240	X	--	--	--	--	X	--
Hydnotrya sp. CS245	X	--	--	--	--	X	--
Helvellaceae							
Balsamia	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Balsamia aff. filamentosa	--	X	X	X	--	--	--
Balsamia aff. latispora	X	--	--	X	X	X	--
Balsamia alba	--	X	--	--	X	--	--
Balsamia quercicola	--	X	--	X	--	--	X
Balsamia setchellii	X	X	--	X	--	--	--
Balsamia sp. CS101	X	--	X	--	--	--	X
Balsamia sp. CS166	X	--	--	X	--	--	--
Morchellaceae							
Leucangium	**X**	**--**	**--**	**X**	**X**	**--**	**--**
Leucangium carthusianum	X	--	--	X	X	--	--
Pezizaceae							
Cazia	**--**	**X**	**--**	**--**	**X**	**--**	**--**
Cazia flexiascus	--	X	--	--	X	--	--
Paragalactinia	**--**	**X**	**--**	**--**	**X**	**X**	**--**
Paragalactinia infossa	--	X	--	--	X	X	--

	Habitat			Season			
Taxon	**Conifer**	**Oak**	**Mixed**	**Winter**	**Spring**	**Summer**	**Autumn**
Sarcosphaera	**X**	**X**	**--**	**--**	**X**	**X**	**--**
Sarcosphaera setchellii	--	X	--	--	X	X	--
Sarcosphaera sp. CS252	X	--	--	--	--	X	--
Pyronemataceae							
Genea	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Genea arenaria	--	X	--	X	X	--	--
Genea sp. CS3	--	--	X	--	--	--	X
Genea sp. CS45	X	--	--	--	X	--	--
Genea sp. CS46	--	X	--	--	X	--	--
Genea sp. CS79	--	X	--	--	X	X	--
Genea sp. CS99	--	X	--	--	--	--	X
Genea sp. CS112	--	--	X	--	--	--	X
Genea sp. CS115	--	X	X	X	--	--	X
Genea sp. CS163	--	X	--	X	--	--	--
Genea sp. CS171	X	--	--	X	X	--	--
Genea sp. CS196	--	X	--	--	X	--	--
Genea sp. CS207	--	X	--	--	X	--	--
Genea sp. CS214	--	X	--	--	X	--	--
Genea sp. CS224	X	--	--	--	X	--	--
Geopora	**X**	**X**	**--**	**X**	**X**	**--**	**X**
Geopora sp. CS95	X	--	--	--	--	--	X
Geopora sp. CS147	X	--	--	X	--	--	--
Geopora sp. CS150	X	--	--	X	--	--	--
Geopora sp. CS189	--	X	--	--	X	--	--
Geopora sp. CS218	--	X	--	--	X	--	--
Tuberaceae							
Choiromyces	**X**	**--**	**--**	**X**	**X**	**X**	**X**
Choiromyces sp. CS5	X	--	--	X	--	X	X
Choiromyces sp. CS61	X	--	--	--	--	X	--
Tuber	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Tuber aff. anniae	X	--	--	X	--	--	X
Tuber beyerlei	X	--	--	--	--	--	X
Tuber candidum	--	X	X	X	X	X	X
Tuber luomae	X	--	--	--	--	--	X
Tuber quercicola	--	X	--	X	X	X	X
Tuber whetstonense	X	--	--	--	--	X	X
Tuber sp. CS22	X	--	--	--	--	--	X

	Habitat			Season			
Taxon	**Conifer**	**Oak**	**Mixed**	**Winter**	**Spring**	**Summer**	**Autumn**
Tuber sp. CS114	--	--	X	--	--	--	X
Basidiomycota							
Agaricales							
Agaricaceae							
Agaricus	**X**	**--**	**--**	**--**	**--**	**X**	**X**
Agaricus cf. inapertus	X	--	--	--	--	X	X
Lepiota	**X**	**--**	**--**	**--**	**--**	**X**	**--**
Lepiota viridigleba	X	--	--	--	--	X	--
Cortinariaceae							
Cortinarius	**X**	**--**	**--**	**--**	**--**	**--**	**X**
Cortinarius pinguis	X	--	--	--	--	--	X
Phlegmacium	**X**	**--**	**--**	**--**	**X**	**X**	**--**
Phlegmacium sublilacinum	X	--	--	--	X	X	--
Hymenogastraceae							
Hymenogaster	**X**	**X**	**X**	**X**	**X**	**X**	**--**
Hymenogaster cf. parksii	X	X	--	X	X	X	--
Hymenogaster cf. thwaitesii	--	X	--	--	X	--	--
Hymenogaster sp. CS193	--	X	--	--	X	--	--
Hymenogaster sp. CS234	--	X	--	--	X	--	--
Boletales							
Boletaceae							
Xerocomellus	**X**	**X**	**X**	**--**	**X**	**--**	**X**
Xerocomellus cf. macmurphyi	X	X	X	--	X	--	X
Coniophoraceae							
Coniophora	**--**	**X**	**--**	**X**	**--**	**--**	**--**
Coniophora sp. CS143	--	X	--	X	--	--	--
Sedecula	**X**	**--**	**X**	**--**	**--**	**--**	**X**
Sedecula pulvinata	X	--	X	--	--	--	X
Paxillaceae							
Alpova	**X**	**--**	**--**	**--**	**--**	**X**	**--**
Alpova trappei	X	--	--	--	--	X	--
Melanogaster	**X**	**X**	**X**	**--**	**X**	**X**	**X**
Melanogaster euryspermus	--	X	--	--	X	X	--
Melanogaster cf. macrocarpus	X	--	--	--	X	--	--
Melanogaster cf. natsii	--	X	--	--	X	--	--
Melanogaster sp. CS249	X	--	--	--	--	X	--
Rhizopogonaceae							

	Habitat			Season			
Taxon	**Conifer**	**Oak**	**Mixed**	**Winter**	**Spring**	**Summer**	**Autumn**
Rhizopogon	**X**	**--**	**X**	**X**	**X**	**--**	**X**
Rhizopogon arctostaphyli	X	--	--	--	--	--	X
Rhizopogon cf. hawkerae	X	--	X	X	--	--	X
Rhizopogon cf. roseolus	X	--	--	--	--	--	X
Rhizopogon sp. CS10	X	--	--	--	--	--	X
Rhizopogon sp. CS174	X	--	--	X	--	--	--
Sclerodermataceae							
Scleroderma	**--**	**X**	**--**	**--**	**X**	**--**	**--**
Scleroderma sp. CS210	--	X	--	--	X	--	--
Geastrales							
Geastraceae							
Geastrum	**X**	**--**	**--**	**--**	**--**	**--**	**X**
Geastrum sp. CS131	X	--	--	--	--	--	X
Geastrum sp. CS140	X	--	--	--	--	--	X
Schenella	**X**	**--**	**X**	**X**	**--**	**X**	**--**
Schenella pityophila	X	--	X	X	--	X	--
Gomphales							
Gomphaceae							
Gautieria	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Gautieria sp. CS16	X	--	--	--	--	--	X
Gautieria sp. CS17	X	--	--	--	--	--	X
Gautieria sp. CS53	X	--	--	--	X	X	--
Gautieria sp. CS56	X	--	--	--	--	X	--
Gautieria sp. CS82	--	X	--	--	X	X	--
Gautieria sp. CS137	X	--	--	--	--	--	X
Gautieria sp. CS169	X	--	--	X	--	--	--
Gautieria sp. CS225	X	--	--	--	X	--	--
Gautieria sp. CS235	--	--	X	--	X	--	--
Hysterangiales							
Hysterangiaceae							
Hysterangium	**X**	**X**	**--**	**X**	**X**	**X**	**X**
Hysterangium cf. setchellii	X	--	--	--	--	--	X
Hysterangium sp. CS38	--	X	--	X	X	X	X
Hysterangium sp. CS63	X	--	--	--	--	X	--
Hysterangium sp. CS76	--	X	--	--	--	X	--
Hysterangium sp. CS97	X	--	--	--	--	--	X
Hysterangium sp. CS215	--	X	--	--	X	--	--

