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Research

Distribution and abundance of the introduced ectomycorrhizal fungus *Amanita phalloides* in North America

Benjamin E. Wolfe[1], Franck Richard[2], Hugh B. Cross[3] and Anne Pringle[1]

[1]Department of Organismic & Evolutionary Biology, Harvard University, Cambridge, MA, USA; [2]Centre d'Ecologie Fonctionnelle et Evolutive (CNRS, UMR 5175), Montpellier, France; [3]State Herbarium, South Australian Department of Environment & Heritage, The University of Adelaide, SA, Australia

Author for correspondence:
Benjamin E. Wolfe
Tel: +1 617 496 9741
Email: bewolfe@fas.harvard.edu

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Summary

- Despite a growing awareness of the global reach of ectomycorrhizal (EM) fungal introductions, little is known about the fate of introduced EM fungi in novel ranges.
- Using herbarium specimens, species distribution models, and field collections of sporocarps, root tips and extramatrical mycelia, we assessed the distribution and abundance of the European species *Amanita phalloides* in North America.
- There are two distinct ranges of the fungus, one along the West Coast (California to British Columbia) and the second on the East Coast (Maryland to Maine). As predicted by a species distribution model, the West Coast range is larger. *Amanita phalloides* is more frequently found in native forests on the West Coast than on the East Coast. At Point Reyes Peninsula in California, *A. phalloides* dominates community sporocarp biomass, and is frequent as root tips. In individual soil cores at Point Reyes, root tips of *A. phalloides* make up 50% of total root tip biomass. Hyphae of *A. phalloides* are frequent, but make up only 2% of total hyphal biomass.
- The contrasting patterns of the distribution and abundance of *A. phalloides* on the East and West Coasts of North America may influence both its future spread and its impacts.

Introduction

Data on the movement, establishment and spread of soil microbes are rare, perhaps because of the cryptic nature of microbial diversity (van der Putten *et al.*, 2007). Data on mutualists are especially uncommon, as the effects of pathogens on animals and plants are more obvious than effects caused by mutualists (Desprez-Loustau *et al.*, 2007). Only recently have there been comprehensive studies of the occurrence of introduced soil microbial mutualists outside of native ranges, including nitrogen-fixing bacteria (Chen *et al.*, 2005; Rodríguez-Echeverría *et al.*, 2007) and mycorrhizal fungi (Vellinga *et al.*, 2009).

One group of soil mutualists that has been frequently introduced to novel ranges includes the ectomycorrhizal (EM) fungi (Vellinga *et al.*, 2009). EM fungal introductions have occurred across the globe and encompass a diverse array of both ascomycete and basidiomycete species. While the number of reports of these introduced EM fungi is rapidly increasing (Díez, 2005; Tedersoo *et al.*, 2007), there are very few data on the fate of introduced species in novel ranges (Selosse *et al.*,1999; Desprez-Loustau *et al.*, 2007; Vellinga *et al.*, 2009). After EM fungi are introduced, do they spread? Are species restricted to a few locations, associated with introduced host trees, or do species shift hosts and become widespread across the landscape? If introduced fungi grow within a native forest community, do the fungi dominate the local fungal community, and will these species have the potential to displace native species?

Amanita phalloides is a widely recognized EM fungus that was introduced to North America and is successfully spreading in its novel range. It was introduced from Europe and is now found on North America's East and West Coasts (Pringle & Vellinga, 2006; Pringle *et al.*, 2009). It is gener-

ally considered to be common and spreading along the West Coast (Pringle & Vellinga, 2006; Pringle *et al.*, 2009), but relatively rare and localized along the East Coast (Tanghe & Simons, 1973; Tanghe, 1983). However, there have been no attempts to systematically quantify the current range of *A. phalloides* in North America, especially outside of California. Moreover, nothing is known about the abundance of *A. phalloides* sporocarps, root tips, or extramatrical mycelia within the forests where it has established. Without these baseline data, it will be impossible to understand the ecology of this species in its new habitats, and difficult to understand interactions between *A. phalloides* and native species.

Using a series of field surveys, species distribution models, and measurements of sporocarp, root tip, and hyphal frequency or biomass, we comprehensively assessed the distribution and abundance of *A. phalloides* in North America across multiple spatial scales. We then compared the abundance of *A. phalloides* to abundances of other *Amanita* species using data from available literature on EM fungal communities. We addressed the following questions:

- What is the current range of *A. phalloides* in North America? Based on species distribution modeling of abiotic variables (temperature and precipitation), what is the potential range of *A. phalloides* in North America?
- What are the frequency and abundance of *A. phalloides* in forests where it has invaded? What proportion of the EM community biomass is *A. phalloides*, both aboveground (as sporocarps) and belowground (as root tips and extramatrical mycelia)?
- What is the composition of the EM fungal community where *A. phalloides* invades and how frequent is *A. phalloides* relative to other species in the EM fungal community?
- Does the frequency of occurrence of *A. phalloides* in its introduced range differ from data for *Amanita* species in their native ranges?

Materials and Methods

Current distribution of *A. phalloides* in North America

To make the best estimate of the distribution of *Amanita phalloides* (Vaill. Ex fr.: Fr.) in North America we relied on three sources of information: recent collections deposited in herbaria; recent collections made by amateur collectors or mycological clubs and given to our laboratory; and our own field surveys in targeted areas where *A. phalloides* had been reported. Collections were considered as independent occurrences when they were found at least 500 m distant from each other; 500 m was chosen as a threshold because preliminary genetic data suggest that genetically similar populations of *A. phalloides* are contained within 300 m (A. Pringle & H. B. Cross, unpublished). Most collections were recent and were made during the past 15 yr. Our own field observations were made over a 3-yr period, from 2005 to 2008. We also used some records from the website Mushroom Observer (http://mushroomobserver.org/). We only used Mushroom Observer records when photographs that were unambiguously *A. phalloides* were given with the record. Identification was based on the color of the cap (olive-green, olive-brown, or yellow-brown), gray-brown scales on the stipe, and the presence of a distinct annulus and volva.

For each unique occurrence of *A. phalloides*, we determined the type of habitat around the collection and considered it to be in one of two categories: natural native forest, where the forest was generated and is maintained with minimal human intervention and is composed of native tree species, or planted forest, where the forest was created by direct human intervention and is planted with either native or nonnative tree species.

We sequenced the nuclear ribosomal internal transcribed spacer (ITS) region of: all poorly preserved herbarium specimens, where morphological confirmation of identity was ambiguous; field collections where only a portion of the sporocarp was collected and given to us; or sporocarps that had unusual features that were not characteristic of typical *A. phalloides* sporocarps (e.g. faded coloration of the cap or lack of odor). The nucleotide sequence of the ITS is nearly identical across all specimens of *A. phalloides* sequenced to date, but is different from that of all other closely related species of Section Phalloideae in *Amanita* (Pringle *et al.*, 2009). Sequencing of the ITS was carried out as described in Pringle *et al.* (2009). ITS sequences were deposited in GenBank as indicated in Supporting Information Table S1. Vouchers of most collections (indicated in Table S1) are kept by the Pringle Laboratory Herbarium and are available upon request.

