Mycorrhiza (2019) 29:97–111
https://doi.org/10.1007/s00572-018-00879-7

ORIGINAL ARTICLE

Plant-mediated partner discrimination in ectomycorrhizal mutualisms

Laura Bogar[1] · Kabir Peay[1] · Ari Kornfeld[2] · Julia Huggins[3] · Sara Hortal[4] · Ian Anderson[4] · Peter Kennedy[3]

Received: 7 September 2018 / Accepted: 26 December 2018 / Published online: 7 January 2019

Abstract

Although ectomycorrhizal fungi have well-recognized effects on ecological processes ranging from plant community dynamics to carbon cycling rates, it is unclear if plants are able to actively influence the structure of these fungal communities. To address this knowledge gap, we performed two complementary experiments to determine (1) whether ectomycorrhizal plants can discriminate among potential fungal partners, and (2) to what extent the plants might reward better mutualists. In experiment 1, split-root *Larix occidentalis* seedlings were inoculated with spores from three *Suillus* species (*S. clintonianus*, *S. grisellus*, and *S. spectabilis*). In experiment 2, we manipulated the symbiotic quality of *Suillus brevipes* isolates on split-root *Pinus muricata* seedlings by changing the nitrogen resources available, and used carbon-13 labeling to track host investment in fungi. In experiment 1, we found that hosts can discriminate in multi-species settings. The split-root seedlings inhibited colonization by *S. spectabilis* whenever another fungus was available, despite similar benefits from all three fungi. In experiment 2, we found that roots and fungi with greater nitrogen supplies received more plant carbon. Our results suggest that plants may be able to regulate this symbiosis at a relatively fine scale, and that this regulation can be integrated across spatially separated portions of a root system.

Keywords Ectomycorrhiza · *Larix occidentalis* · Partner choice · *Pinus muricata* · Stable isotope enrichment · *Suillus*

Introduction

Ectomycorrhizal fungi play critical roles in forest ecosystems, both as major drivers of nutrient cycling and carbon storage in soils (Averill et al. 2014; Clemmensen et al. 2015) and as key mediators of plant community dynamics (Nara and Hogetsu 2004; Bennett et al. 2017). Given the plant costs involved in the symbiosis (in terms of root occupancy, immune activation, plant carbon, or other resources) (Smith and Read 2008; Van Wees et al. 2008; Kennedy 2010; Corrêa et al. 2012; Pringle 2016), hosts should prefer to invest in more beneficial ectomycorrhizal mutualists when presented with several possible partners. This scenario has been well documented in the arbuscular mycorrhizal symbiosis, where plants can reward greater nutrient-providing symbionts with increased carbon supplies (Bever et al. 2009; Kiers et al. 2011; Argüello et al. 2016), and in the legume-rhizobial mutualism, where plants can punish ineffective nitrogen fixers, apparently by withholding oxygen (Kiers et al. 2003). These mechanisms—rewards and punishment—likely fall along a continuum of host control strategies that encompass many possible mechanisms (Kiers and Denison 2008).

Because mechanisms of host control should only be required when a symbiotic interaction is costly to the plant, the cost of the ectomycorrhizal mutualism has been the subject of much debate. Since the fungi themselves principally benefit from host-derived carbon, this resource is often assumed to be the main cost to the plant host (Smith and Read 2008; Pringle 2016). This view has been challenged recently by the finding that carbon is an excess resource for plants (Corrêa et al.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00572-018-00879-7) contains supplementary material, which is available to authorized users.

✉ Laura Bogar
lbogar@stanford.edu

[1] Department of Biology, Stanford University, 371 Serra Mall, Stanford, CA 94305, USA

[2] Department of Global Ecology, Carnegie Institution for Science, Stanford, CA 94305, USA

[3] Department of Plant & Microbial Biology, University of Minnesota, Saint Paul, MN 55108, USA

[4] Hawkesbury Institute for the Environment, Western Sydney University, Hawkesbury Campus, Locked Bag 1797, Penrith, NSW 2751, Australia

2012), and that carbon allocation does not always directly correspond to nitrogen provisioning from the fungi (Corrêa et al. 2008; Valtanen et al. 2014; Hasselquist et al. 2016; Hortal et al. 2017). Carbon may still be important, however, when considering the potential costs of ectomycorrhizal mutualism. It might be most realistic to think of the "cost" of supporting any given ectomycorrhizal fungus as an *opportunity* cost: although the plant is typically not carbon-limited, any carbon that goes to one fungus is carbon that could have been invested in another, potentially superior, mutualistic partner. Similarly, any root tip occupied by one fungus is a root tip that could have been occupied by another. A host should, hypothetically, allow an interaction to continue only while it is receiving mutualistic services that are at least as beneficial as the average mutualist in the available species pool (Johnstone and Bshary 2008), although recent work has demonstrated that environmental fluctuations can induce hosts to support lower-quality mutualists (Moeller and Neubert 2016). Maintaining this kind of control over the fungi should require an ectomycorrhizal plant to adjust its investment in mutualists according to their relative performances, even when carbon is not limiting.

Although discrimination among partners by plants has been demonstrated in other root symbioses, the ectomycorrhizal mutualism may be controlled in different ways. Unlike arbuscular mycorrhizal and rhizobial mutualisms, the ectomycorrhizal symbiosis is consistently extracellular and involves dozens of independently evolved fungal lineages (Smith and Read 2008; Tedersoo and Smith 2013). Additionally, the magnitude of variation in partner quality may be more extreme: Ectomycorrhizal fungi vary dramatically in enzymatic capabilities, biomass production, and environmental tolerances (Agerer 2001; Jones et al. 2003; Talbot et al. 2013). Arbuscular mycorrhizal fungi, by contrast, may contain less functional variation within their monophyletic guild (Powell et al. 2009), although the full extent of their functional diversity has yet to be adequately characterized (Chagnon et al. 2013; Behm and Kiers 2014). Similarly, while there has been well-documented variation in nitrogen provisioning by different rhizobial strains (Friesen 2012), they provide a single nutrient compared to the more diverse services provided in mycorrhizal symbioses (such as multiple nutrients, water acquisition, and protection from heavy metals and pathogens) (Colpaert et al. 2011; Bennett et al. 2017). Given the phylogenetic diversity and the breadth of symbiotic benefits provided by ectomycorrhizal fungi, their plant hosts may need to regulate them using different mechanisms from those employed by arbuscular mycorrhizal or rhizobial hosts.

Plant hosts have at least two opportunities to discriminate among fungal partners. Prior to initiating an interaction, a plant may respond to fungal signals and accept or reject potential partners based on their identities or anticipated benefits. This phenomenon is well documented in rhizobial symbioses (Oldroyd et al. 2011), and may also be important in mycorrhizal associations (Schmitz and Harrison 2014; Garcia et al. 2015). In ectomycorrhizal associations, the strongest evidence for pre-interaction screening by plants is the phenomenon of host specificity. Among fungi, many are specific to particular families or genera of plants (Molina and Horton 2015); among plants, although strict specificity to one or a few species of fungi is almost entirely restricted to mycoheterotrophs (Bruns et al. 2002), the suite of symbionts a plant supports often varies predictably with plant taxonomy (Molina and Horton 2015). In many cases, this specificity may be exerted at the spore germination stage: Host-specific fungi will not germinate until appropriate roots are present, despite being capable of broader associations as hyphae (Massicotte et al. 1994; Lofgren et al. 2018). Additionally, recent research into ectomycorrhizal fungal gene expression has identified many small secreted peptides that appear to be expressed at the initiation of symbiosis, and whose identities can vary considerably among fungal taxa (Plett et al. 2011; Liao et al. 2016). This points strongly to pre-interaction signaling as an important checkpoint in this symbiosis.