	Habitat			Season			
Taxon	**Conifer**	**Oak**	**Mixed**	**Winter**	**Spring**	**Summer**	**Autumn**
Phallogastraceae							
Phallogaster	**X**	**--**	**--**	**--**	**X**	**--**	**--**
Phallogaster phillipsii	X	--	--	--	X	--	--
Phallales							
Trappeaceae							
Trappea	**X**	**--**	**--**	**--**	**--**	**--**	**X**
Trappea sp. CS127	X	--	--	--	--	--	X
Russulales							
Albatrellaceae							
Leucogaster	**X**	**--**	**--**	**X**	**--**	**--**	**X**
Leucogaster cf. odoratus	X	--	--	--	--	--	X
Leucogaster rubescens	X	--	--	--	--	--	X
Leucogaster sp. CS18	X	--	--	--	--	--	X
Leucophleps	**X**	**--**	**X**	**--**	**--**	**X**	**X**
Leucophleps sp. CS113	--	--	X	--	--	--	X
Leucophleps sp. CS247	X	--	--	--	--	X	--
Russulaceae							
Lactarius	**X**	**X**	**--**	**X**	**X**	**X**	**X**
Lactarius sp. CS40	--	X	--	X	X	X	--
Lactarius sp. CS123	X	--	--	--	--	--	X
Russula	**X**	**X**	**--**	**X**	**X**	**X**	**X**
Russula cf. similaris	X	--	--	X	--	--	X
Russula sp. CS77	--	X	--	--	--	X	--
Russula sp. CS81	--	X	--	--	--	X	--
Russula sp. CS205	--	X	--	--	X	--	--
Glomeromycota							
Glomerales							
Glomeraceae	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Glomus	**--**	**X**	**--**	**--**	**X**	**--**	**--**
Glomus sp. CS195	--	X	--	--	X	--	--
Mucoromycota							
Endogonaceae							
Jimgerdemannia	**X**	**X**	**--**	**X**	**--**	**X**	**--**
Jimgerdemannia sp. CS155	X	--	--	X	--	--	--

Note that except for eleven species that could be confidently identified in the field (*Agaricus inapertus, Cazia flexiascus, Cortinarius pinguis, Genea arenaria, Leucangium carthusianum,* *Paragalactinia infossa, Sarcosphaera setchellii, Schenella pityophila, Sedecula pulvinata, Tuber candidum,* and *T. quercicola*), all species presences listed here are based on evidence from sequenced collections only. As such, this is a conservative record of where they occur as we did not analyze DNA for every sporocarp unearthed. Genera were consistently identified in the field and represent a broader understanding of where and when these taxa produce truffles.

FIGURE LEGENDS

Figure 1: Map of the site locations included in truffle surveys from October 2022 to July 2024 (A) and a photograph of Rye, the truffle dog, locating a truffle (B). Sites are colored by dominant habitat type, although multiple habitats may have been present (Appendix S1: Figure S1). Numbers indicate multiple site visits. Satellite imagery from ESRI. Photograph by Heather Dawson.

Figure 2: Number of truffles found in two years of sampling (by genus). A) All truffle genera across seasons. B-E represent finds within a single season colored by the habitats of that season's finds. Numbers above each bar indicate the total number of truffles of that genus found. Colors indicate the habitats each genus is found in (green = conifer, red-brown = oak, tan = both conifer and oak.

Figure 3. Examples of CSNM truffle diversity from conifer habitat (A-E) and oak habitat (F-J). A.) *Tuber* aff. *anniae*. B.) *Hydnotrya* sp. CS245. C.) *Xerocomellus* cf. *macmurphyi*. D.) Parasitized *Choiromyces* sp. E.) *Lactarius* sp. CS123. F.) *Melanogaster* cf. *euryspermus*. G.) *Cazia flexiascus*. H.) *Sarcosphaera setchellii*. I.) *Elaphomyces* sp. CS50. J.) *Tuber quercicola*. All photographs by Heather Dawson.

Figure 4. *Balsamia quercicola*. Photograph by Heather Dawson.

Figure 5. A.) *Sedecula pulvinata,* B.) the novel truffle *Coniophora* sp. CS143. Photograph A by Jonathan Frank; photograph B by Heather Dawson.

Figure 6. *Lepiota viridigleba.* Photograph by Jonathan Frank.

Figure 7. *Genea* truffles cut in half to show a chambered interior. A.) *Genea arenaria*, a brown species of *Genea*. B.) *Genea.* sp. CS99, an undescribed species with a black peridium. Both photographs by Heather Dawson.