To estimate the current range size of *A. phalloides* in North America, we determined the area of a minimum convex polygon created around occurrence data for the East and West Coasts using Hawth's Tools (http://www.spatialecology.com/htools/tooldesc.php) in ArcMap 9.3 (ESRI, Inc., Redlands, CA, USA).

Potential distribution of *A. phalloides* in North America

To estimate the potential distribution of *A. phalloides* in North America based on abiotic parameters, we developed a species distribution model (SDM). Using this approach we determined the primary environmental variables associated with the occurrence of *A. phalloides* in its native range in Europe, and then projected its range to North America based on areas where these variables were most similar. We used the program maxent version 3.2.19 (Phillips *et al.*, 2006) because it has been shown to perform better than some other SDM algorithms when presence-only data are

used (Elith *et al.*, 2006) and because it has been successfully used to model the distribution of macrofungi (Wollan *et al.*, 2008).

Occurrence records from Europe consisted of herbarium specimens, our own collections, and records in databases (Table S2). We used occurrence records that were distributed throughout the known range of *A. phalloides* in Europe and only included recent records; most specimens or records were collected in the past 15–20 yr. If a specimen or record did not have a specific latitude and longitude associated with it, we inferred these data from information provided on the accession using Google Earth (Version 5.0; Google Inc., Mountainview, CA, USA). Of the 224 occurrence records, 168 (75%) were randomly selected for use in training the SDM, and the remaining 56 (25%) were used to test the SDM.

Environmental layers in the native and introduced ranges were obtained from WorldClim data sets (Hijmans *et al.*, 2005). We used 19 bioclimatic variables, as well as altitude, at 2.5 arc-minutes resolution as indicated in Table S3. We did not include information on hosts or other biotic variables in the model and instead targeted the potential abiotic niche of *A. phalloides* in North America. A future paper will build on this abiotic model and address the role of hosts in the range expansion of *A. phalloides*.

To determine model performance, we used the area under the curve (AUC) of a receiver-operating characteristic (ROC) analysis. Values range from 0.5 to 1.0, with 0.5 indicating poor model performance (not better than random) and 1.0 indicating perfect model performance. Values of AUC > 0.9 indicate high performance of the model (Pearce & Ferrier, 2000). We used the minimum training presence threshold (Phillips *et al.*, 2006) as a cut-off to delineate areas in North America that are abiotically suitable for *A. phalloides*. Jackknife tests were used to determine the relative importance of different variables for the prediction power of the SDM.

Local abundance of *A. phalloides*

Preliminary data from herbarium specimens pointed to the Point Reyes Peninsula of Marin County, California as a region with dense populations of *A. phalloides*. In this county the species is invading native coast live oak (*Quercus agrifolia*) forests (Pringle *et al.*, 2009), and subsequent work focused on these invaded habitats. We also included two sites on the East Coast for root tip sampling, as a comparison to our results from California.

Plot set-up To determine the abundance of *A. phalloides* relative to other fungi, we measured *A. phalloides* aboveground and belowground as sporocarps, EM root tips and extraradical hyphae. We established two plots on the Point Reyes Peninsula, hereafter called Drake's Landing and Heart's Desire. Plots were chosen from sites where sporocarps of *A. phalloides* are commonly observed and have been found for at least the last 15 yr (Drake's Landing) or 30 yr (Heart's Desire), according to herbarium collections (Drake's Landing: collection by Tom Horton currently in the Pringle Laboratory Herbarium; Heart's Desire: SFSU collection # HDT 28598). The Drake's Landing plot was 20 m × 20 m while the Heart's Desire plot was 25 m × 10 m. Plots were divided into 5 m × 5 m subplots for EM root tip sampling. The plots were different sizes because of their orientation with roads, trails and natural features of the landscape (dead trees, slopes, etc.). The dominant trees species in both plots were *Quercus agrifolia* and either *Pseudotsuga menziesii* (at Drake's Landing) or *Pinus muricata* (at Heart's Desire). There are no introduced plants found within either plot, nor are introduced plants common at either site. More details on these plots are given in the Supporting Information Part 3.

Sporocarps All fleshy epigeous sporocarps of EM fungi (both Ascomycota and Basidiomycota) within each plot were harvested once a month during the peak fruiting period (October–March) from 2007 to 2008. Sporocarp surveys were conducted during periods of high sporocarp production following periods of sustained moisture. Sporocarps were dried at 35°C for 24 h on a mushroom dryer and then weighed. To separate EM fungi from non-EM fungal biomass, we identified each sporocarp to the genus level. We did not attempt to identify the non-*A. phalloides* sporocarps to species, but instead lumped their biomass together as 'non-*A. phalloides* biomass'. We sampled sporocarps in only one season and for this reason we cannot present quantitative data on the temporal variability of sporocarp biomass. However, we have worked at these sites for over 5 yr, and our observations of *A. phalloides* sporocarp production across these years were similar to those in the time period when we collected the sporocarp biomass data.

EM fungal root tips Within each plot, two soil cores were taken to a depth of 12 cm from randomly sampled points within each 5 m × 5 m subplot. Sampling occurred in the first week of December in 2007. At Drake's Landing we collected 32 cores and at Heart's Desire we collected 20 cores. We chose a sampling depth of 12 cm because preliminary coring at these sites had shown that the highest concentration of root tips was found from the soil surface to this depth. Cores were placed on ice and roots were extracted and washed with water within 2 d of sampling. Root tips with a morphology similar to that of *A. phalloides* (smooth mantle with creamy color and velvety hoarfrost appearance; dichotomous branching) were removed from each core sample and separated from the other root tips of the samples. We were generous with our working definition

of the morphology of *A. phalloides* so that we were sure to capture all root tips that could be *A. phalloides*. Root tips were lyophilized within 2 d of processing for storage. DNA was extracted from root tips using a modified cetyltrimethyl ammonium bromide (CTAB) chloroform:isoamyl alcohol extraction procedure as described in the Supporting Information Part 3.