Once an interaction has been initiated, the plant may also be able to reward or sanction its partner according to the benefits it provides. This is often cited as a key prerequisite for mutualism stability (Hoeksema and Kummel 2003; Frederickson 2013), since pre-interaction signaling may not truthfully convey the quality of the potential partner. It is far from clear, however, to what extent and on what basis ectomycorrhizal plants may discriminate among fungal partners according to their symbiotic benefit. Theoretical work predicts that plants in nitrogen-limited systems should reward nitrogen-providing fungi with carbon resources (Franklin et al. 2014; Moeller and Neubert 2016). This would be consistent with the way that plants distribute resources among non-symbiotic roots, investing resources to encourage the growth of nitrogen-providing roots into patches of valuable soil resources (Chen et al. 2018). Importantly, these soil resources may be available to both roots and soil fungi, creating competition between the symbionts (Peay 2016). These competitive interactions could complicate rewards for cooperation, as hosts may direct photosynthate to portions of the root system that provide nitrogen, regardless of their symbiotic status. Adding to this complexity, analysis of theoretical models suggests that, even with plant preferential allocation, a stable community of fungi on a root system may include some non-mutualists, assuming that the plant will invest initially in fungi of unknown quality (Christian and Bever 2018). Thus, the degree of plant discrimination that exists in any given symbiosis may be important in explaining the diversity of ectomycorrhizal fungi that coexist on a fine scale.

Reflecting this complexity, evidence for plant rewards of cooperative ectomycorrhizal fungi has thus far been equivocal. Hasselquist et al. (2016) found idiosyncratic effects of

nitrogen addition and shading on resource trading between ectomycorrhizal fungi and host trees in a boreal forest, suggesting that rates of carbon and nitrogen exchange are context-dependent and may not always benefit host plant growth. In particular, fungal retention of nitrogen may be greatest when little nitrogen is present, with transfer to host plants increasing as more becomes available (Näsholm et al. 2013). Comparing three species of *Pisolithus* associated with *Eucalyptus grandis*, Hortal et al. (2017) did not find evidence for plant carbon rewards of fungal nitrogen supply under conditions where nitrogen was not limiting. As soil nitrogen declines, plants have been shown to invest more in mycorrhizas, but they do not necessarily experience greater nitrogen transfer to their own tissues with greater fungal colonization (Corrêa et al. 2008). Taken together, it appears that plants may, under certain conditions, reward fungal nitrogen provisioning with carbon resources, but the relationship between fungal nitrogen transfer rates and plant investment in the fungus is complex.

To examine the importance of pre-colonization screening (based on fungal identity) and post-colonization selection (for symbiotic benefit) in mediating plant discrimination among ectomycorrhizal fungi, we performed two experiments. Both used host plants in the Pinaceae and fungi in the Pinaceae-specific genus *Suillus*. In Experiment 1, we grew split-root *Larix occidentalis* seedlings associated with each of three *Suillus* species, in single- or pair-wise combinations, to investigate whether the presence of another fungus elsewhere in the root system would affect the timing and extent of colonization from spores in an isolated root compartment. We hypothesized that, if the plant could discriminate prior to associating with a fungus, the initiation of colonization would vary depending upon the presence and identity of an indirect competitor elsewhere on the root system, and on the anticipated quality of each partner. In experiment 2, we grew paired split-root *Pinus muricata* seedlings with isolated genotypes of *Suillus brevipes* that varied only in access to organic nitrogen. We then tracked plant photosynthate investment in fungi and roots of artificially varying nutritional qualities using ^{13}C labeling. If rewards for nutrient provisioning were important in this system, we expected nitrogen-providing roots and fungi to receive more photosynthate from the plant than those with access to fewer nitrogen resources.

Materials and methods

Experiment 1: *Larix occidentalis-Suillus* spp.

Plant propagation

In March 2014, *Larix occidentalis* seeds (Silva Seed Company, Roy, WA, USA) were soaked in distilled water for 24 h and then stratified at 4 °C for 4 weeks. The seeds were germinated in Petri dishes containing moistened filter paper and then transferred into 19.5 cm × 19 cm × 0.5 cm plexiglass chambers. Each chamber was filled with 180 ml of a twice-autoclaved mix of forest soil, peat moss, and sand (2:2:1 by volume). We added 10 germinated seeds to the upper soil surface. The microcosms were placed in a growth chamber at 21 °C with a 16:8-h light-dark cycle and watered regularly with distilled water. After 4 weeks, the chambers were opened and the roots of each seedling were pruned to facilitate division into two primary root segments. The chambers were closed and the seedlings were grown for another 8 weeks under the same conditions prior to their transfer into the experimental microcosms.

Microcosm set-up

The experimental microcosms included the same soil mix, but consisted of two separate square 10 cm × 10 cm × 1 cm Petri dishes that were glued together (Fig. S1). Using a soldering tool, a small hole was notched into the upper sides of the Petri dishes to plant each half of the seedling's root system inside each of the two Petri dishes while the shoot grew above them. This allowed isolation of the two halves of the root system for each seedling into the separate but immediately adjacent sides of the microcosm. A 1-mL pipette tip was inserted in a second small hole in each Petri dish, which allowed for controlled addition of water while preventing cross contamination between the two sides of the microcosm. For each seedling, both halves of the root system were checked for viability and size equivalency during transfer. If necessary, some roots were trimmed to make the two halves of the root system as equal as possible. Because the seedlings were not completely uniform in size, different sized seedlings were evenly distributed across treatments (i.e., all treatments received a similar range of seedling sizes).

Experimental treatments

To investigate how single- versus two-species ectomycorrhizal fungal inoculation affected both fungal colonization patterns and plant performance, we added inoculum of one of three *Larix*-associated *Suillus* species on one side of the microcosm: *S. clintonianus*, *S. grisellus*, or *S. spectabilis*. All three species exhibit ecological specificity to host plants in the genus *Larix* (Finlay 1989; Molina and Horton 2015; Rineau et al. 2016; Kennedy et al. 2018). *S. clintonianus* and *S. spectabilis* are commonly encountered in younger forests and on seedlings (Leski and Rudawska 2012; Kennedy et al. 2018), and often fruit prolifically (pers. obs.), while *S. grisellus* prefers mature forest habitats (Rineau et al. 2016) and makes smaller and fewer fruit bodies than the other two species (pers. obs.). The ectomycorrhizal species inoculum was added as homogenized single-species spore slurries at a concentration of 5×10^5

spores/ml of soil, which falls within the range of previous assays that have observed consistent ectomycorrhizal colonization (Kennedy and Bruns 2005). Spores were obtained from multiple sporocarps collected in Minnesota, USA, in fall 2013 (see Kennedy et al. (2011) for additional details on spore inoculum preparation). In half of the microcosms, a second species was inoculated at the same time on the other side of the microcosm. The resulting treatments, which contained six replicate microcosms initially, were as follows: (1) *S. clintonianus*/non-ectomycorrhizal, (2) *S. grisellus*/non-ectomycorrhizal, (3) *S. spectabilis*/non-ectomycorrhizal, (4) *S. clintonianus/S. grisellus*, (5) *S. clintonianus/S. spectabilis*, (6) *S. grisellus/S. spectabilis*. In order to prevent algal growth and shield the roots and fungi from light, each microcosm was wrapped in aluminum foil. The seedlings were grown in the microcosms for 20 weeks under the same light and temperature conditions used prior to inoculation, watering to saturation two or three times per week.