FIGURES

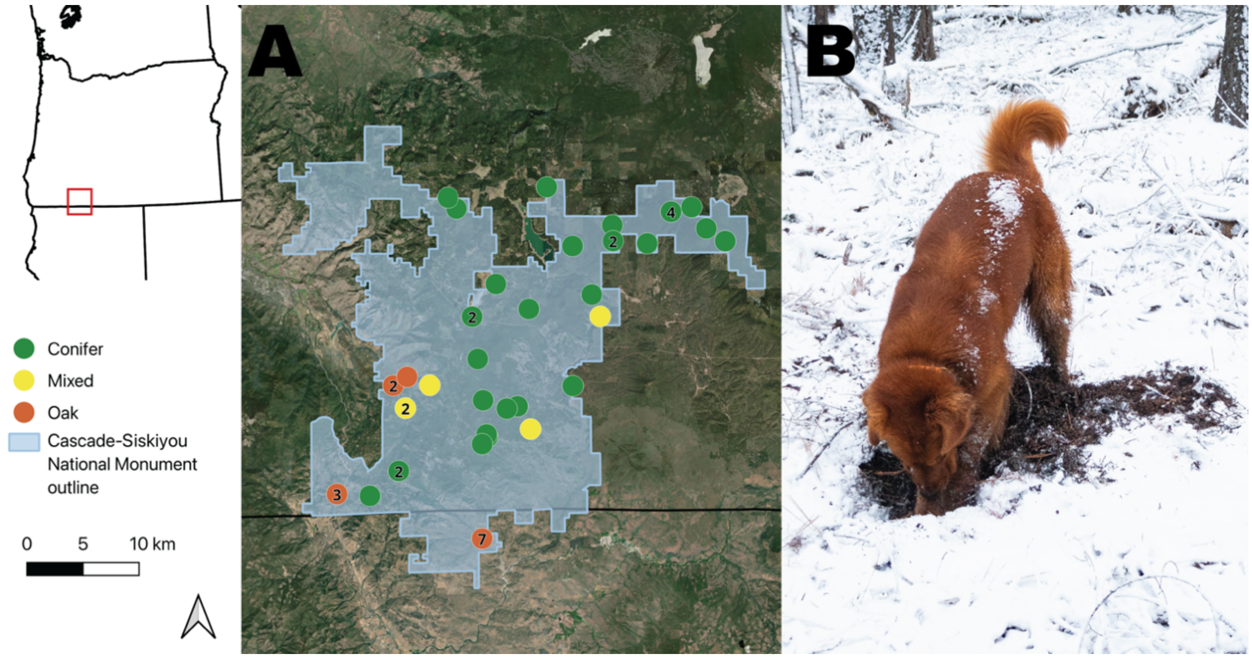


Figure 1

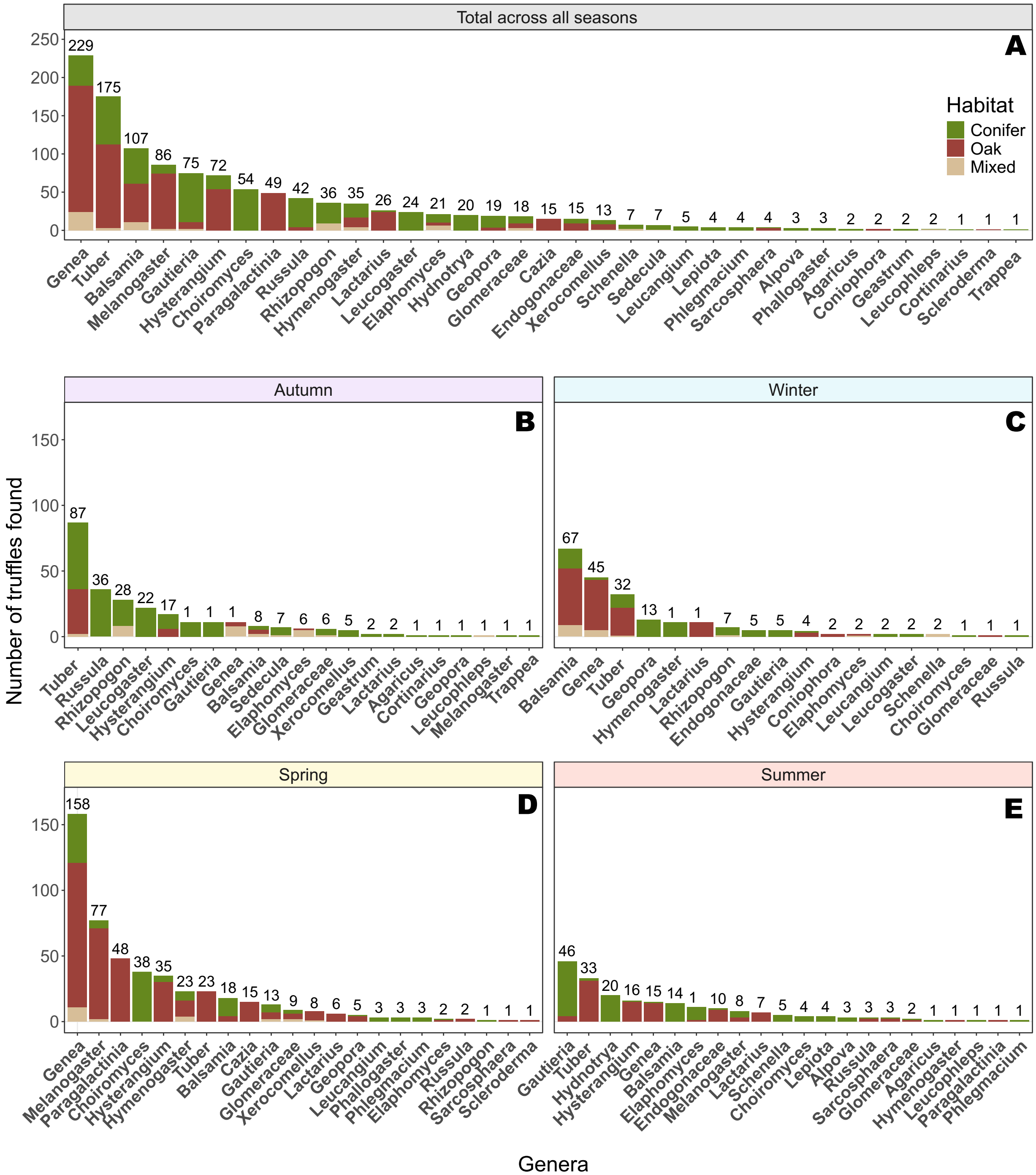
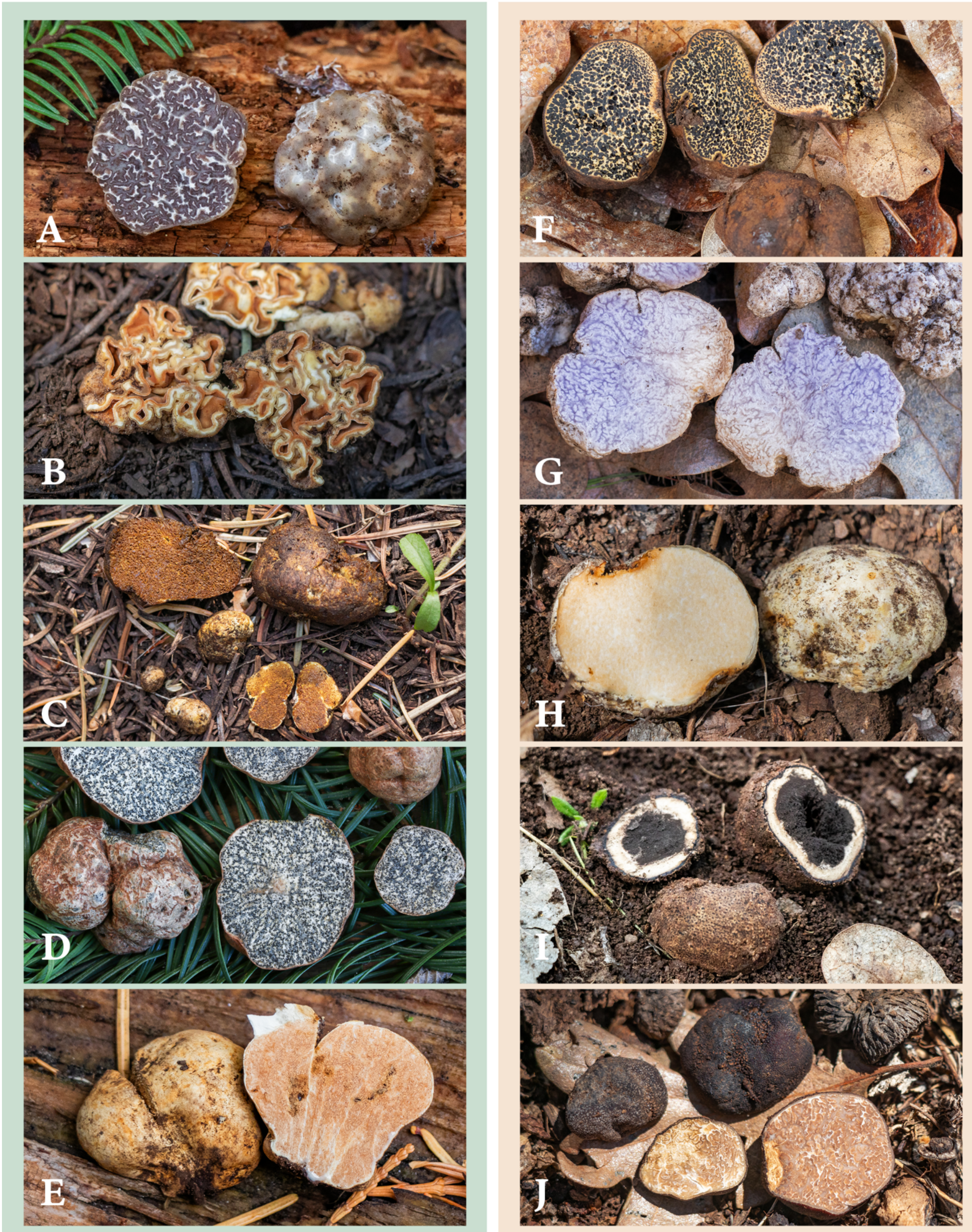

Total across all seasons
A
Habitat
Conifer
Oak
Mixed
Autumn
B
Winter
C
Spring
D
Summer
E
Number of truffles found
Genera
Genea Tuber Balsamia Melanogaster Gautieria Hysterangium Choiromyces Paragalactinia Russula Rhizopogon Hymenogaster Lactarius Leucogaster Elaphomyces Hydnotrya Geopora Glomeraceae Cazia Endogonaceae Xerocomellus Schenella Sedecula Leucangium Lepiota Phlegmacium Sarcosphaera Alpova Phallogaster Agaricus Coniophora Geastrum Leucophleps Cortinarius Scleroderma Trappea
229 175 107 86 75 72 54 49 42 36 35 26 24 21 20 19 18 15 15 13 7 7 5 4 4 4 3 3 2 2 2 2 1 1 1
Tuber Russula Rhizopogon Leucogaster Hysterangium Choiromyces Gautieria Genea Balsamia Sedecula Elaphomyces Glomeraceae Xerocomellus Geastrum Lactarius Agaricus Cortinarius Geopora Leucophleps Melanogaster Trappea
87 36 28 22 17 1 1 1 8 7 6 6 5 2 2 1 1 1 1 1 1
Balsamia Genea Tuber Geopora Hymenogaster Lactarius Rhizopogon Endogonaceae Gautieria Hysterangium Coniophora Elaphomyces Leucangium Leucogaster Schenella Choiromyces Glomeraceae Russula
67 45 32 13 1 1 7 5 5 4 2 2 2 2 2 1 1 1
Genea Melanogaster Paragalactinia Choiromyces Hysterangium Hymenogaster Tuber Balsamia Cazia Gautieria Glomeraceae Xerocomellus Lactarius Geopora Leucangium Phallogaster Phlegmacium Elaphomyces Russula Rhizopogon Sarcosphaera Scleroderma
158 77 48 38 35 23 23 18 15 13 9 8 6 5 3 3 3 2 2 1 1 1
Gautieria Tuber Hydnotrya Hysterangium Genea Balsamia Elaphomyces Endogonaceae Melanogaster Lactarius Schenella Choiromyces Lepiota Alpova Russula Sarcosphaera Glomeraceae Agaricus Hymenogaster Leucophleps Paragalactinia Phlegmacium
46 33 20 16 15 14 1 10 8 7 5 4 4 3 3 3 2 1 1 1 1 1

Figure 2



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603 Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

APPENDIX 1: **Supplementary tables and figure**

Table S1. List of sites and characteristics. Elevation data from Ficks and Hijmans (2017).