To rapidly separate *A. phalloides* root tip morphotypes from all other EM morpotypes, we screened the root tips with a PCR primer pair specific to *A. phalloides*. A PCR reaction with the *A. phalloides*-specific primer ITSphl5F (ATTTATATGGATGGGGACAACT) designed by our laboratory was used in combination with ITS4B (Gardes & Bruns, 1993) to obtain a band ~200 bp in length when root tips were *A. phalloides*. This primer pair amplifies a portion of the ITS of *A. phalloides*, but not root tips of closely related *Amanita* or other EM fungal species (Table S6, Fig. S2). For all root tips, we also performed a PCR with the primer pair ITS1F and ITS2 (White *et al.*, 1990; Gardes & Bruns, 1993) to obtain one band *c.* 350 bp in length to confirm that lack of amplification with ITSphl5F-ITS4B for root tips was not a result of poor DNA quality or the presence of inhibitors.

We also conducted root tip surveys in plots at two sites where *A. phalloides* occurs on the East Coast of North America: Durand-Eastman Park in Irondequoit, New York and Jake's Landing Road in Dennisville, New Jersey. The Durand-Eastman Park plot contains planted conifers including *Pinus* spp., *Tsuga canadensis*, and *Abies* spp. The Jake's Landing Road plot contains a plantation of *Pinus strobus*. These sites are two of several sites where *A. phalloides* first detected on the East Coast in the 1960s and 1970s (Tanghe & Simons, 1973; Tanghe, 1983). Plots were 20 m × 20 m at each of these sites. Soil cores were sampled in mid-October of 2007. The same protocols used for collecting and processing root tips in California were used for these root tips.

Extramatrical mycelia To estimate the abundance of extramatrical mycelia (EMM) of *A. phalloides* in soils where it has invaded at Point Reyes, we used the mesh bag approach that has been widely used in other studies of EM fungal EMM in the field (Wallander *et al.*, 2001). We chose to use mesh bags filled with sand for several reasons. First, these bags only contain EMM of fungi that are actively growing during the time that the mesh bags were incubated in the soil; they do not contain spore banks or senescent hyphae of the surrounding soils. Secondly, using sand as opposed to sterile field soil promotes the growth of EM fungi over saprotrophic fungi because there is no organic matter in the sand. Previous studies have shown that most sequences (generally > 80%) obtained from mesh bags are of EM fungi (Kjøller, 2006; Parrrent & Vilgalys, 2007; Hedh *et al.*, 2008). We acknowledge that the composition of EM fungi in mesh bags probably does not match the composition of EM fungi in surrounding soils, because of differences in the substrates and because of the different growth rates of fungi. However, we did not collect data on diversity and were not focused on questions of diversity and instead targeted the spatial frequency of *A. phalloides* EMM across a site, and the relative abundance of *A. phalloides* EMM inside the mesh bags.

In November 2007, we installed 25 mesh bags in the Heart's Desire Plot described above. We chose this site because it had a substantial density of *A. phalloides* sporocarps and had the highest species richness of EM fungal sporocarps of sites surveyed at Point Reyes (B. Wolfe, pers. obs.). Mesh bags were 4 cm × 8 cm × 3 cm, made with a 48-μm nylon mesh, and filled with 40 g of 50-μm particle size acid-washed sand.

We made small holes in the soil to a depth of *c.* 10 cm so that the top of the bag was at the surface of the soil. The bag crossed from the organic to the mineral horizon of the soil. In November 2008, the mesh bags were retrieved from the soil and stored at −4°C. Twenty-one bags were successfully retrieved and processed.

Soil was carefully removed from the outside of the bag to avoid contamination. Hyphae were extracted from the sand by floating and decanting the contents of the bag in sterile Petri dishes with sterile water. DNA was extracted from the EMM using the protocol described above for root tip DNA extractions. To determine the presence of *A. phalloides* in a mesh bag, we used the same PCR screening approach outlined above for the root tips with the ITSphl5f and ITS4B primer pair.

In mesh bags where *A. phalloides* EMM were detected, we used real-time quantitative PCR (qPCR) to determine the abundance of *A. phalloides* relative to other fungi. To determine *A. phalloides* biomass, we used the primer pair ITSphl5f and ITS4 (White *et al.*, 1990). For total fungal biomass, we used the primer pair ITS1f-5.8s as previously used for qPCR by Fierer *et al.* (2005). Details on validation of the qPCR approach and these primers are given in the Supporting Information Part 2. PCRs were run on an ABI 7900HT Fast Real-Time PCR System (Applied BioSystems, Carlsbad, CA, USA). Each 25-μl reaction contained 0.5 μl of each primer (10 mM), 12.5 μl of Power SYBR® Green PCR Mater Mix, and 9.5 μl of DNA-free water. Thermocycling conditions were 10 min at 95°C, 35 cycles at 95°C for 30 s, 55°C for 30 s and 72°C for 1 min, followed by a meting curve temperature profile of 52°C for 15 s, 99°C for 15 s and 95°C for 15 s. All PCR reactions were replicated three times. PCR reactions of the same type (total fungal or *A. phalloides* specific) were run on the same plate with an appropriate standard. A standard curve for *A. phalloides* biomass was generated by conducting a qPCR on DNA extracted from mycelium of a known mass of a pure culture of *A. phalloides* isolated from Lebanon, New

Jersey, USA and making a 10-fold dilution series from 10^{-1} to 10^{-5}. A standard curve for total fungal biomass was similarly generated by extracting DNA from a pool of mixed species mycelium of known mass removed from the mesh bags for use in a 10-fold dilution series from 10^{-1} to 10^{-5}. The cycle number at which the PCR amplification reaches the threshold for the exponential phase of the amplification curve (C_t) was plotted against known biomass of mycelia for standards. These regression equations were used to determine the quantity of mycelial biomass in unknown samples of DNA extracts from mesh bags.

EM fungal community diversity at a site invaded by *A. phalloides*

In December 2006, we conducted a study at the Drake's Landing site described above to determine the diversity of the root tips of the EM fungal community at that site and the frequency of occurrence of *A. phalloides* in that community. We collected a soil core (5 cm wide, 10 cm deep) at every meter along a 30-m transect for a total of 30 cores. The transect started at the edge of the forest and ran into the forest, perpendicular to the road. The forest edge starts *c.* 5 m from the edge of one of the main roads in Point Reyes National Seashore (Limantour Road). From each core, we sorted root tips into morphotypes based on color and texture of the mantle, presence of emanating hyphae, and ramification type. Root tip features were determined under a stereomicroscope. From each morphotype, we chose several root tips for DNA extraction using the methods described above, except that ITS1F and ITS4 were the primers used for PCR and sequencing.

Morphotype ITS sequences were edited in SEQUENCHER 4.8 (Gene Codes Corp., Ann Arbor, MI, USA). We used SEQUENCHER to group similar sequences into an operational taxonomic unit (OTU) with 97% similarity. We used BLASTn searches to assign each OTU to a taxonomic rank based on sequence similarity. We were able to assign most sequences to at least the family level.