Data collection

After a total of 32 weeks of growth, seedlings were harvested to quantify ectomycorrhizal fungal colonization and seedling performance. During harvest, the stem of each seedling was cut immediately above the point where the root system split into two halves. All parts of above this splitting point were designated as shoot biomass and everything below as root biomass. Shoots were dehydrated at 65 °C for 72 h and then weighed. Needles from each seedling were also analyzed for leaf nitrogen content. For that analysis, 2 mg of leaf material was ground to a fine powder by shaking at 1500 rpm for 5 min in 2-mL screw-cap tubes with a Tungsten bead on a Geno/Grinder 2010 (SPEX, Mutchen, NJ, USA). The percent leaf nitrogen by mass was quantified on an elemental analyzer (Vario PyroCube, Elementar, Mt. Laurel, NJ, USA) at the University of Minnesota. The root systems of each seedling were rinsed free of adhering soil and stored in tap water at 4 °C until scoring for ectomycorrhizal colonization (all treatments were processed within 10 days of harvest). Prior to scoring, each root sample was cut into 1-cm segments and mixed to equalize ectomycorrhizal root tip density across the sample. We then selected pieces of the root sample at random and visually scored ectomycorrhizal fungal colonization under a dissecting microscope. For each root system sample, a minimum of 200 root tips were assessed. After scoring, all portions of the root sample (i.e., scored and un-scored) were dehydrated at 65 °C for 72 h and weighed for dry biomass. Because the microcosms were constructed with clear plastic and their depths were thin enough that much of the root system was visible, we were also able to visually inspect each microcosm and record the presence of ectomycorrhizal root tips without disturbing the root systems or the fungi during the course of the experiment. To quantify time to initial ectomycorrhizal fungal colonization, we unwrapped the microcosms every 2 weeks and searched each side for ectomycorrhizal root tips. We note that these time measurements are low-resolution estimates because initial colonization could have happened any time in the 2-week window before the observations were recorded.

Statistical analyses

We used two-way analyses of variance (ANOVA) to compare differences across ectomycorrhizal fungal species identity and competitor treatments in (a) % ectomycorrhizal colonization at the final harvest and (b) time with ectomycorrhizal fungal colonization. We performed another two-way ANOVA to compare compartment root mass across treatments, as a proxy for plant investment in the fungus. (To validate this method, we performed a one-tailed Welch two sample *t* test to confirm that uncolonized root compartments had lower root mass than colonized ones.) We also assessed differences in the benefits provided by each fungus by comparing plant biomass and % leaf nitrogen by mass using two distinct two-way ANOVAs. Following each significant ANOVA, differences among treatment means were determined using Tukey's HSD tests. Prior to interpreting the ANOVAs, models were checked for adherence to homoscedasticity and normality assumptions, and data were log-transformed if necessary.

Experiment 2: *Pinus muricata-Suillus brevipes*

Plant and fungal propagation

Pinus muricata seeds were obtained from cones collected at Point Reyes National Seashore, CA, USA, in November 2013. Fungal inoculum was produced using 12 *Suillus brevipes* cultures that were originally isolated from sporocarps collected in Yosemite National Park and Mendocino County, CA, USA, as well as Alberta, Canada (Branco et al. 2015). Each plant received just one of these isolates to ensure genetic homogeneity of fungi across the root system, while allowing us to capture intraspecific variation in fungal behavior across seedlings with different fungi (Table 1). We cultivated these isolates on modified Melin-Norkrans medium (Marx 1969) with sterile cellophane membranes for transfer to seedlings. Pine seeds were surface-sterilized, germinated on moist filter paper, and planted into twice-autoclaved soil (50% sand, 50% low-ectomycorrhizal inoculum soil from Point Reyes) (Peay 2018) in February 2014. Throughout the experiment, seedlings were maintained in a growth room with a 16:8-h light:dark cycle at a temperature between 20 and 27 °C. In late 2014 (July through December), seedlings were gently uprooted, rinsed in deionized water, and moved into ectomycorrhizal synthesis chambers consisting of clean zip-top plastic bags containing Ingestad solution-moistened paper

Table 1 Ectomycorrhizal fungal isolates used in Experiment 2 (*Pinus muricata–Suillus brevipes*), and their distribution across replicates in different experimental treatments. Replicates are organized by nitrogen treatment: S/S = sand/sand; N/S = nitrogen/sand; N/N = nitrogen/nitrogen. Replicates are further divided between two quantities of nitrogen: 0.5 g or 1 g casein per treated root compartment. Numbers represent the number of harvested seedlings that were inoculated with a particular isolate in each nitrogen treatment. Isolate is indicated for seedlings which were successfully colonized; all others appear in the "no fungus" category

		Replicates					
		0 g	0.5 g		1 g		
Isolate	Origin	S/S	N/S	N/N	N/S	N/N	Total
No fungus	NA	12	7	7	5	7	38
Sb001	Yosemite	1	1	0	1	1	4
Sb015	Yosemite	1	0	0	1	0	2
Sb018	Yosemite	1	1	0	0	0	2
Sb073	Alberta, Canada	1	0	0	1	0	2
Sb100	Alberta, Canada	2	1	2	0	0	5
Total		18	10	9	8	8	53

towels (Nylund and Wallander 1989). Cellophane membranes covered in *Suillus brevipes* mycelium, representing 8–12 weeks of growth from a plug, were gently pressed against the roots, and the bags were zipped closed (roots inside, shoot outside) and wrapped in foil to prevent light penetration. Seedlings remained in synthesis chambers for 1 week, and then were transplanted (with adhering mycelia) into a twice-autoclaved artificial soil containing 50% sieved perlite, 40% vermiculite, and 10% peat by volume, in which they grew until harvest in early March (total 12–32 weeks). Uninoculated seedlings were rinsed in deionized water and then replanted into artificial soil, skipping the synthesis chambers step. Although the staggered mycorrhization schedule meant that seedlings were not at a uniform age when fungi were introduced, all mycorrhizal seedlings used in the experiment had been colonized for at least 12 weeks and were observed to have mature ectomycorrhizas in several places on their root systems when they were transplanted into split-root microcosms.

Microcosm set-up

In March 2015, all seedlings were uprooted, checked for colonization, and replanted into split-root microcosms (two 631-mL Anderson Tree Band pots taped together) containing the same sterile artificial soil medium (Fig. S1). The root system of each seedling (inoculated with a single genotype) was divided in two halves. Each half of the root system was planted in one of the two pots of the microcosm with the shoot above them. Seedlings were watered to saturation once per week with deionized water during the four-month period during which they grew in the split-root microcosms.

Nitrogen treatments to manipulate fungal partner quality

During the replanting of seedlings into the split-root microcosms, we planted nitrogen-containing mesh bags into both compartments of each microcosm (ANKOM technology, NY: R510 bags, 50-μm pore size). These bags allowed us to manipulate the symbiotic quality of the genetically identical isolates on either side of a given seedling's root system, by changing the amount of nitrogen available to them. Bags were filled with twice-autoclaved sand mixed with casein (Thermo Fisher Scientific product S25238), an extract of nitrogen-rich milk proteins, in three concentrations: no casein, 0.5 g casein, or 1 g casein per bag, creating a gradient of nitrogen availability to plants and fungi. Seedlings were divided into three nitrogen treatment groups: Sand on each side of the root system (sand/sand), an identical amount of casein on each side (nitrogen/nitrogen), and sand on one side, casein on the other (nitrogen/sand) (Table 1). The sand/sand (S/S) and nitrogen/nitrogen (N/N) treatments allowed the genetically identical isolates on each side of a given root system to perform as plant symbionts of similar quality. The nitrogen/sand (N/S) treatments were intended to force one of the genetically identical isolates (provided with only a sand bag) into the role of a putatively less effective symbiont than its competitor (provided with casein) on the other side of the root system. Because the plant had the opportunity to associate with symbionts of different quality, we called this the "choice" treatment.

^{13}C labeling

We used stable isotope enrichment to track plant allocation of carbon resources to roots and genetically identical isolates on each side of their root systems. In July 2015, plants were placed in CO_2 enrichment chambers at the Oxford Tract greenhouses at the University of California, Berkeley (Herman et al. 2012). We used an automated system to introduce $^{13}CO_2$ into the chamber each time the plants drew down chamber CO_2 below 400 ppm, maintaining CO_2 concentrations between 400 and 800 ppm during the 6-h labeling period. Plants were labeled in five batches, one per day, due to space limitations within the labeling chambers. The first batch was labeled using 99% $^{13}CO_2$ (Sigma-Aldrich product 364592). For subsequent batches, we used a 10% enriched $^{13}CO_2$ source (Sigma-Aldrich product 600180). (See "Statistical Analysis" for details on our treatment of batch effects introduced by different ^{13}C concentrations.) Plants were harvested exactly seven days after ^{13}C enrichment, and harvested root tissues were stored up to a week in cold tap water before dissection and drying.