		Habitats present at site			Number of site visits per season			
Site	Elevation (m)	Mixed	Conifer	Oak	Autumn	Winter	Spring	Summer
40-4E-12.1	1222	--	X	--	1	--	--	--
Baldy Mountain	1072	X	X	--	--	1	--	--
Beaver Creek Rd	1441	--	X	--	--	--	1	--
Bluejay Creek	1323	--	X	--	1	--	--	--
Buck Prairie Snow Park	1696	--	X	--	--	--	1	--
Buck Rock	900	X	X	X	--	2	1	--
Chinquapin Mtn Rd	1511	--	X	--	--	1	--	--
Conde Creek	1580	--	X	--	--	1	--	--
Copco Rd	1122	--	X	--	--	--	1	--
Horseshoe Ranch	821	--	X	X	--	2	4	1
Hyatt Lake	1574	--	X	--	1	--	1	--
Jenny Creek	1154	--	X	X	1	--	--	--
Keene Creek Ridge	1603	--	X	X	1	--	--	--
Keno Access Lower	1433	--	X	--	--	1	--	--
Mariposa Meadows	1066	--	--	X	1	1	--	1
Mill Creek Rd	1603	--	X	--	1	--	--	--
North Hyatt Lake	1560	--	X	--	--	1	--	--
PCT	1439	--	X	--	1	--	--	--
PCT Pilot Rock	1514	X	X	X	1	--	1	--
PCT South	1324	--	X	--	1	--	--	--
PCT Big Draw	1710	--	X	--	--	--	--	1
Parsnip Lakes	1373	--	X	--	1	--	--	--
Pilot Rock Trail	1419	X	X	X	--	--	1	--
Randcore Pass	1323	X	--	X	1	--	--	--
Rock Slide	1939	--	X	--	--	--	--	1
Site 1	975	X	--	--	1	1	--	--
Skookum Creek Rd	1568	X	X	X	1	--	--	--
Surveyor	1622	--	X	--	2	--	1	1
Surveyor Campground	1601	--	X	--	1	--	--	--
Surveyor Lower	1498	--	X	--	--	1	1	--
Surveyor Peak	1824	--	X	--	--	--	1	--

		Habitats present at site			Number of site visits per season			
Site	Elevation (m)	Mixed	Conifer	Oak	Autumn	Winter	Spring	Summer
T-Forks	1383	--	X	--	--	--	1	--
Tyler Road	825	--	--	X	--	1	--	--

Table S2. Truffles with conservation assessments located in the Cascade-Siskiyou National Monument during this study.

Species	IUCN Red List rank	ORBIC rank
*Balsamia alba**	–	Rank 1
Balsamia quercicola	–	Rank 3
Balsamia setchellii	–	Rank 3
Cazia flexiascus	–	Rank 3
Choiromyces alveolatus	Proposal/Assessment	Rank 3
Elaphomyces decipiens	–	Rank 4
Hydnotryopsis setchellii	Proposal/Assessment	–
Lepiota viridigleba	Data deficient	Rank 1
Leucogaster odoratus	–	Rank 3
Melanogaster natsii	–	Rank 3
Phallogaster phillipsii	Proposal/Assessment	–
Sedecula pulvinata	Proposal/Assessment	Rank 3
Tuber luomae	–	Rank 3
Tuber quercicola	–	Rank 3
Tuber whetstonense	–	Rank 1

Notes: Assessments were from the International Union for Conservation of Nature (IUCN) and Oregon Biodiversity Information Center (ORBIC). ORBIC ranks correspond with Rank 1 = Critically imperiled, 2 = Imperiled, 3 = Rare, uncommon or threatened, but not immediately imperiled, 4 = Cause for long-term concern, 5 = Demonstrably widespread, abundant, and secure. *We collected this taxon from roughly 3 km south of the Oregon border in California

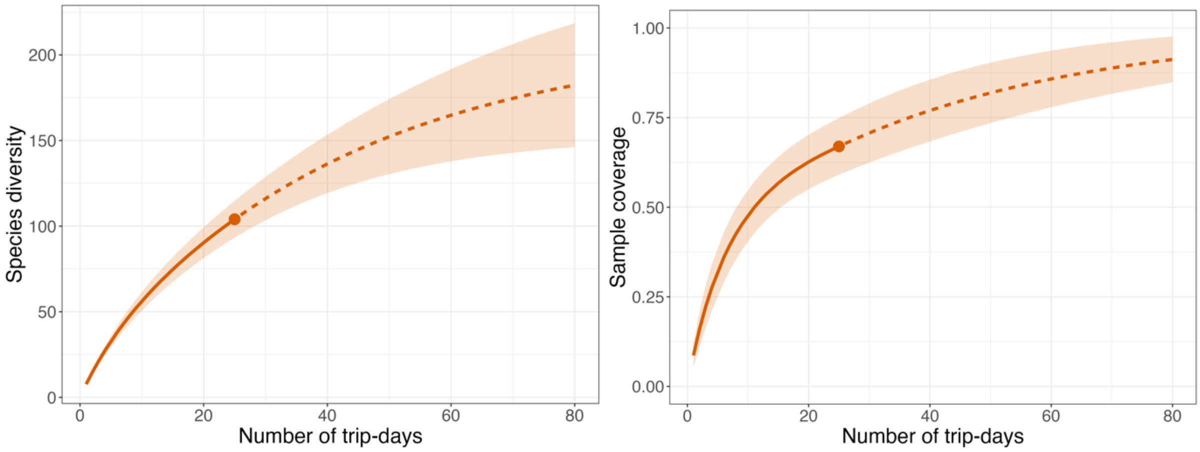

Figure S1. Rarefaction curve illustrating a) extrapolated species diversity and b) sampling completeness. Solid line indicates species accumulation curve to date across both years of sampling. Dotted line indicates extrapolated accumulation. Solid shape indicates 95% confidence interval.

APPENDIX S1 REFERENCES

Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37:4302–4315.

Collection _nr	Species	iNaturalist_Nr	iNaturalist_URL	GenBank_ Nr_ITS	GenBank_ Nr_LSU	Sequencing _method
CS1	Elaphomyces	140850376	https://www.inaturalist.org/observations/140850376	--	--	--
CS2	Elaphomyces sp. CS2	140850469	https://www.inaturalist.org/observations/140850469	PQ408974	PV061897	PacBio
CS3	Genea sp. CS3	140850747	https://www.inaturalist.org/observations/140850747	PQ408975	PV061898	PacBio
CS4	Genea sp. CS3	140850754	https://www.inaturalist.org/observations/140850754	PQ408976	PV061899	PacBio
CS5	Choiromyces sp. CS5	140880401	https://www.inaturalist.org/observations/140880401	PQ408977	--	PacBio
CS6	Russula cf. similaris	140880274	https://www.inaturalist.org/observations/140880274	PQ408978	PV061900	PacBio
CS7	Russula cf. similaris	140880149	https://www.inaturalist.org/observations/140880149	PQ408979	PV061901	PacBio
CS8	Russula cf. similaris	140924605	https://www.inaturalist.org/observations/140924605	PQ408980	PV061902	PacBio
CS9	Leucogaster cf. odoratus	140851929	https://www.inaturalist.org/observations/140851929	PQ408981	PV061903	PacBio
CS10	Rhizopogon sp. CS10	140851903	https://www.inaturalist.org/observations/140851903	PQ408982	PV061904	PacBio
CS11	Choiromyces sp. CS5	140851877	https://www.inaturalist.org/observations/140851877	PQ408983	--	PacBio
CS12	Tuber aff. anniae	140851834	https://www.inaturalist.org/observations/140851834	PQ408984	PV061905	PacBio
CS13	Tuber aff. anniae	140851729	https://www.inaturalist.org/observations/140851729	PQ408985	PV061906	PacBio
CS14	Tuber aff. anniae	140851707	https://www.inaturalist.org/observations/140851707	PQ408986	PV061907	PacBio
CS15	Choiromyces sp. CS5	140851644	https://www.inaturalist.org/observations/140851644	PQ408987	PV061908	PacBio
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CS18	Leucogaster sp. CS18	140851439	https://www.inaturalist.org/observations/140851439	PQ408990	PV061911	PacBio
CS19	Tuber whetstonense	140851168	https://www.inaturalist.org/observations/140851168	PQ408991	PV061912	PacBio
CS20	Tuber aff. anniae	140850961	https://www.inaturalist.org/observations/140850961	PQ408992	PV061913	PacBio
CS21	Hysterangium cf. setchellii	140850854	https://www.inaturalist.org/observations/140850854	PQ408993	PV061914	PacBio
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CS24	Tuber aff. anniae	140881643	https://www.inaturalist.org/observations/140881643	PQ408996	PV061916	PacBio
CS25	Tuber beyerlei	140881564	https://www.inaturalist.org/observations/140881564	--	--	--
CS26	Choiromyces	140881631	https://www.inaturalist.org/observations/140881631	--	--	--
CS27	Tuber beyerlei	140881435	https://www.inaturalist.org/observations/140881435	PQ408997	PV061917	PacBio
CS28	Genea	148081024	https://www.inaturalist.org/observations/148081024	--	--	--
CS29	Balsamia	148080976	https://www.inaturalist.org/observations/148080976	--	--	--
CS30	Balsamia aff. filamentosa	148124803	https://www.inaturalist.org/observations/148124803	PQ408998	PV061918	PacBio
CS31	Balsamia	148099278	https://www.inaturalist.org/observations/148099278	--	--	--