Frequency and dominance of *A. phalloides* EM root tips and sporocarps in North American forests compared with *Amanita* species in their native ranges

To determine how the patterns of abundance that we observed for *A. phalloides* in North America compare with abundance patterns of root tips of *Amanita* species in native ranges, we compiled data on the abundance of *Amanita* species from all studies published where an *Amanita* was detected as an EM root tip using molecular approaches. We used data for *Amanita* species from the three biogeographic regions where *A. phalloides* occurs: the West Coast of North America, the East Coast of North America (east of the Mississippi River), and Europe. From each paper we determined the frequency of occurrence of *Amanita*, which we define as the number of samples with the focal *Amanita* species divided by the total number of samples. If sporocarps of *Amanita* were found in plots that were sampled, but root tips of *Amanita* were not detected, we included these studies and considered the frequency of root tip occurrence to be zero.

We also compiled data from the literature on the biomass of *Amanita* sporocarps in their native ranges to compare to our measurements of *A. phalloides* sporocarp biomass at Point Reyes. We used the same approach for searching the literature as described for root tips and included studies that reported a measure of both dry total EM sporocarp biomass and dry biomass of *Amanita* species. From these data we calculated the per cent of the total EM biomass that was comprised of each *Amanita* species.

Results

Current distribution of *A. phalloides* in North America

Amanita phalloides has spread across a larger range on the West Coast of North America as compared to the East Coast, with 87 confirmed sites on the West Coast and 25 on the East Coast (Fig. 1). Using a minimum convex polygon to estimate range boundaries, the West Coast range is 356 000 km^2, while the East Coast range is 278 000 km^2.

On the West Coast, *A. phalloides* occurs along a 1700-km stretch from the Los Angeles area of Southern California northward to Vancouver Island, British Columbia, Canada. The highest density of confirmed occurrences is around the San Francisco and San Pablo Bays of California. Of those specimens that had an indication of forest type, 47 (58%) were collected from native forests. While some of these collections are from sites along trails and roads, at forest edges, *A. phalloides* was also collected from deep within native forests, especially across Marin County, California.

On the East Coast, *A. phalloides* occurs along a 750-km stretch from the Atlantic Coastal Plain of Maryland northward to the White Mountains of New Hampshire, and east to the coastal islands of Maine. Of those specimens that had an indication of forest type, only four (19%) were collected from native forests. In all four cases, fungi were found along the edges of trails or roads.

Predicted distribution of *A. phalloides* in North America

The species distribution model developed with environmental data from Europe predicts a larger range for *A. phalloides* on the West Coast than on the East Coast (Fig. 2). The model had high predictive power, with an AUC of

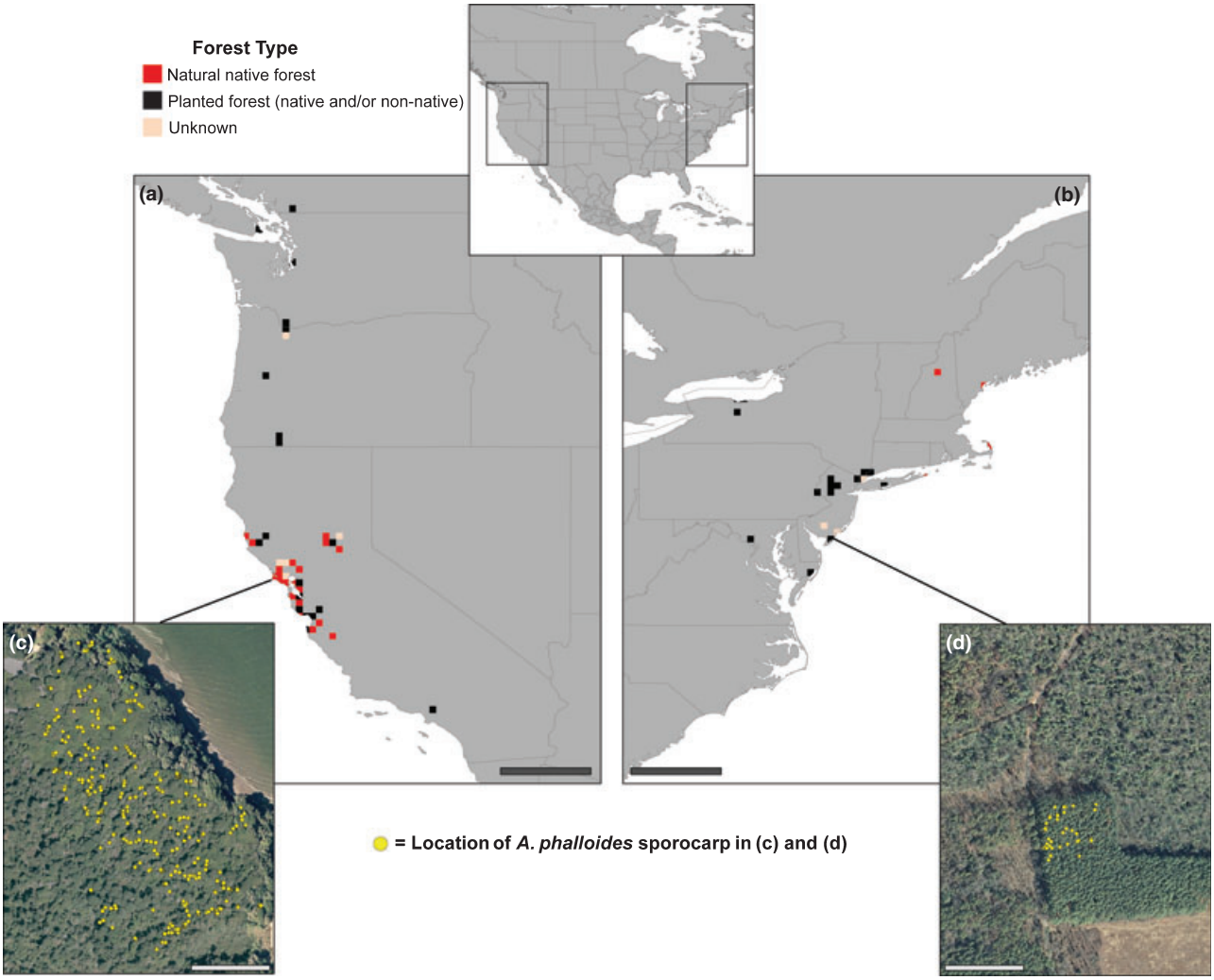

Fig. 1 Confirmed occurrences of *Amanita phalloides* on the (a) West Coast and (b) East Coast of North America. In (a) and (b), colored boxes cover 20 km × 20 km squares where *A. phalloides* has recently been collected; each box may cover multiple populations of *A. phalloides*. The squares are color-coded according to the most frequent forest type where *A. phalloides* has been collected within that square. Gray scale bars are 300 km. In (c) and (d), aerial photographs of forests at Heart's Desire (Point Reyes Peninsula, California) and Jake's Landing Road (Dennisville, New Jersey) show the density of sporocarps of *A. phalloides* during the main period of sporocarp production (28–29 November 2007 for Heart's Desire and 21 October 2006 for Jake's Landing Road). Bars, 100 m.