Data collection

At the end of the experiment, we harvested 53 labeled seedlings. Many plants were not successfully colonized by fungi, so replication of uncolonized plants was greater in all treatments. These included 3 colonized and 14 uncolonized nitrogen/nitrogen plants, 6 colonized and 12 uncolonized nitrogen/sand plants, and 6 colonized and 12 uncolonized sand/sand plants. Twelve additional plants, which had been in the growth room with other plants, but had not received ^{13}C label, were harvested as unenriched controls (3 uncolonized and 1 colonized nitrogen/nitrogen plants, 3 uncolonized and 1 colonized nitrogen/sand plants, and 3 uncolonized and 1 colonized sand/sand plants). (See Table 1 for a summary of the distribution of replicates across nitrogen levels and fungal isolates.) For each seedling, we removed the shoot, separated the root compartments, and thoroughly rinsed the roots from each compartment separately in tap water. After clipping the roots from each compartment into 2-cm fragments, we used randomly selected pieces to determine the ectomycorrhizal colonization (counting at least 100 fine roots per plant). For colonized plants, we collected both ectomycorrhizas and uncolonized fine roots (< 1-mm diameter) from the fragments for ^{13}C concentration measurements; because uncolonized plants had no ectomycorrhizas, we collected only uncolonized fine root tissue. All plant and fungal tissues were oven-dried and weighed as in experiment 1. Dried uncolonized fine roots were homogenized into a fine powder in a Minibeadbeater (Biospec products, Inc., Bartlesville, OK, USA) for stable isotope analysis; due to low mass of available tissue per plant, ectomycorrhizas were analyzed without grinding (approximately 15–25 whole ectomycorrhizas per sample tin, typically 1–3 mg dry mass). Most colonized plants yielded enough ectomycorrhizal dry mass for mass spectrometry, but three nitrogen/sand, two nitrogen/nitrogen, and one sand/sand plant did not, bringing replication for ectomycorrhizal tissue down to 12 plants total. Elemental (carbon/nitrogen) and stable isotope analysis were performed using a Costech Elemental Analyzer (Costech Analytical Technologies, Valencia, CA, USA) coupled with a Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) (Thermo Fisher Scientific, Waltham, MA, USA) at the Carnegie Institute of Global Ecology, Stanford, California, USA. The signal was processed as described in Kornfeld et al. (2012).

Data processing

^{13}C label concentration was calculated from IRMS data as mmol ^{13}C excess per mol ^{12}C, relative to unenriched pine seedlings (Slater et al. 2001). Using these concentration values, we calculated a log-ratio carbon allocation metric to express differences in the amount of ^{13}C allocated by the plant to each half of its root system (Ji and Bever 2016), allowing us to consider the distribution of ^{13}C in a root system with respect to a compartment of interest. A positive allocation metric indicates greater [^{13}C] in the focal compartment relative to the other half of the root system, while a negative value indicates a lesser [^{13}C] than in the other half. This ratio was calculated separately for each tissue of interest (ectomycorrhizas and uncolonized fine roots). When root systems were divided between nitrogen (casein) and non-nitrogen (sand) compartments, we used the nitrogen-provisioning half of the root system as the focal compartment, calculating the allocation metric as $\ln([^{13}C]_{N\ compartment}/[^{13}C]_{Sand\ compartment})$. Otherwise, when nitrogen treatments on each side were identical, the numerator position was assigned at random to the value from one of the compartments, with the value from the other compartment serving as the denominator (i.e., $\ln([^{13}C]_A/[^{13}C]_B)$), resulting in values arbitrarily assigned to positive or negative quantities. When the mmol ^{13}C excess value was zero or slightly negative (one value, − 0.03 mmol ^{13}C excess per mol ^{12}C in the mycorrhizas of a nitrogen/nitrogen plant), we forced it to a very small positive value for log-transformation (+ 0.000001 mmol ^{13}C/mol ^{12}C). To compare the nitrogen contents of ectomycorrhizas or fine root tissues in paired root compartments, we employed the same log-ratio procedure as we did for ^{13}C allocation, comparing the percent nitrogen contents of each tissue across paired root compartments.

Statistical analyses

To assess differences in $^{13}CO_2$ enrichment across labeling batches, we examined patterns in overall enrichment and in the carbon allocation metric. This analysis included only fine roots from uncolonized plants, a reference tissue that was well represented in all labeling batches. To examine overall enrichment, we performed a Kruskal-Wallis test with a post hoc Dunnett test to compare the mean mmol ^{13}C excess in fine roots across the two halves of each split-root system. To test for batch effects in the carbon allocation metric, we used an analysis of variance (ANOVA). To determine whether total nitrogen supplied to the plant (0 g, 0.5 g, or 1 g times the number of nitrogen-containing bags planted near the root system), average % ectomycorrhizal colonization across the root system, or their interaction affected 1) seedling biomass, or 2) fine root nitrogen concentration, we built two linear models (after log-transformation to improve homoscedasticity and normal distribution of residuals). We performed a two-way ANOVA to see how nitrogen treatment (N/S, N/N, or S/S) and addition level (0 g, 0.5 g, of 1 g casein) affected carbon allocation to root tissues. For this analysis, we used data from the primary nutrient-absorbing organ in colonized and uncolonized plants: ectomycorrhizas for colonized plants, and fine roots from uncolonized plants. To see how these nitrogen treatments may have affected tissue nitrogen

concentrations, we performed the same set of tests using the log-transformed ratio of nitrogen concentrations in paired root compartments as the dependent variable. We performed a Tukey HSD test following each significant ANOVA to identify the pair-wise differences among groups. (In each case, if a factor did not significantly predict the dependent variable, we did not include it in the figures or Tukey analysis. This process led us to pool replicates from different nitrogen addition levels within N/N, N/S, S/S treatments.) To examine how root nitrogen provisioning might have influenced carbon allocation, we performed linear regression predicting the log carbon allocation ratio (the difference in carbon quantities allocated to each side of a split-root system) to the log-transformed nitrogen concentration ratio (the difference in tissue nitrogen concentrations between sides of a split-root system), including root tissue type (ectomycorrhiza vs. uncolonized fine root) as a covariate. All statistical analyses for both experiments were conducted in R v.3.3.3 (R Core Team 2017) and considered significant at $p < 0.05$.

Results

Experiment 1: Can *Larix* discriminate among three *Suillus* species?

All the uninoculated halves of the one-species treatment microcosms remained non-mycorrhizal throughout the experiment. As such, we are confident the ectomycorrhizal root tip colonization observed was the result of our spore inoculation and that there was no cross contamination between sides of the microcosms. The extent of colonization by each *Suillus* species depended upon the interaction between fungal species identity and competitor identity (two-way interaction: $F_{3, 34} = 3.475$, $p = 0.027$; Table S1a. When growing alone, all three species colonized seedling root systems to similar extents, occupying on average 38% of available roots per seedling (Fig. 1a). When a second species was present on the other side of the microcosm, *S. clintonianus* and *S. grisellus* colonized the root systems to a similar extent as when growing alone (Fig. 1a). Colonization by *S. spectabilis*, however, was sensitive to the presence of a second species: the fungus occupied a much smaller proportion of the root system in the two-species treatments (0.9%) than in the single-species treatment (34.7%).

The timing of root system colonization by the three *Suillus* species also varied by treatment, although with statistical significance that was marginal (two-way interaction: $F_{3, 34} = 2.363$, $p = 0.088$; Table S1b). Without an ectomycorrhizal fungal competitor, all three *Suillus* species colonized *Larix* seedlings at approximately the same time (Fig. 1b). When a second species was present on the other side of the microcosm, however, *S. spectabilis* established much later, forming ectomycorrhizas only in the last 1–3 weeks of the experiment. In contrast, time with ectomycorrhizal colonization by *S. clintonianus* or *S. grisellus* was approximately the same whether growing alone or when a second species was present (Fig. 1b).