CS32	Balsamia setchellii	148099497	https://www.inaturalist.org/observations/148099497	PQ408999	PV061919	PacBio
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CS102	Tuber luomae	185906873	https://www.inaturalist.org/observations/185906873	PQ409020	PV061944	PacBio
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CS107	Leucogaster	185911750	https://www.inaturalist.org/observations/185911750	--	--	--
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CS109	Choiromyces sp. CS5	185912458	https://www.inaturalist.org/observations/185912458	--	--	--
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CS111	Unknown	185914693	https://www.inaturalist.org/observations/185914693	--	--	--
CS112	Genea sp. CS112	186002972	https://www.inaturalist.org/observations/186002972	PQ409027	PV061951	PacBio
CS113	Leucophleps sp. CS113	194708748	https://www.inaturalist.org/observations/194708748	PQ409028	PV061952	PacBio
CS114	Tuber sp. CS114	186003027	https://www.inaturalist.org/observations/186003027	PQ409029	PV061953	PacBio
CS115	Genea sp. CS115	186003135	https://www.inaturalist.org/observations/186003135	PQ409030	PV061954	PacBio
CS116	Balsamia quercicola	186003339	https://www.inaturalist.org/observations/186003339	PQ409031	PV061955	PacBio
CS117	Balsamia sp. CS101	186003383	https://www.inaturalist.org/observations/186003383	PQ409032	PV061956	PacBio
CS118	Rhizopogon cf. hawkerae	186003461	https://www.inaturalist.org/observations/186003461	PQ409033	PV061957	PacBio
CS119	Tuber beyerlei	186003481	https://www.inaturalist.org/observations/186003481	PQ409034	PV061958	PacBio
CS120	Hysterangium cf. setchellii	186003562	https://www.inaturalist.org/observations/186003562	PQ409035	PV061959	PacBio
CS121	Tuber whetstonense	186004101	https://www.inaturalist.org/observations/186004101	PQ409036	PV061960	PacBio
CS122	Russula	186004160	https://www.inaturalist.org/observations/186004160	--	--	--
CS123	Lactarius sp. CS123	186004186	https://www.inaturalist.org/observations/186004186	PQ409037	PV061961	PacBio
CS124	Tuber whetstonense	186005214	https://www.inaturalist.org/observations/186005214	PQ409038	PV061962	PacBio
CS125	Tuber beyerlei	186005256	https://www.inaturalist.org/observations/186005256	PQ409039	PV061963	PacBio
CS126	Tuber beyerlei	186005399	https://www.inaturalist.org/observations/186005399	PQ409040	PV061964	PacBio
CS127	Trappea sp. CS127	186005594	https://www.inaturalist.org/observations/186005594	PQ409041	PV061965	PacBio
CS128	Tuber beyerlei	186005688	https://www.inaturalist.org/observations/186005688	PQ409042	PV061966	PacBio
CS129	Xerocomellus cf. macmurphyi	186005844	https://www.inaturalist.org/observations/186005844	PQ409043	PV061967	PacBio
CS130	Rhizopogon cf. roseolus	186005941	https://www.inaturalist.org/observations/186005941	PQ409044	PV061968	PacBio
CS131	Geastrum sp. CS131	186005967	https://www.inaturalist.org/observations/186005967	PQ409045	PV061969	PacBio
CS132	Choiromyces sp. CS5	186006021	https://www.inaturalist.org/observations/186006021	PQ409046	PV061970	PacBio
CS133	Lactarius sp. CS123	186006064	https://www.inaturalist.org/observations/186006064	PQ409047	PV061971	PacBio

CS134	Hysterangium sp. CS38	188337954	https://www.inaturalist.org/observations/188337954	PQ409048	PV061972	PacBio
CS135	Hysterangium sp. CS38	188338047	https://www.inaturalist.org/observations/188338047	PQ409049	PV061973	PacBio
CS136	Tuber whetstonense	188338253	https://www.inaturalist.org/observations/188338253	PQ409050	PV061974	PacBio
CS137	Gautieria sp. CS137	188338580	https://www.inaturalist.org/observations/188338580	PQ409051	PV061975	PacBio
CS138	Russula cf. similaris	188338813	https://www.inaturalist.org/observations/188338813	PQ409052	PV061976	PacBio
CS139	Tuber whetstonense	188339003	https://www.inaturalist.org/observations/188339003	PQ409053	PV061977	PacBio
CS140	Geastrum sp. CS140	188339035	https://www.inaturalist.org/observations/188339035	PQ409054	PV061978	PacBio
CS142	Genea sp. CS115	191557183	https://www.inaturalist.org/observations/191557183	PQ409055	PV061979	PacBio
CS143	Coniphora sp. CS143	191560197	https://www.inaturalist.org/observations/191560197	PQ409056	PV061980	PacBio
CS144	Glomeraceae	191560228	https://www.inaturalist.org/observations/191560228	--	--	--
CS145	Balsamia quercicola	191557728	https://www.inaturalist.org/observations/191557728	PQ409057	PV061981	PacBio
CS146	Tuber candidum	191557760	https://www.inaturalist.org/observations/191557760	PQ409058	PV061982	PacBio
CS147	Geopora sp. CS147	191557919	https://www.inaturalist.org/observations/191557919	PQ409059	PV061983	PacBio
CS148	Choiromyces sp. CS5	191557992	https://www.inaturalist.org/observations/191557992	PQ409060	PV061984	PacBio
CS149	Rhizopogon cf. hawkerae	191558034	https://www.inaturalist.org/observations/191558034	PQ409061	PV061985	PacBio
CS150	Geopora sp. CS150	191558121	https://www.inaturalist.org/observations/191558121	PQ409062	PV061986	PacBio
CS151	Rhizopogon cf. hawkerae	191558152	https://www.inaturalist.org/observations/191558152	PQ409063	PV061987	PacBio
CS152	Balsamia	191558300	https://www.inaturalist.org/observations/191558300	--	--	--
CS153	Balsamia aff. latispora	191558326	https://www.inaturalist.org/observations/191558326	PQ409064	PV061988	PacBio
CS154	Balsamia aff. latispora	191558476	https://www.inaturalist.org/observations/191558476	PQ409065	PV061989	PacBio
CS155	Jimgerdemannia sp. CS155	191559962	https://www.inaturalist.org/observations/191559962	PQ409067	--	PacBio
CS156	Balsamia setchellii	197860967	https://www.inaturalist.org/observations/197860967	PQ409068	PV061991	PacBio
CS157	Balsamia setchellii	197861036	https://www.inaturalist.org/observations/197861036	PQ409069	PV061992	PacBio
CS158	Genea arenaria	197861109	https://www.inaturalist.org/observations/197861109	PQ409070	PV061993	PacBio
CS159	Balsamia quercicola	197861358	https://www.inaturalist.org/observations/197861358	PQ409071	PV061994	PacBio
CS160	Lactarius sp. CS40	197862873	https://www.inaturalist.org/observations/197862873	PQ409072	PV061995	PacBio
CS161	Schenella pityophila	197863019	https://www.inaturalist.org/observations/197863019	PQ409073	PV061996	PacBio
CS162	Lactarius sp. CS40	197863149	https://www.inaturalist.org/observations/197863149	PQ409074	PV061997	PacBio
CS163	Genea sp. CS163	197863243	https://www.inaturalist.org/observations/197863243	PQ409075	PV061998	PacBio
CS164	Balsamia quercicola	197863274	https://www.inaturalist.org/observations/197863274	PQ409076	PV061999	PacBio
CS165	Hymenogaster cf. parksii	197863300	https://www.inaturalist.org/observations/197863300	PQ409077	PV062000	PacBio
CS166	Balsamia sp. CS166	197863516	https://www.inaturalist.org/observations/197863516	PQ409078	PV062001	PacBio
CS167	Balsamia	197863775	https://www.inaturalist.org/observations/197863775	PQ409079	PV062002	PacBio