0.978 for the training data and 0.950 for the test data. The variables that best explained the predicted ranges were: temperature seasonality (standard deviations of weekly mean temperatures expressed as a percentage of the annual mean temperature), which explained 34.3% of the variability; temperature annual range (the difference between the maximum temperature of the warmest period and the minimum temperature of the coldest period), which explained 19.5% of the variability; and precipitation of the coldest quarter (the total amount of precipitation over the coldest 3-month period of the year), which explained 11.4% of the variability. These three variables were also shown to be the most important in jackknife tests (Table S3).

Moreover, when we used the model to predict the area where the fungus could grow along the West Coast (using a default threshold in the MAXENT model of 2.330; $P < 0.001$), the predicted area of eventual occupied habitat includes over 470 000 km^2. By contrast, when the model was used to predict the suitable area in northeastern North America, only small areas of Cape Cod and Nantucket Island, less than 1500 km^2, are above the threshold for predicted suitable habitat.

We also used the SDM to extract data for predicted probabilities of occurrence from the geographic locations where *A. phalloides* currently occurs. We used these data to compare the probabilities of occurrence from the East and West Coasts. Mean probabilities from the model were higher on the West Coast as compared with the East Coast (West Coast: 4.57 ± 0.96; East Coast: 0.293 ± 0.05; t-value = 2.46, P = 0.016).

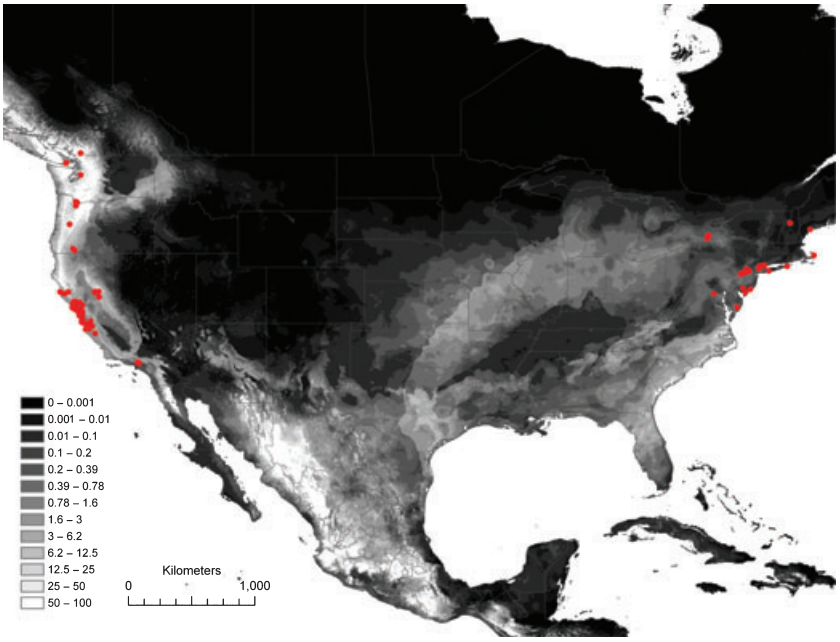

Fig. 2 Predicted distribution of *Amanita phalloides* in North America based on a species distribution model developed with occurrence data from European collections of *A. phalloides*. Red dots indicate known occurrences of *A. phalloides*. Shading indicates probability of *A. phalloides* occurring in a pixel, with lighter shading indicating a higher probability of occurrence.

Local abundance of *A. phalloides*

Sporocarps of *A. phalloides* dominated the local EM fungal community at the Point Reyes plots, reaching high densities (Fig. 1) and producing abundant biomass (Fig. 3). The biomass of *A. phalloides* sporocarps was 85% and 62% of the total sporocarp biomass at Drake's Landing and Heart's Desire, respectively.

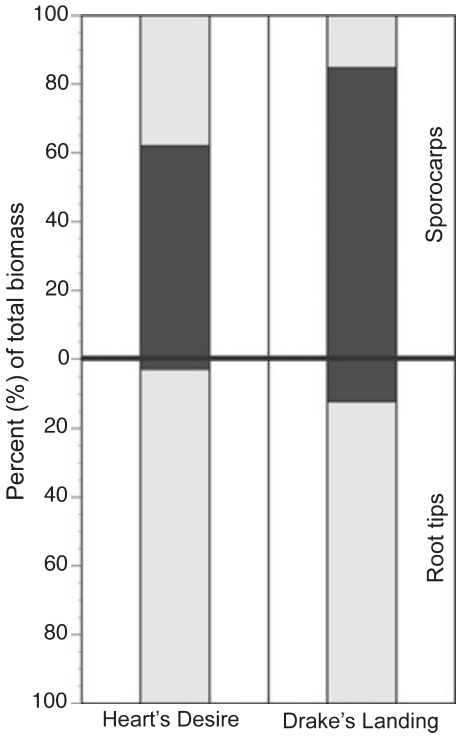

Fig. 3 Sporocarp and root tip biomass of *Amanita phalloides* (dark grey bars) and all other ectomycorrhizal fungal species (light grey bars) in two forests on Point Reyes Peninsula, California, USA.

Root tips of *A. phalloides* were also frequent, but made up a small portion of the total community biomass. Root tips were encountered in 13% of the cores taken from Drake's Landing, and 20% of the cores of Heart's Desire. Although root tips colonized by *A. phalloides* were only a small portion of the total root tip biomass across whole forest stands (12% at Drake's Landing and 3% at Heart's Desire), in cores where *A. phalloides* was present, it was 57 ± 18% of the total root tip biomass at Drake's Landing and 45 ± 15% at Heart's Desire. On the East Coast, EM root tips of *A. phalloides* were less frequent (3% at Jake's Landing Road and 6% at Durand-Eastman Park) and made up a smaller proportion of total root tip biomass (0.2% at Jake's Landing Road and 0.5% at Durand-Eastman Park; Table 1).

Amanita phalloides was positively identified from six of 21 (30%) in-growth mesh bags (only 21 of 25 bags were recovered from the site). In the quantitative PCR assay, both *A. phalloides* and total fungal standard dilutions produced robust standard curves (Fig. S3), and an analysis of the melting curve profile indicated a single amplification product for the *A. phalloides*-specific primer (data not shown). In the six bags where *A. phalloides* EMM was detected, qPCR assays indicated that *A. phalloides* biomass made up on average 2% (range: 0.1–5.6%) of the total fungal biomass (Table 1).