The tested fungal species appeared to be equally effective mutualists, producing plants with statistically indistinguishable biomass and foliar nitrogen fractions in single-species treatments (Table 2; Table S2). Competitor identity was a significant predictor of seedling biomass, but a post hoc Tukey test revealed that this pattern was driven entirely by low biomass in the no competitor ("None") treatments (Table 2; Table S2). Foliar N was not significantly affected by focal species, competitor identity, or their interaction. We also observed no significant differences in root mass among *Larix* seedlings root compartments with different fungal treatments (Table S1c), although uncolonized compartments did have significantly lower root mass than colonized compartments ($t = -2.015$, df = 25.186, $p = 0.027$).

Experiment 2: Can *Pinus* reward N-provisioning roots and fungi with carbon resources?

Colonization by *Suillus brevipes* isolates ranged from 0 to 86% across all root compartments, with a median of 17% among colonized plants. Although the mesh bags were intended to make added nitrogen available only to fungi, they did not completely exclude roots. Nitrogen from casein, then, was available both to plants and to fungi through direct contact as well as possible leaching from the bags into the surrounding soil during watering. Although nitrogen addition level (0 g, 0.5 g, or 1 g casein ingrowth bags) significantly affected fine root nitrogen concentrations (i.e., mean % nitrogen across the two halves of a split-root system) (Table S3a), % ectomycorrhizal colonization had only a marginally significant effect, and neither factor had a significant impact on plant biomass (Table S3b). All labeled plants were substantially enriched for ^{13}C (range −20.2 to + 358‰, with mean ± standard deviation (sd) = +54.8 ± 88.4 ‰, compared to unenriched plant tissues with a range of − 30.3 to − 27.1‰, mean ± sd = −28.1 ± 1.0‰). The extent of fine root enrichment (mean mmol ^{13}C excess across the two halves of a split-root system) in uncolonized plants was significantly predicted by labeling day (Kruskal-Wallis: $X^2 = 28.015$, df = 3, $p < 0.001$), but this batch effect disappeared when we analyzed the log-ratio carbon allocation metric (ANOVA: $F_{3,35} = 0.396$, $p = 0.756$).

Nitrogen treatment (N/N, S/S, or N/S), but not nitrogen addition level (0 g, 0.5 g, or 1 g casein), significantly affected plant carbon allocation to the primary absorptive belowground tissue: fine roots in uncolonized plants, and ectomycorrhizas in colonized plants. The "choice" treatment (sand(S)/nitrogen(N)) exhibited the most pronounced preferential allocation (most positive allocation metrics towards the nitrogen

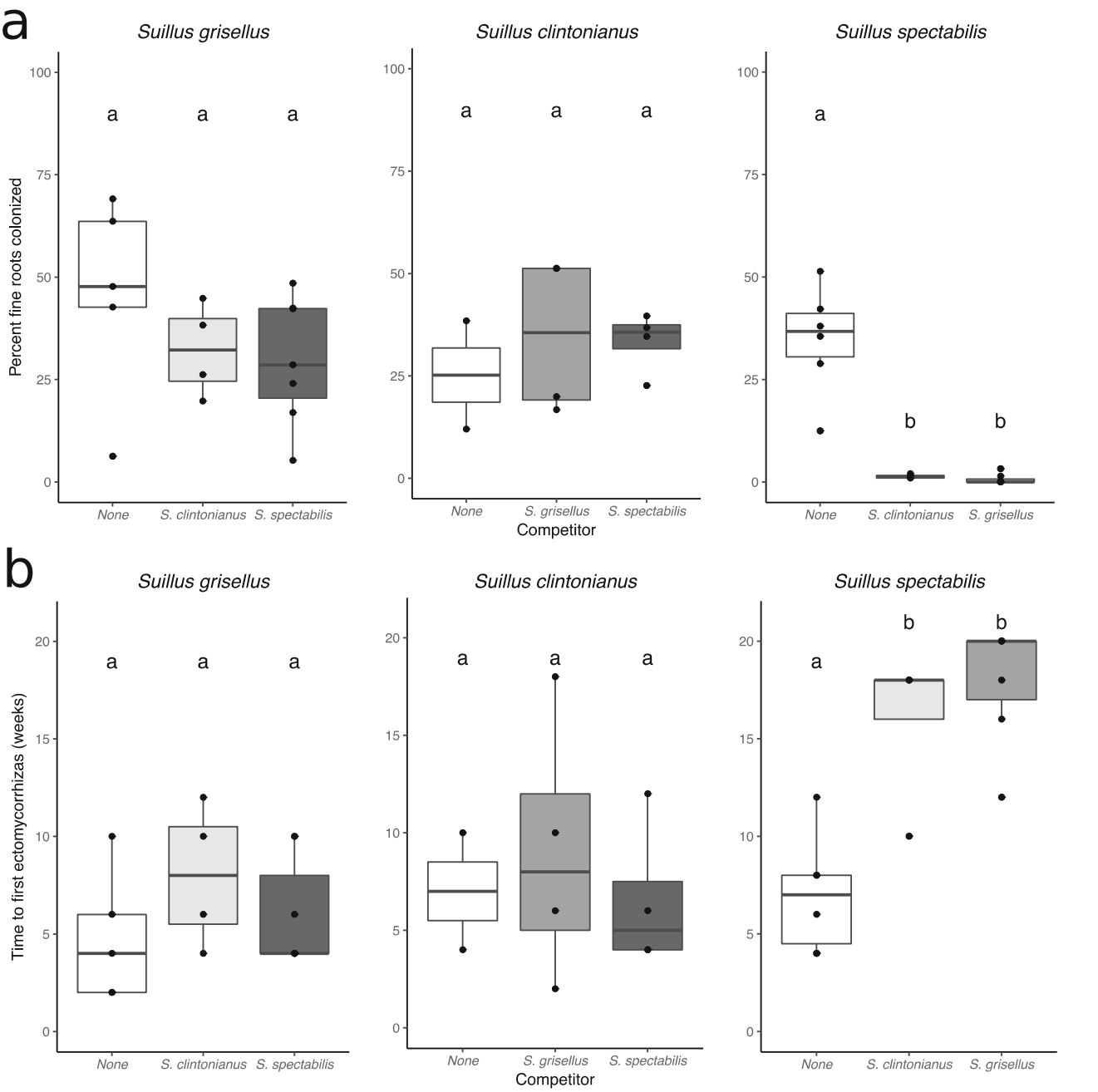

Fig. 1 Extent of ectomycorrhizal percent (%) colonization (**a**) and timing of ectomycorrhizal fungal colonization (**b**) of *Larix occidentalis* seedlings varies based on the interaction between fungal species identity and competitor treatment. Lowercase letters indicate significant differences identified among the interacting factors (fungus and competitor identity) by a post hoc Tukey HSD test on the two-way ANOVA results. The midline of each box represents the mean value for that fungus and competitor combination, hinges illustrate the boundaries of the first and third quartiles, and whiskers extend to the most extreme points no more than one and a half times the interquartile range away from the hinge. All data are plotted as individual points overlain on the boxplots

compartment), with a median carbon allocation value of + 0.83 towards the nitrogen compartment (Fig. 2a; Table 3). This pattern was mirrored by the effect of nitrogen treatment on log-transformed ratios of tissue nitrogen concentrations in paired root compartments (Fig. 2b; Table 3), with a median nitrogen metric value of + 0.22 (representing higher tissue nitrogen in the nitrogen-amended compartment). There was also a strong positive association between the log-transformed ratio of ^{13}C allocation to root tissues and the log-transformed ratio of percent nitrogen detected in those tissues. Specifically, as the difference (log ratio) in percent nitrogen between tissues in paired root compartments

Table 2 *Larix* seedling biomass and foliar % nitrogen from experiment 1. Competitor identity significantly affected biomass, with the no competitor treatment ("None") having significantly less mass than when any other fungus was present on the roots (*$p < 0.05$). Foliar N was not significantly affected by focal species, competitor identity, or their interaction. Values presented are mean plus or minus one standard deviation

		Focal species:		
		S. clintonianus	*S. grisellus*	*S. spectabilis*
	Competitor species:			
Total biomass (g)	*S. clintonianus*	NA		
	S. grisellus	1.553 ± 0.299	NA	
	S. spectabilis	1.603 ± 0.217	1.584 ± 0.212	NA
	None*	1.285 ± 0.049	1.268 ± 0.248	1.257 ± 0.114
Foliar nitrogen (%)	*S. clintonianus*	NA		
	S. grisellus	1.490 ± 0.107	NA	
	S. spectabilis	1.338 ± 0.096	1.327 ± 0.142	NA
	None	1.335 ± 0.163	1.434 ± 0.206	1.282 ± 0.162

increased, so did the difference in ^{13}C allocation to those paired tissues. Root tissue type—ectomycorrhiza, from colonized plants, or uncolonized fine root, from uncolonized plants—was not a significant predictor of the carbon allocation metric (Fig. 3 and Table S4: adjusted $r^2 = 0.8155$, df = 49, $p < 0.001$).