CS168	Jimgerdemannia sp. CS155	197863836	https://www.inaturalist.org/observations/197863836	PQ409080	PV062003	PacBio
CS169	Gautieria sp. CS169	197863999	https://www.inaturalist.org/observations/197863999	PQ409081	PV062004	PacBio
CS170	Geopora sp. CS147	197864056	https://www.inaturalist.org/observations/197864056	PQ409082	PV062005	PacBio
CS171	Genea sp. CS171	197864085	https://www.inaturalist.org/observations/197864085	PQ409083	PV062006	PacBio
CS172	Russula cf. similaris	197864126	https://www.inaturalist.org/observations/197864126	PQ409084	PV062007	PacBio
CS173	Geopora sp. CS147	197864202	https://www.inaturalist.org/observations/197864202	PQ409085	PV062008	PacBio
CS174	Rhizopogon sp. CS174	197864261	https://www.inaturalist.org/observations/197864261	PQ409086	PV062009	PacBio
CS175	Tuber aff. anniae	197864401	https://www.inaturalist.org/observations/197864401	PQ409087	PV062010	PacBio
CS176	Rhizopogon	198584286	https://www.inaturalist.org/observations/198584286	PQ409088	PV062011	PacBio
CS178	Genea arenaria	198581978	https://www.inaturalist.org/observations/198581978	PQ409089	PV062012	PacBio
CS187	Genea	203532814	https://www.inaturalist.org/observations/203532814	--	--	--
CS188	Genea arenaria	203533251	https://www.inaturalist.org/observations/203533251	PQ409090	PV062013	PacBio
CS189	Geopora sp. CS189	203533540	https://www.inaturalist.org/observations/203533540	PQ409091	PV062014	PacBio
CS190	Hysterangium sp. CS38	203534211	https://www.inaturalist.org/observations/203534211	PQ409092	PV062015	PacBio
CS191	Genea	203534589	https://www.inaturalist.org/observations/203534589	--	--	--
CS192	Lactarius sp. CS40	203534847	https://www.inaturalist.org/observations/203534847	PQ409093	PV062016	PacBio
CS193	Hymenogaster sp. CS193	203535168	https://www.inaturalist.org/observations/203535168	PQ409094	PV062017	PacBio
CS194	Balsamia alba	203535368	https://www.inaturalist.org/observations/203535368	PQ409095	PV062018	PacBio
CS195	Glomus sp. CS195	203535694	https://www.inaturalist.org/observations/203535694	PQ409096	PV062019	PacBio
CS196	Genea sp. CS196	203535789	https://www.inaturalist.org/observations/203535789	PQ409097	PV062020	PacBio
CS197	Gautieria sp. CS82	203536087	https://www.inaturalist.org/observations/203536087	PQ409098	PV062021	PacBio
CS198	Tuber candidum	203536256	https://www.inaturalist.org/observations/203536256	PQ409099	PV062022	PacBio
CS199	Glomeraceae	203533048	https://www.inaturalist.org/observations/203533048	--	--	--
CS200	Melanogaster sp. 'euryspermus'	214221787	https://www.inaturalist.org/observations/214221787	PQ409101	PV062024	PacBio
CS201	Genea sp. CS79	214222205	https://www.inaturalist.org/observations/214222205	PQ409102	PV062025	PacBio
CS202	Gautieria sp. CS82	214225726	https://www.inaturalist.org/observations/214225726	PQ409104	PV062027	PacBio
CS203	Cazia flexiascus	214224095	https://www.inaturalist.org/observations/214224095	PQ409105	PV062028	PacBio
CS204	Paragalactinia infossa	214224343	https://www.inaturalist.org/observations/214224343	PQ409106	PV062029	PacBio
CS205	Russula sp. CS205	219046309	https://www.inaturalist.org/observations/219046309	PQ409107	PV062030	PacBio
CS206	Hymenogaster cf. parksii	219046182	https://www.inaturalist.org/observations/219046182	PQ409108	PV062031	PacBio
CS207	Genea sp. CS207	219046376	https://www.inaturalist.org/observations/219046376	PQ409109	PV062032	PacBio
CS208	Lactarius sp. CS40	219046443	https://www.inaturalist.org/observations/219046443	PQ409110	PV062033	PacBio
CS209	Glomeraceae	219046640	https://www.inaturalist.org/observations/219046640	--	--	--

CS210	Scleroderma sp. CS210	219046675	https://www.inaturalist.org/observations/219046675	PQ409111	PV062034	PacBio
CS211	Balsamia aff. latispora	219048278	https://www.inaturalist.org/observations/219048278	PQ409112	PV062035	PacBio
CS212	Glomeraceae	219048410	https://www.inaturalist.org/observations/219048410	--	--	--
CS213	Hymenogaster cf. thwaitesii	219048518	https://www.inaturalist.org/observations/219048518	PQ409113	PV062036	PacBio
CS214	Genea sp. CS214	219048565	https://www.inaturalist.org/observations/219048565	PQ409114	PV062037	PacBio
CS215	Hysterangium sp. CS215	219048654	https://www.inaturalist.org/observations/219048654	PQ409115	PV062038	PacBio
CS216	Hysterangium sp. CS215	219048754	https://www.inaturalist.org/observations/219048754	PQ409116	PV062039	PacBio
CS217	Gautieria sp. CS82	219048820	https://www.inaturalist.org/observations/219048820	PQ409117	PV062040	PacBio
CS218	Geopora sp. CS218	219048897	https://www.inaturalist.org/observations/219048897	PQ409118	PV062041	PacBio
CS219	Melanogaster cf. natsii	219048969	https://www.inaturalist.org/observations/219048969	PQ409119	PV062042	PacBio
CS220	Melanogaster cf. natsii	219049005	https://www.inaturalist.org/observations/219049005	PQ409120	PV062043	PacBio
CS221	Xerocomellus cf. macmurphyi	219049078	https://www.inaturalist.org/observations/219049078	PQ409121	PV062044	PacBio
CS222	Xerocomellus cf. macmurphyi	219049149	https://www.inaturalist.org/observations/219049149	--	--	--
CS223	Genea	219049246	https://www.inaturalist.org/observations/219049246	--	--	--
CS224	Genea sp. CS224	219049434	https://www.inaturalist.org/observations/219049434	PQ409122	PV062045	PacBio
CS225	Gautieria sp. CS225	219049506	https://www.inaturalist.org/observations/219049506	PQ409123	PV062046	PacBio
CS226	Genea sp. CS171	219049600	https://www.inaturalist.org/observations/219049600	PQ409124	PV062047	PacBio
CS227	Choiromyces	219049755	https://www.inaturalist.org/observations/219049755	PQ409125	PV062048	PacBio
CS228	Gautieria sp. CS53	219049813	https://www.inaturalist.org/observations/219049813	PQ409126	PV062049	PacBio
CS229	Elaphomyces sp. CS64	219049873	https://www.inaturalist.org/observations/219049873	PQ409127	PV062050	PacBio
CS230	Phlegmacium sublilacinum	219049971	https://www.inaturalist.org/observations/219049971	PQ409128	PV062051	PacBio
CS231	Glomeraceae	219050026	https://www.inaturalist.org/observations/219050026	--	--	--
CS232	Melanogaster cf. macrocarpus	219050083	https://www.inaturalist.org/observations/219050083	PQ409129	PV062052	PacBio
CS233	Rhizopogon	219050212	https://www.inaturalist.org/observations/219050212	PQ409130	PV062053	PacBio
CS234	Hymenogaster sp. CS234	219050557	https://www.inaturalist.org/observations/219050557	PQ409131	PV062054	PacBio
CS235	Gautieria sp. CS235	219050758	https://www.inaturalist.org/observations/219050758	PQ409132	PV062055	PacBio
CS236	Phallogaster phillipsii	219051186	https://www.inaturalist.org/observations/219051186	PQ409133	PV062056	PacBio
CS237	Melanogaster sp. 'euryspermus'	219051409	https://www.inaturalist.org/observations/219051409	PQ409134	PV062057	PacBio
CS238	Xerocomellus cf. macmurphyi	219051442	https://www.inaturalist.org/observations/219051442	PQ409135	PV062058	PacBio
CS240	Hydnotrya sp. CS240	232200121	https://www.inaturalist.org/observations/232200121	PQ409170	--	Sanger
CS241	Gautieria sp. CS56	232200199	https://www.inaturalist.org/observations/232200199	PQ409171	--	Sanger
CS242	Hydnotrya	232200329	https://www.inaturalist.org/observations/232200329	--	--	--
CS243	Elaphomyces	232200368	https://www.inaturalist.org/observations/232200368	--	--	--