EM fungal community diversity at a site invaded by *A. phalloides*

Amanita phalloides was the most frequently encountered species along the Drake's Landing transect (Fig. 4), occurring in six of the 30 cores. It occurred most often in

Table 1 Biomass of *Amanita phalloides* sporocarps, root tips and hyphae detected in this study

	California (Point Reyes)				East Coast			
	Heart's Desire		Drake's Landing		Durand-Eastman Park		Jake's Landing	
	Total[1] (g)	Standardized[2] (g m^{-2})	Total	Standardized (g m^{-2})	Total	Standardized (g m^{-2})	Total	Standardized (g m^{-2})
Sporocarps: *Amanita phalloides*	284.0	1.136	175.5	0.439	–	–	–	–
Sporocarps: other EM fungi	174.0	0.694	31.5	0.079	–	–	–	–
Root tips: *Amanita phalloides*	0.019	5.212 ± 2.910	0.076	15.026 ± 10.063	0.006	0.999 ± 0.696	0.005	0.718 ± 0.718
Root tips: other EM Fungi	0.659	176.740 ± 44.660	0.548	107.461 ± 28.516	1.246	198.458 ± 30.068	2.423	385.087 ± 45.066
Hyphae: *Amanita phalloides*	0.0002	0.086 ± 0.036	–	–	–	–	–	–
Hyphae: other (EM)[3] fungi	0.1	40.456 ± 9.694	–	–	–	–	–	–

[1]Total biomass is the sum of all biomass of a specific fraction of fungus detected across the entire plot for all sampling units.
[2]Standardized biomass is the biomass detected per sampling unit (plot, core, or mesh bag) standardized to the area sampled. There are no error estimates for sporocarps because sporocarps were harvested from the entire plot area. Error bars represent one standard error.
[3]It is assumed that most of the fungi growing in the sand-filled in-growth bags were ectomycorrhizal (EM) fungi as most studies that identify all fungal species in mesh bags find that the majority are EM fungi (see text for references). However, it is likely that a small portion of the mesh bag biomass contained saprotrophic fungi.

cores taken nearest to the road, but was also found up to 24 m into the forest. The transect included a total of 45 different ITS OTUs (Table S4).

The species richness of EM fungi per core was low (2.21 ± 0.27). There was no difference in EM fungal richness per core among cores with and cores without *A. phalloides* (with *A. phalloides*, 3.00 ± 0.37; without *A. phalloides*, 2.00 ± 0.32; *t*-value = −1.57, $P = 0.130$).

Other *Amanita* species, including *A. muscaria* and *A. gemmata*, were observed within or nearby the transect as sporocarps, but were not detected as root tips.

Frequency and dominance of *A. phalloides* EM root tips and sporocarps in North American forests compared with *Amanita* species in their native ranges

Root tips colonized by *Amanita* species are generally infrequent in studies of EM fungal communities; dominance of an EM fungal community by an *Amanita* species is rare (Fig. 5). Among 103 root tip records of 49 *Amanita* species collected from 36 different studies of EM fungal communities, mean root tip frequency of *Amanita* species across all studies was 3.94 (± 0.74)% (Table S5). In many cases, root tip frequency was zero; 42 of the records report *Amanita* sporocarps and not the matching root tips.

Mean root tip frequency was not the same across all three biogeographic regions; the West Coast had higher *Amanita* root tip frequency (8.49 ± 1.74%) than the East Coast (1.36 ± 0.33%) or Europe (1.25 ± 0.37%) (Fig. 5). Several studies at Point Reyes showed high frequencies of other *Amanita* species, including *A. muscaria* and *A. franchetii*.

The mean frequency of root tips colonized by *A. phalloides* at Point Reyes, based on data from our plots at Drake's Landing and Heart's Desire, was 17.5 (± 2.5%). This relatively high root tip frequency contrasts with other data from California, and data from the East Coast of North America and Europe. In a different *Quercus*-dominated ecosystem of California, Morris *et al.* (2008) found *A. phalloides* as root tips in only one of 64 cores collected and found no *A. phalloides* sporocarps. Our data suggest that root tips on the East Coast are similarly rare (Table 1; Fig. 5). Moreover, three different studies at three European sites (in Corsica, France, and Denmark) where *A. phalloides* sporocarps are found have never detected a root tip colonized by *A. phalloides* (Fig. 5; Table S5).

In contrast to dominance of *A. phalloides* sporocarps at Point Reyes, sporocarps of other *Amanita* species in their native ranges tend to make up a small portion of total EM sporocarp biomass. With the exception of another study at Point Reyes where the combined biomass of three *Amanita* species made up almost half of the EM sporocarp biomass (Gardes & Bruns, 1996), *Amanita* sporocarps most frequently made up less than 1% of total EM sporocarp biomass (Table 2).

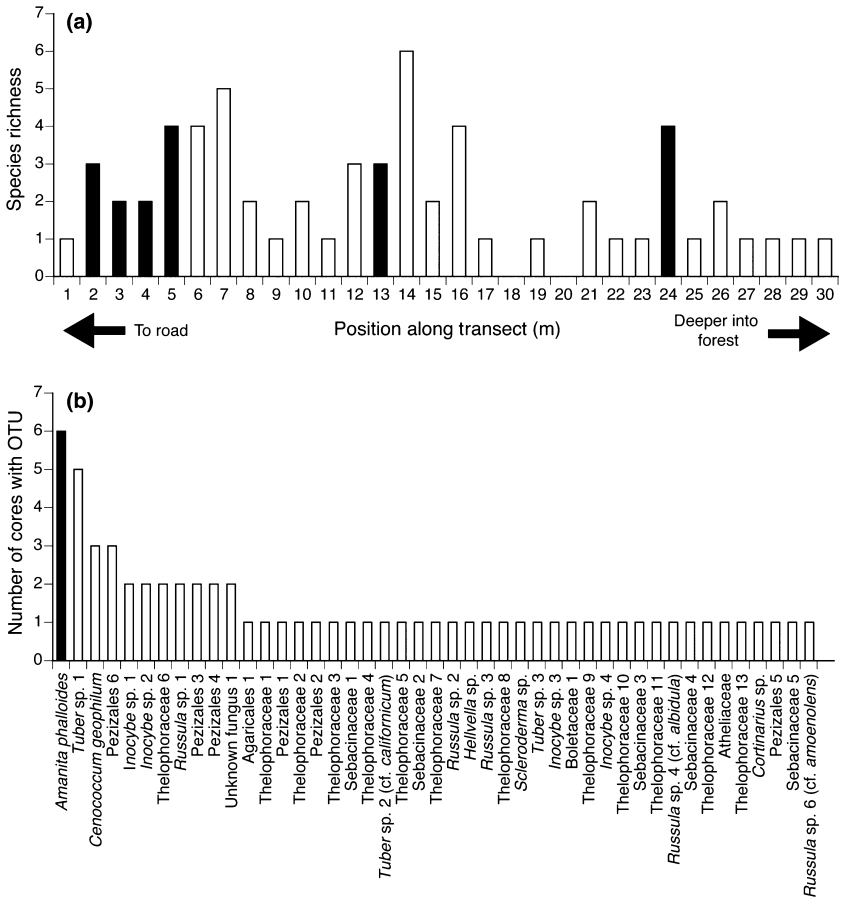

Fig. 4 (a) Ectomycorrhizal (EM) fungal species richness and (b) rank abundance curve of the EM community sampled at Drake's Landing (New Jersey, USA). In (a), black bars indicate those cores where *Amanita phalloides* were present. GenBank accession information for taxa is given in Supporting Information Table S4.