Discussion

Collectively, our results suggest that ectomycorrhizal plants have the ability to discriminate among potential fungal partners both prior to colonization (experiment 1) and during a symbiotic interaction (experiment 2). In the first experiment, we found that *Larix occidentalis* seedlings associated less with *Suillus spectabilis* when presented with an alternative ectomycorrhizal symbiont than when no alternative was available (Fig. 1). That is, the extent of *S. spectabilis* colonization was heavily reduced in the two-species treatments relative to when it colonized alone. This likely reflected delayed onset of root colonization by this fungus (Fig. 1b), suggesting the mechanism at work acted prior to an active symbiosis with *S. spectabilis*. While our results do not identify the specific mechanism, we believe they are most consistent with some kind of pre-colonization signaling. One potential mechanism could be the activation of plant defense genes prompted by fungal signals, as Hortal et al. (2017) recently demonstrated that defense genes can be upregulated when multiple ectomycorrhizal fungal species colonize *Eucalyptus* seedlings. Specifically, they found only the least effective ectomycorrhizal mutualist triggered defense gene activation, suggesting that plants may have localized control on colonization. When the least effective mutualist was the only fungus available for colonization, however, they showed that defense genes were not upregulated against that symbiont, consistent with our observations in the present experiment. This effect need not solely reflect plant control of colonization, however, as it is equally plausible that both *S. clintonianus* and *S. grisellus*, but not *S. spectabilis*, induced a plant immune response across the entire root system as they colonized the host roots. This activation of defense pathways could have inhibited colonization by *S. spectabilis*, but not either of the other fungi. If plant defense genes were responsible for inhibiting colonization by *S. spectabilis* in our system, however, they were only active for a limited period of time. After 20 weeks, seedlings with high colonization by *S. grisellus* or *S. clintonianus* on one side of the microcosm were eventually colonized (at low levels) by *S. spectabilis* on the other side. Since there was no nutrient supplementation throughout the experiment, it is possible that local depletion of nutrients on the initially colonized side created an incentive for host plants to allow *S. spectabilis* onto their roots in order to exploit additional nutrients on the other side of the microcosms. Or, perhaps, nutrient depletion could have weakened colonization on the initially colonized side, leading to a downregulation of the plant's defense response. Future work that carefully manipulates soil nutrient content and subsequently tracks resource movement and plant defenses would be required to explore this scenario further. This type of indirect priority effect may be important in the assembly of ectomycorrhizal fungal communities (Fukami 2015), and understanding its mechanisms could provide valuable ecological insight.

Although our results could be consistent with other mechanisms, we think these alternative explanations are less likely to be true. One is that this effect was mediated by plant resource allocation. For example, the earlier-establishing fungus may have siphoned plant resources to its side of the split-root microcosm, resulting in the *S. spectabilis* side accumulating fewer spore-germination cue molecules simply because it received fewer plant resources. We believe this possibility is unlikely, however, as all three *Suillus* species were equivalently effective mutualists in terms of plant biomass gain and leaf nitrogen content, and root biomass was equivalent across all three fungal species (Table S1c). Furthermore, even if *S. spectabilis* were an inferior

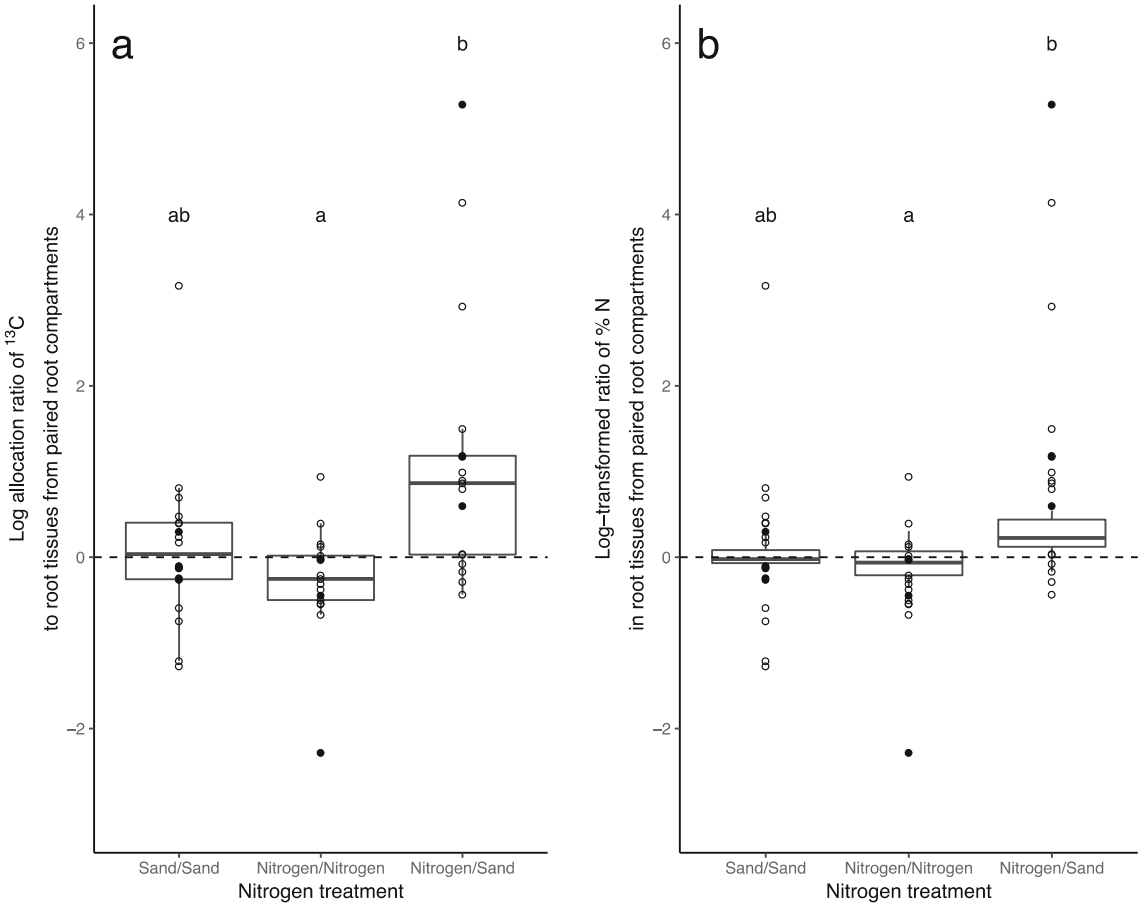

Fig. 2 **a** The log-transformed ^{13}C allocation ratio, representing carbon allocation to across the split-root systems of *Pinus muricata* seedlings, was significantly higher in the choice (nitrogen/sand) treatment than in the nitrogen/nitrogen treatment (significance determined by a post hoc Tukey test). This result mirrors the pattern in **b**: the log-transformed ratio of nitrogen content in root tissues—whether absorptive roots or ectomycorrhizas—was greatest in the choice treatment. Lowercase letters indicate significant differences identified among nitrogen treatments by a post hoc Tukey HSD test after an ANOVA (different nitrogen concentrations were pooled, as indicated in Methods). The midline of each box represents the mean value for that fungus and competitor combination, hinges illustrate the boundaries of the first and third quartiles, and whiskers extend to the most extreme points no more than one and a half times the interquartile range away from the hinge. All data are plotted as individual points overlain on the boxplots. Closed circles illustrate data from ectomycorrhizal tissue; open circles indicate values from uncolonized fine root tissue. The dashed line illustrates a log ratio of 0 (no difference between paired root compartments)

mutualist, the plant did not have much opportunity to withhold resources, because that fungus did not establish until the end of the experiment in the presence of a competitor. A second possibility is fungal control on root colonization. In order to respond to a reduction in host quality—for instance, if the host shifted photosynthate to the competing fungus—*S. spectabilis* would need to be engaged in active symbiosis with a root as the competitor became established. In our experiment, we detected no active symbiosis until very late in the trial. It also seems unlikely that an obligate symbiont such as *S. spectabilis* would reduce its own growth so profoundly, in the absence of a suitable alternative host. Taken together, the hypothesisthat *S. spectabilis* was actively discriminating against *L. occidentalis* is also an unlikely explanation for our results.