CS244	Melanogaster	232200889	https://www.inaturalist.org/observations/232200889	--	--	--
CS245	Hydnotrya sp. CS245	232200950	https://www.inaturalist.org/observations/232200950	PQ409172	--	Sanger
CS246	Alpova trappei	232200999	https://www.inaturalist.org/observations/232200999	PV061183	PV062059	PacBio
CS247	Leucophleps sp. CS247	232201322	https://www.inaturalist.org/observations/232201322	PV061184	PV062060	PacBio
CS249	Melanogaster sp. CS249	232201743	https://www.inaturalist.org/observations/232201743	PQ409174	PV062061	PacBio (LSU)
CS250	Hydnotrya	232201782	https://www.inaturalist.org/observations/232201782	--	--	--
CS251	Tuber whetstonense	232202084	https://www.inaturalist.org/observations/232202084	PQ409175	--	Sanger
CS252	Sarcosphaera sp. CS252	232202139	https://www.inaturalist.org/observations/232202139	PQ409176	PV062062	PacBio (LSU)

Appendix S3: Additional truffle genera of note

Ascomycetes

Elaphomyces: The genus *Elaphomyces* is well-described in Europe and covered extensively by Paz et al. (2017), and taxa from North America have historically been given European names but most are now understood to be different species. North American *Elaphomyces* with a black peridium have been called the European name *E. anthracinus*, a species now established not to occur in North America (Castellano and Stephens 2017). In May 2023, we located an unusual black *Elaphomyces* truffle (*Elaphomyces* sp. CS50) fruiting in oak savanna near the southern boundary of CSNM (Figure 3i). The truffle had a coarsely warty black outer cortex coated in a brown hyphal mat, a whitish inner cortex, and a powdery black spore mass. On a return trip to the same location a month later, we found a fragment of another black *Elaphomyces* nearby, visually similar in appearance (*Elaphomyces* sp. CS72). Based on morphology, we assumed that the two collections were the same species, but sequencing revealed that the ITS pairwise identity of the two was only 92%. Attempts to locate additional fruiting bodies have been unsuccessful. While several black *Elaphomyces* species are described from the east coast of North America (Castellano and Stephens 2017, Castellano et al. 2021, Castellano 2022), few records of any black *Elaphomyces* have been made from the U.S. west coast, and none have been formally described. Although the few existing Oregon *E. 'anthracinus'* collections (included in regional conservation lists of rare taxa) have not been DNA sequenced, we anticipate that our collection will not match any of them. The distinct morphology of the outer cortex does not match the description of *E. anthracinus*, which is described as "finely warty" (Trappe et al. 2009). Both ITS sequences show that our *E.* sp. CS50 and *E.* sp. CS72 collections are near the European species *E. aculeatus* (94% and 97% similarity respectively), which displays a morphology similar to ours. This species is in the same group as *E. anthracinus* (*Elaphomyces* subsection *Sclerodermei*) (Paz et al. 2017), and has been assessed as 'Critically Endangered' in Europe (https://redlist.info/iucn/species_view/206042). Three species of black *Elaphomyces* from the east coast of North America have also been placed in this group: *E. loebiae* (Castellano and Stephens 2017), *E. dunlapii* (Castellano et al. 2021), and *E. gouldhoytii* (Castellano 2022). The two former collections have been sequenced and are clearly distinct from our CSNM *Elaphomyces*. *E. gouldhoytii* has not been sequenced but has significantly larger spores (34.9 µm vs. ours ~18 µm). A possible record of *E. aculeatus* was made from Mexico (Gómez-Reyes et al. 2012), but without molecular data for these collections, we cannot determine if this European taxon is also found in Mexico (https://redlist.info/iucn/species_view/206042). Given the similar morphology and habitat of our collections and the *E. 'aculeatus'* from Mexico, we consider the possibility that they are conspecific and closely related to the European taxon. Based on the lack of regional collection data for black *Elaphomyces* species that resemble these as well as our many dog surveys in oak habitat within and outside of CSNM, we suspect that these truffles are rare. We intend to formally describe them once we have collected additional material.

Sarcosphaera: The rare and little-known truffle species *Sarcosphaera setchellii* (formerly *Hydnotryopsis setchellii*) has few recent records and has been proposed for IUCN Global Red List assessment as several of the records are historic (https://redlist.info/iucn/species/236504/). Sequences representing multiple *Hydnotryopsis* collections were included in a recent treatment of the genus *Sarcosphaera*, and the phylogenetic data showed that all of these sequences should be combined with *Sarcosphaera* (Borovička et al, 2024). While the species *Sarcosphaera setchellii* is the only formally described truffle species in this group known from North America, there is at least one other *Sarcosphaera* truffle species known from this region with even fewer collection records. We collected two distinct *Sarcosphaera* truffles: *Sarcosphaera setchellii* from the southern boundary of CSNM in oak habitat in late spring/summer (Figure 3h) and one from high elevation conifer habitat in summer that showed some molecular similarity to a *Hydnotryopsis 'gilkeyae'* ITS sequence (Genbank AY927853). Both finds represent new location records for these rare and understudied taxa, and contribute to our understanding of preferred habitat and fruiting patterns of *Sarcosphaera* truffles.

Choiromyces: We found an unexpected abundance of *Choiromyces* (Tuberaceae) truffles in the CSNM, primarily in high elevation conifer forests with an apparent increase in abundance around snowmelt. While widespread and largely common in Europe (Gógán Csorbainé et al. 2008), this genus is considered rare in North America, with *C. alveolatus* and *C. meandriformis* listed as sensitive species ([ORBIC] Oregon Biodiversity Information Center 2023). We sequenced eight collections of 54 *Choiromyces* sporocarps found during our surveys, all with the macro morphology of *C. alveolatus*. Seven of our collections align well with each other with few nucleotide differences, but one collection (*C.* sp. CS61) is distinct from the others. There is enough variation between our collections and sequences called *C. alveolatus* on GenBank to suggest some cryptic diversity under this name, so further investigation into this taxon is warranted. Based on the dissimilarity in the sequence data, we have not formally called our collections *C. alveolatus*, but as the morphology fits with the species description, we consider our collections close enough to the described taxon to group them with a current understanding of species occurrence and distribution.While *C. alveolatus* is considered rare (Natural Heritage rank "Imperiled in Oregon", [ORBIC]), our observations suggest that the populations we encountered within the CSNM are productive and widespread in high elevation mixed conifer forest.

Tuber: The "true truffle" genus *Tuber* is well-represented within the CSNM as our second most common genus (n = 175). From the collections that we sequenced, we determined that we collected eight different species: *T.* aff. *anniae* (species complex; Figure 3a), *T. beyerlei*, *T. candidum, T. luomae*, *T. quercicola* (Figure 3j), *T. whetstonense*, and two species with no matches on GenBank, *T.* sp. CS22 and *T.* sp. CS114. While we were not able to identify all *Tuber* species in the field, we confidently identified both *T. candidum* and *T. quercicola*, which comprise at least 40.1% and 18.8% respectively of all our *Tuber* finds.