Fig. 5 Abundance of *Amanita* root tips from ectomycorrhizal community studies on the West Coast of North America, the East Coast of North America, and Europe. Data points for *Amanita phalloides* are indicated as black bars; other *Amanita* species, white bars. Arrows indicate the three European studies where the frequency of *A. phalloides* root tips was zero. The number after each species matches the record number in Supporting Information Table S5.

Table 2 Percentage of total ectomycorrhizal community sporocarp biomass of various *Amanita* species

Amanita species	% of total[1]	Study system	Study
A. phalloides – Heart's Desire	62.0	Point Reyes Peninsula, California, USA; *Pinus muricata-Quercus agrifolia* forest	This study
A. phalloides – Drake's Landing	85.0	Point Reyes Peninsula, California, USA; *Pseudotsuga menziesii-Quercus agrifolia* forest	This study
A. franchetii	31.0	Point Reyes National Seashore, California, USA; *Pinus muricata* forest	Gardes & Bruns (1996)
A. magniverrucata	13.5		
A. gemmata/A. pantherina	3.5		
A. aspera, *A. muscaria*, *A. pantherina*, and *A. porphyria* combined	< 1	Willamette National Forest, Oregon, USA; *Pseudotsuga menziesii* forest	Smith *et al.* (2002)
A. constricta sensu lato	0.4	*Tsuga heterophylla-Pseudotsuga menziesii* forest, Olympic National Park, Washington, USA	O'Dell *et al.* (1999)
A. franchetii	0.2		
A. porphyria	2.5		
A. silvicola	0.6		
A. constricta	1.3	*Picea sitchensis-Tsuga heterophylla* forest, Olympic National Park, Washington	Edmonds & Lebo (1998)
A. fulva	0.4	*Picea abies* forest, Billingen, Sweden	Dahlberg *et al.* (1997)
A. porphyria	0.5		
A. citrina	< 1	*Pinus* forests, Lleida, Catalonia, Spain,	Martínez de Aragón *et al.* (2007)
A. ovoidea	2.5		
A. pantherina	< 1		
A. solitaria	< 1		
A. verna	1.3		
A. muscaria	0.2	*Pinus taeda* plantation, Santa Catarina, southern Brazil[2]	Giachini *et al.* (2004)
A. pantherina var. *multisquamosa*	0.1		

[1]% of total EM fungal sporocarp biomass. < 1 means that a study reported sporocarps present, but did not give a value for the biomass of that species because it had a very low biomass.
[2]In this study, the *Amanita* species were probably nonnative because these were plantations of nonnative hosts.

Discussion

Current and potential distribution of *A. phalloides* in North America

Our surveys establish a wider geographic range for *A. phalloides* on the West Coast of North America as compared with the East Coast. On the East Coast, *A. phalloides* appears most often in planted forests of native or nonnative trees, but in California it is commonly found in natural native forests. Although it is likely that populations of *A. phalloides* from both regions were not detected during our continental-scale survey, the effort we used to search for records on both coasts was similar; the absence of populations of basidiomycete fungi is difficult to confirm because of the cryptic habit of soil mycelia and ephemeral nature of sporocarps.

We did not explicitly collect data to test among the potential mechanisms that would cause the differences in the range size and habitat of *A. phalloides* on the different coasts. However, our species distribution model (SDM) predicted a substantially larger area of suitable habitat on the West Coast than on the East Coast. The two variables that explained over 50% of the SDM's prediction were related to temperature, suggesting that temperature is a significant control on the distribution of *A. phalloides*. Similarly, in a recent study that developed an SDM specifically for *A. phalloides* in Norway, temperature in May had the greatest relative contribution to that model's predictive power (Wollan *et al.*, 2008). In Europe, *A. phalloides* is common in regions that have a Mediterranean climate with mild winters (Neville & Poumarat, 2004). The colder winters of the Northeastern USA may limit the spread of *A. phalloides* in the Northeast. A large part of the Southeastern USA, as well as Mexico, appear to have suitable bioclimatic environments for *A. phalloides*, and these may be locations where future range expansions of *A. phalloides* will occur. Past reports of *A. phalloides* occurring in Mexico have not been verified (Pringle & Vellinga, 2006).

The number and timing of original introductions may also explain the different range sizes of *A. phalloides* on the East and West Coasts. The first Californian sporocarps of *A. phalloides* were collected in the early 1940s (Pringle *et al.*, 2009), but the first confirmed collections of *A. phalloides* on the East Coast were not made until the early 1970s (Tanghe & Simons, 1973; Tanghe, 1983). There may be a considerable lag between the introduction of an EM fungus and sporocarp production (Vellinga *et al.*, 2009) and if *A. phalloides* arrived in California earlier than it arrived at the East Coast, it may have had more time to spread on the West Coast.

Biotic interactions between *A. phalloides* and resident fungi, pathogens and plant hosts may also contribute to the differences in the range sizes of *A. phalloides* in North America. Unfortunately, we have no data on these interactions and cannot confirm their role in limiting or facilitating the spread of *A. phalloides*.

Local abundance of *A. phalloides*

At Point Reyes, we measured the local abundance of *A. phalloides* as sporocarps, root tips and extramatrical mycelia. As sporocarps, *Amanita phalloides* was a dominant EM species and made up the majority of the aboveground sporocarp biomass. As root tips, the total biomass of *A. phalloides* was low across the forest stand, but the spatial frequency of *A. phalloides* was relatively high and *A. phalloides* dominated individual core biomass where it was present. The frequency of extramatrical mycelia of *A. phalloides* within one forest stand was relatively high, but within individual mesh bag samples, *A. phalloides* made up only a small portion of the total fungal biomass.