In experiment 2, we found that *Pinus muricata* seedlings directed recent photosynthate to roots that contained more nitrogen, but made no distinction between ectomycorrhizal roots of colonized plants and roots of uncolonized plants (Fig. 3). Although it is well established that resource-providing roots receive greater investment from a plant (Eissenstat et al. 2015; Chen et al. 2016; Cheng et al. 2016), this is to our knowledge the first demonstration of carbon investment tracking tissue nitrogen concentrations equally well in mycorrhizal and uncolonized fine roots. This mechanism may allow for plant rewards of resource-providing fungi, a phenomenon known to occur in the arbuscular mycorrhizal symbiosis (Kiers et al. 2011). The design of our experiment, however, makes it difficult to establish the time frame in which plant rewards may be important: The ^{13}C data represent allocation after a week-long chase period, while the nitrogen concentration data likely reflect a long-term average of nitrogen flux through the tissue. Despite operating on different time scales, the close correspondence between differences in tissue nitrogen and recent carbon concentrations across the

Table 3 Two-way ANOVA and post hoc Tukey results for experiment 2, relating the log-transformed carbon allocation ratio (A, B) or log-transformed fine root nitrogen concentration ratios (C, D) to nitrogen treatment (N/N, N/S, S/S) and addition level (0 g, 0.5 g, 1 g casein). Asterisks highlight significant results (*$p < 0.05$)

A) Log-transformed carbon allocation ratio

	Factor	Df	Sum sq	Mean sq	*F* value	*p*
Two-way ANOVA	Nitrogen treatment	2	12.28	6.142	3.565	0.036*
	Nitrogen addition level	1	0.14	0.139	0.081	0.777
	Treatment:addition level	1	0.01	0.006	0.003	0.955
	Residuals	48	82.7	1.723		

B) Tukey results: treatment effects on carbon allocation ratio

Comparison	Diff	Lwr	Upr	*p* adj
Casein/casein-sand/sand	− 0.3790605	− 1.4526894	0.6945684	0.6716385
Casein/sand-sand/sand	0.7790913	− 0.2790889	1.8372715	0.1869057
Casein/sand-casein/casein	1.1581518	0.08452286	2.2317807	0.0318151*

C) Log-transformed fine root nitrogen concentration ratio

	Factor	Df	Sum sq	Mean sq	*F* value	*p*
Two-way ANOVA	Nitrogen treatment	2	2.005	1.0025	4.444	0.017*
	Nitrogen addition level	1	0.016	0.0158	0.070	0.792
	Treatment:addition level	1	0.101	0.1011	0.448	0.506
	Residuals	48	10.828	0.2256		

D) Tukey results: treatment effects on fine root nitrogen concentration ratio

Comparison	Diff	Lwr	Upr	*p* adj
Casein/casein-Sand/sand	− 0.1492725	− 0.5377522	0.2392071	0.6246249
Casein/sand-sand/sand	0.3177271	− 0.0651627	0.7006168	0.1214721
Casein/sand-casein/casein	0.4669996	0.07851994	0.8554793	0.0149384*

split-root microcosms indicates that these resources may be tightly coupled. In contrast with our results, however, Valtanen et al. (2014) found that these resources may not to be strictly coupled over the long-term in diverse fungal communities. Future work should aim to clarify the influence of experimental time frame and mutualist diversity on the coupling of plant and fungal resource exchange in this symbiosis. Finally, we note that if fine spatial carbon-nitrogen coupling (root tip to root tip) does exist, this would allow the plant to regulate its own root foraging efforts while also encouraging cooperative fungi, suggesting that fundamental plant processes could reward cooperation even without symbiosis-specific mechanisms (Frederickson 2013).

Although the patterns we observed provide intriguing evidence that ectomycorrhizal competition can be strongly influenced by host plant signaling, modifications to and expansion upon the work presented here would bolster our understanding of this system. Including independent replicates for each fungus/competitor combination, for example, would further increase our confidence in the interpretation of the experiment 1 results. With regard to experiment 2, the nitrogen source we used (casein protein) was not solely fungus-accessible, although it was contained in mesh bags that partially inhibited root growth. To fully disentangle the influence of fungal partner quality from the effects of competition between plant roots and soil fungi, it would be necessary to fully separate roots from the nitrogen source and render it solely accessible to the fungi, for example by introducing an air gap (He et al. 2005; Fellbaum et al. 2014). Using a labeled nitrogen source would also illuminate resource trading dynamics that we could not capture here. It would also would be useful to be able to directly control the rates of ^{13}C uptake across plants (rather than tracking allocation metrics in split-root plants), which would require stricter control of label concentration and timing than we were able to achieve in this study. Furthermore, future work should examine the extent to which the fungi, themselves, exert control over the symbiosis. Because individual fungi may associate with several plants simultaneously in common mycelial networks, they may direct nutrients preferentially towards some hosts over others, a process which has been shown to occur in the arbuscular mycorrhizal symbiosis (Kiers et al. 2011; Walder et al. 2012; Fellbaum et al. 2014) and may also be important in the ectomycorrhizal mutualism (e.g., Ek et al. 1996). To our

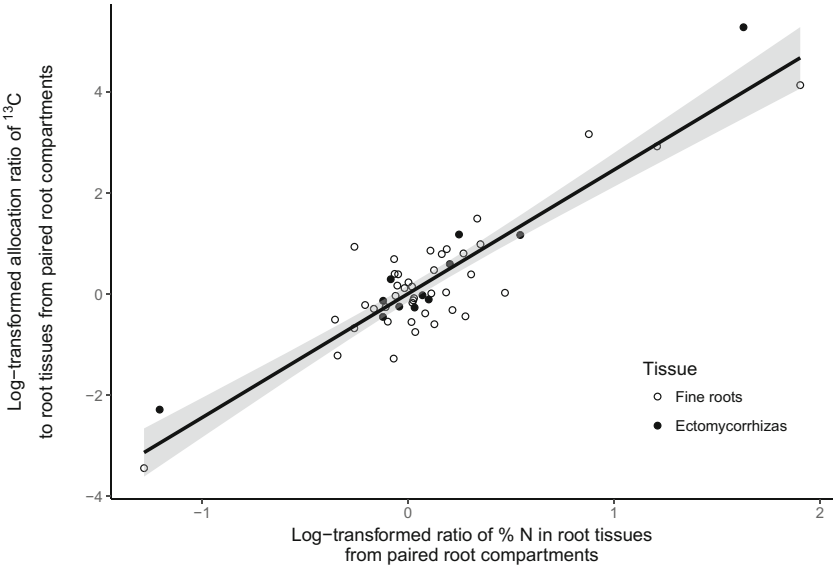

Fig. 3 The difference in nitrogen content within a tissue type in paired root compartments, measured as a log ratio, was significantly correlated with the difference in ^{13}C allocation to those tissues, measured as the log-ratio ^{13}C allocation metric described in Materials and Methods (adjusted $r^2 = 0.8155$, df = 49, $p < 0.001$). When comparing paired root compartments A and B from a single microcosm, the metric is calculated as $\ln([^{13}C]_A/[^{13}C]_B)$, so that a negative value indicates more ^{13}C on side B, while a positive value indicates more ^{13}C on side A. Open circles illustrate values for uncolonized fine root tissue; closed circles are from ectomycorrhizas. The line illustrates the predicted relationship between the variables as modeled by linear regression, with shading indicating the 95% confidence interval. Although percent nitrogen represents a long-term average of nitrogen flux through a root, while the ^{13}C allocation measures carbon flux over the course of the week-long chase period, the amount of carbon these plants would allocate to their root tissues—whether ectomycorrhizas or absorptive roots—was related to the amount of nitrogen those roots contained

knowledge, however, the extent to which ectomycorrhizal fungi might control nutrient exchange has not yet been thoroughly addressed. We hope that future research can develop a more holistic understanding of how plants and fungi jointly control these symbiotic interactions. Finally, it will be valuable to expand this work to more diverse systems involving fungi with broader host range than *Suillus* species: Generalist fungi may interact differently with host plants than specialists, and exploring more diverse systems will generate ecologically relevant insight into how partner choice and resource exchange work in the field.