Through all of the surveys, *T. candidum* and *T. quercicola* were the only oak-associated *Tuber* species. These taxa were also the only *Tuber* species we found fruiting in the spring, and most of our *Tuber* records are from autumn under conifers. While *T. whetstonense* is described from oak habitat close to the CSNM (Frank et al. 2006), we only recorded this species from high elevation conifer habitat, where it seems to be relatively abundant. We sequenced eight *Tuber* collections that all cluster with the type and additional collections of *T. whetstonense.*

We sequenced two putatively novel *Tuber* taxa, *T.* sp. CS22 and *T.* sp. CS114. *T.* sp. CS22 appears to be a new *Tuber* in the Maculatum clade, and, despite no close vouchered collections on GenBank, there are a handful of near matches in environmental and root tip sequences, including an environmental sample from British Columbia (KM403038) and an orchid mycorrhizal root sample from New York, USA (AY634172). We only found one fruiting body of this new taxon. Two collections of *Tuber* sp. CS114 were made from mixed ponderosa pine and white and black oak forest. The appearance and aroma of this truffle is similar to *T. quercicola*, but the ITS region differs by 4-5% to other *T. quercicola* sequences. Based on close matches to two root tip sequences from ponderosa pine (MH038070 and JQ393153), it is likely that this species is a pine associate.

<u>Basidiomycetes</u>

Xerocomellus: We collected several hypogeous *Xerocomellus* truffles from a single survey in *Abies concolor* forest in October 2023, and again under *Quercus garryana* in May 2024 in two locations (Figure 3c). These collections appear similar to *Xerocomellus macmurphyi*, but an initial comparison of ITS sequences with two sequenced herbarium collections (GenBank

AY918953 and KJ882289) showed significant differences. Upon further examination of the PacBio sequence data for our first collection, three dominant read variants were noted in the sample. The least abundant variant is distinct from the other two and is a better match to the *X. macmurphyi* sequences, in particular due to sharing a 12-13 bp region with these herbarium collections that is distinct from our other sequences (the two most abundant read variants in our conifer forest collection, and the single read variants from our two sequenced oak collections). The presence of this single read variant provides support that our collections may in fact be *X. macmurphyi*, with the caveat of additional differences in the alignment. We expect that nucleotide variation in the ITS may be common in this species, as the ITS sequences of the two herbarium collections only align with a pairwise identity of 95.5%. Based on this evaluation, we are calling our collections *Xerocomellus* cf. *macmurphyi* and highlight the presence of this unique sequestrate species in several locations in the CSNM. In particular, its presence in conifer habitat in the fall is noteworthy, as *X. macmurphyi* is otherwise known only from hardwoods in the winter and spring (Smith et al. 2018).

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Appendix S4: Truffle parasites

We noted a prevalence of *Choiromyces* ascomata infected with a fungal parasite tentatively identified as *Melanospora* sp. (found with at least seven sporocarps; Figure 3d and S1A-C). This type of truffle infection has been recorded in several truffle species, including a historic record involving *Choiromyces meandriformis,* and more recently seen in *Tuber* spp. from China, with the new species *Melanospora subterranea* described from *T. huidongense* and *T. indicum* (Fan et al. 2012). All *Choiromyces* truffles that we encountered with this type of infection had a dark "peppered" appearance to what is typically a creamy-yellow gleba, but no outward signs of premature decay. The aroma did not differ from uninfected specimens, and mature *Choiromyces* spores were found in abundance along with the supposed *Melanospora* parasite spores. Both the *Choiromyces* and parasite spores appeared healthy, suggesting that the parasite is not inhibiting the truffle's reproductive ability. Our attempts to sequence the dark-spored parasite found fruiting in *Choiromyces* truffles in the CSNM have been unsuccessful, so species identification remains inconclusive pending further examination.

During a November 2023 survey in mixed conifers, we collected a gray, marbled truffle that superficially appeared to be a *Tuber* species at the time of collection. PacBio sequencing of this specimen revealed two fungal species present in similar quantities in the DNA extraction, one being *Balsamia latispora* and the other *Micoascus* sp. (Figure E.1.d). The genus *Microascus* (Ascomycota) is more broadly known as a saprotroph and occasional tissue pathogen in humans (Sandoval-Denis et al. 2016), and not commonly known as a truffle parasite. However, a western North American treatment of *Balsamia* mentioned a visually similar instance of a parasitized *Balsamia* truffle (Southworth et al. 2018; Figure 4b).

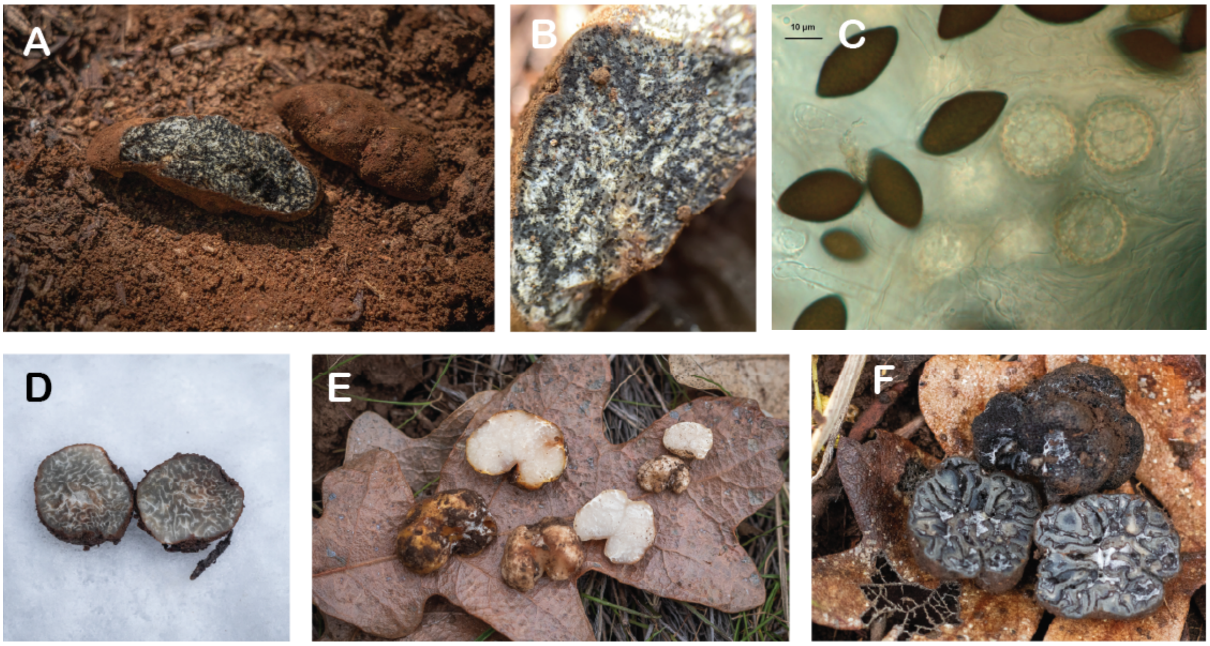

Figure S1. Photographs of parasitized truffles a) *Choiromyces meandriformis* sporocarp with dark marbling from *Melanospora* sp., b) *C. meandriformus* and *Melanospora* sp. At 10x magnification, c) mature spores from *C. meandriformis* and *Melanospora* sp., d) *Balsamia latispora* and *Microascus* sp., e) *Tuber candidum* and *Entoloma* sp., f) *Genea* sp. with *Entoloma* sp. Photographs by Heather Dawson.

Another instance of parasitism, though less visually distinctive, was a white *Entoloma* sp. "mold" on *Tuber candidum* and *Genea* sp. (Figures E.1.e-f). The *Entoloma* sp. may have caused the *T. candidum* to produce odor prematurely, as the parasitized sporocarps were found in early spring and no mature *Tuber* were found fruiting during that survey. The *Entoloma* sp. may have also discolored the *T. candidum*'s typically brown peridium, as these specimens were unusually yellow in color. The parasitized *Genea* sp. truffles were found later in the spring and appeared otherwise healthy apart from a white mycelium covering the internal cavities. Both *Entoloma* spp. parasites appear to be near or conspecific with *Entoloma undatum.* The *Genea* sp. parasite is a close match for several *E. undatum* vouchers, while the *T. candidum* parasite seems to be distinct without close matches on GenBank.

Most *Entoloma* are saprotrophic fungi that produce classic "mushroom" sporocarps; however, there are a few known *Entoloma* truffles that are ectomycorrhizal and a handful of mycoparasitic *Entoloma* fungi. *E. parasiticum* parasitizes *Cantharellus* mushrooms, and *E. abortivum* parasitizes mushrooms in the genus *Armillaria* (Czederpiltz et al. 2001). However, we are not aware of any known *Entoloma* species that targets truffles.

APPENDIX S4 REFERENCES

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