We compiled previously published data on the biomass of *Amanita* sporocarps and the frequency of *Amanita* root tips to put our data on *A. phalloides* at Point Reyes and the East Coast in a global context. These data demonstrated three patterns: root tips colonized by *A. phalloides* are more frequently encountered within forests at Point Reyes than root tips of most other species of *Amanita* in their native ranges (Fig. 5); at three different sites in Europe where *A. phalloides* sporocarps have been found, corresponding root tips have never been found (Fig. 5b; Table S5); and in general, root tips of *Amanita* species are more frequent within forests on the West Coast than on the East Coast and in Europe. At Point Reyes, native *Amanita* species have been shown to be relatively abundant as both sporocarps and EM root tips (Gardes & Bruns, 1996; Horton & Bruns, 1998; Taylor & Bruns, 1999). *Amanita* species were similarly abundant in a recent study of Southern Californian *Quercus agrifolia* stands (Querejeta *et al.*, 2009). Abiotic or biotic factors common across West Coast forests may favor the dominance of *Amanita* species at these sites, making them ideal habitats for the establishment and spread of *A. phalloides*.

It is important to note that the high frequency of root tips colonized by *A. phalloides* observed at Point Reyes may not be a general pattern across the entire West Coast range of *A. phalloides*. In fact, a recent study along the foothills of the Sierra Nevada Mountains in California by Morris *et al.* (2008) found a low frequency of root tips colonized by *A. phalloides* (1.6%). Moreover, sporocarps of *A. phalloides* were not collected at this site, suggesting that *A. phalloides* is not well established in this system. More surveys of invaded North American EM fungal communities are needed to determine how commonly this species achieves the high frequencies observed at Point Reyes.

While the biomass of root tips colonized by *A. phalloides* was low across entire forest stands, *A. phalloides* dominated individual soil cores when it was present, making up *c.* 50% of the EM fungal root tip biomass in invaded cores at both

sites. The dominance of EM fungal root tip communities at small spatial scales (within individual cores) by a single species has been observed for other EM fungi in their native ranges (e.g. Lian *et al.*, 2006). Because no root tips of *A. phalloides* have been found at sites where it occurs in Europe, it is unclear if *A. phalloides* can also be dominant at small spatial scales in its native range.

Within individual forest stands at Point Reyes, EM root tip biomass is low, but the biomass of *A. phalloides* sporocarps is very high. Discrepancies between belowground and aboveground abundances are a common feature of EM fungi (Gardes & Bruns, 1996; Peter *et al.*, 2001; Richard *et al.*, 2005). The mechanisms causing aboveground–belowground discrepancies are largely unknown. Genets of *Amanita* species tend to be small (Redecker *et al.*, 2001; Bagley & Orlovich, 2004; Liang *et al.*, 2005) and small genets formed around sporocarps should lead to the formation of localized clusters of *Amanita* root tips. The observation of a low abundance of root tips across a forest, but locally high abundance of root tips within a core, would suggest that the genet sizes of *A. phalloides* are also small. Preliminary observations support this prediction (A. Pringle & H. B. Cross, unpublished). *Amanita phalloides* may also be very efficient at obtaining carbon from hosts, and require a minimal number of root tips to form a functional symbiosis. Many EM fungi that form ectomycorrhizal root tips with plants are known to have saprotrophic capabilities (as reviewed in Koide *et al.*, 2008). *Amanita phalloides* may also possess some saprotrophic capabilities, leading to a decreased dependence on host plants for carbon.

Potential impacts of *A. phalloides*

Amanita phalloides may have multiple impacts on local forest communities. For example, the dominance of *A. phalloides* could have effects on the biogeochemistry of forests. Ectomycorrhizal fungi contribute considerable amounts of photosynthetically derived carbon to forest soils (Högberg & Högberg, 2002). When a new EM fungal species with different carbon acquisition and allocation strategies invades a forests and grows to dominate the local EM fungal community, it has the potential to alter carbon cycling. Carbon derived from hosts and allocated to sporocarps may be diverted from a relatively recalcitrant pool (tree roots with limited turnover) to a labile pool (fungal sporocarps that rapidly decompose). A similar impact was inferred by Chapela *et al.* (2001); introduced EM fungi appear to drain carbon from soils of Ecuadorian grasslands.

Many invasive species achieve dominance through strong competitive effects (Levine *et al.*, 2003; Lockwood *et al.*, 2007), and there is evidence for strong competitive interactions among EM fungi (Koide *et al.*, 2005; Kennedy *et al.*, 2007). *Amanita phalloides* may compete with other native EM fungal species for carbon from host plants. If *A. phalloides* does not provide the same level of resources to hosts in exchange for photosynthates, this may lead to a breakdown of the mutualism between *A. phalloides* and its host plants. *In situ* studies that measure the flow of resources between hosts and *A. phalloides* are needed to mechanistically assess the potential impacts of *A. phalloides* on native forest ecosystems.

Summary

We have established baseline data on the distribution and abundance of the European EM fungus *A. phalloides* in North America. *Amanita phalloides* has established across two separate North American ranges, although the sizes of the ranges are different; *A. phalloides* has a larger range on the West Coast. In California, *A. phalloides* is more likely to grow within native forests and can be the dominant EM fungus at some sites where it has established. Although the species' ability to influence local fungal biodiversity or function as a mutualist is unknown, its high density and dominance in the forests around Point Reyes Peninsula, California suggests that it has the potential to impact native fungal and plant communities.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Supporting Information, Part 1

Table S1 Confirmed occurrences of *Amanita phalloides* in North America.

Table S2 European occurrences of *Amanita phalloides* used to build the species distribution model.

Fig. S1 Distribution of *Amanita phalloides* in Europe predicted by the species distribution.

Table S3 Environmental variables used to create the species distribution model and their contribution to model performance.

Table S4 Ectomycorrhizal fungi detected as root tips in the study at Drake's Landing.

Table S5 Studies used to compile data on *Amanita* root tip abundance.

Supporting Information, Part 2

Table S6 A portion of an alignment of the ITS region of *Amanita phalloides* and several closely related *Amanita* species from Section Phalloideae.

Fig. S2 PCR amplicons produced using the *Amanita phalloides* specific primer set ITSphl5f-ITS4b or the general fungal primers ITS1f-5.8s.

Fig. S3 Standard curve for *Amanita phalloides* gDNA dilution series generated using the primers sets ITSphl5f and ITS4 and ITS1f and 5.8s.

Fig. S4 Standard curves used to quantify (a) biomass of total fungal extrametrical mycelia and (b) biomass of *Amanita phalloides* extrametrical mycelia using qPCR on gDNA extracted from the mesh bags.

Table S7 Raw data for biomass of extramatrical mycelium in mesh bags.

Supporting Information, Part 3

Methods & Notes S1 Sites descriptions for Point Reyes Peninsula plots, DNA extraction of ectomycorrhizal root tips, and a note on compilation of root tip data.

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