In aggregate, our results support the hypothesis that ectomycorrhizal host plants, like those engaged in other root symbioses, have the ability to discriminate among potential microbial partners (Bever et al. 2009; Kiers et al. 2011; Argüello et al. 2016; Hortal et al. 2017). Further, our results suggest that plant-based discrimination can occur both prior to symbiosis, perhaps based on signals relevant to fungal identity, as well as after symbiosis has begun, based on the services provided by a fungal partner. Although signaling has been suggested to be an important factor regulating partner recognition in this system (Plett et al. 2014), our results suggest that fungal-plant signaling can affect the outcomes of co-colonization among ectomycorrhizal fungi. While our isotope-based results apply equally well to roots associated with fungal partners and to uncolonized roots, they are consistent with plant allocation patterns that would encourage symbiotic cooperation. By influencing which fungi succeed and in which contexts, a host plant may not only affect its own performance, but also alter the pool of symbionts available to nearby plants. This process may in turn influence the way that the forest community develops and how quickly resources like carbon and nitrogen move through the system.

Acknowledgements We thank Nhu Nguyen for collecting and logistical assistance with experiment 1. We thank Ford Denison, Tad Fukami, members of the Peay lab, Thomas Kuyper, and four anonymous reviewers for feedback on prior versions of this manuscript. Thanks also to Don Herman, who provided essential assistance with the isotope enrichment part of experiment 2.

Authors' contributions P.K. designed experiment 1, which was conducted by S.H. and J.H. L.B. designed and carried out experiment 2 with support from K.P. and A.K. L.B. analyzed the data and composed the manuscript, with writing and editing assistance from P.K., and additional revisions from all co-authors.

Funding information S. Hortal received a Hawkesbury Institute for the Environment Research Exchange Program grant. L. Bogar received support for this research from a National Science Foundation (NSF) Graduate Research Fellowship Program grant, scholarships from the Mycological Society of San Francisco and the Phi Beta Kappa Northern California Association, and a predoctoral fellowship from the Stanford Center for Computational, Evolutionary, and Human Genomics. Further support was provided by an NSF-Division of Environmental Biology grant (1554375) to P. Kennedy.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Agerer R (2001) Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114. https://doi.org/10.1007/s005720100108

Argüello A, O'Brien MJ, van der Heijden MGA et al (2016) Options of partners improve carbon for phosphorus trade in the arbuscular mycorrhizal mutualism. Ecol Lett 19:648–656. https://doi.org/10.1111/ele.12601

Averill C, Turner BL, Finzi AC (2014) Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature 505:534–545. https://doi.org/10.1038/nature12901

Behm JE, Kiers ET (2014) A phenotypic plasticity framework for assessing intraspecific variation in arbuscular mycorrhizal fungal traits. J Ecol 102:315–327. https://doi.org/10.1111/1365-2745.12194

Bennett JA, Maherali H, Reinhart KO, Lekberg Y, Hart MM, Klironomos J (2017) Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. Science (80-) 355:181–184. https://doi.org/10.1126/science.aai8212

Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M (2009) Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. Ecol Lett 12:13–21. https://doi.org/10.1111/j.1461-0248.2008.01254.x

Branco S, Gladieux P, Ellison CE, Kuo A, LaButti K, Lipzen A, Grigoriev IV, Liao HL, Vilgalys R, Peay KG, Taylor JW, Bruns TD (2015) Genetic isolation between two recently diverged populations of a symbiotic fungus. Mol Ecol 24:2747–2758. https://doi.org/10.1111/mec.13132

Bruns TD, Bidartondo MI, Taylor DL (2002) Host specificity in ectomycorrhizal communities : what do the exceptions tell us? Integr Comp Biol 42:352–359

Chagnon PL, Bradley RL, Maherali H, Klironomos JN (2013) A trait-based framework to understand life history of mycorrhizal fungi. Trends Plant Sci 18:484–491. https://doi.org/10.1016/j.tplants.2013.05.001

Chen W, Koide RT, Adams TS, DeForest JL, Cheng L, Eissenstat DM (2016) Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. Proc Natl Acad Sci U S A 113:8741–8746. https://doi.org/10.1073/pnas.1601006113

Chen W, Koide RT, Eissenstat DM (2018) Nutrient foraging by mycorrhizas: from species functional traits to ecosystem processes. Funct Ecol 12:3218–3221. https://doi.org/10.1111/1365-2435.13041

Cheng L, Chen W, Adams TS, Wei X, Li L, McCormack ML, DeForest JL, Koide RT, Eissenstat DM (2016) Mycorrhizal fungi and roots are complementary in foraging within nutrient patches. Ecology 97:2815–2823. https://doi.org/10.1002/ecy.1514

Christian N, Bever JD (2018) Carbon allocation and competition maintain variation in plant root mutualisms. Ecol Evol 8:1–9. https://doi.org/10.1002/ece3.4118

Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA, Lindahl BD (2015) Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. New Phytol 205:1525–1536. https://doi.org/10.1111/nph.13208

Colpaert JV, Wevers JHL, Krznaric E, Adriaensen K (2011) How metal-tolerant ecotypes of ectomycorrhizal fungi protect plants from heavy metal pollution. Ann For Sci 68:17–24. https://doi.org/10.1007/s13595-010-0003-9

Corrêa A, Strasser RJ, Martins-Loução MA (2008) Response of plants to ectomycorrhizae in N-limited conditions: which factors determine its variation? Mycorrhiza 18:413–427. https://doi.org/10.1007/s00572-008-0195-0

Corrêa A, Gurevitch J, Martins-Loução MA, Cruz C (2012) C allocation to the fungus is not a cost to the plant in ectomycorrhizae. Oikos 121:449–463. https://doi.org/10.1111/j.1600-0706.2011.19406.x

Eissenstat DM, Kucharski JM, Zadworny M, Adams TS, Koide RT (2015) Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. New Phytol 208:114–124. https://doi.org/10.1111/nph.13451

Ek H, Andersson S, Söderström B (1996) Carbon and nitrogen flow in silver birch and Norway spruce connected by a common mycorrhizal mycelium. 465–467. doi: https://doi.org/10.1007/s005720050148

Fellbaum CR, Mensah JA, Cloos AJ, Strahan GE, Pfeffer PE, Kiers ET, Bücking H (2014) Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. New Phytol 203:646–656. https://doi.org/10.1111/nph.12827

Finlay RD (1989) Functional aspects of phosphorus uptake and carbon translocation in incompatible ectomycorrhizal associations between *Pinus sylvestris* and *Suillus grevillei* and *Boletinus cavipes*. New Phytol 112:185–192. https://doi.org/10.1111/j.1469-8137.1989.tb02373.x

Franklin O, Näsholm T, Högberg P, Högberg MN (2014) Forests trapped in nitrogen limitation - an ecological market perspective on ectomycorrhizal symbiosis. New Phytol 203:657–666. https://doi.org/10.1111/nph.12840

Frederickson M (2013) Rethinking mutualism stability: cheaters and the evolution of sanctions. Q Rev Biol 88:269–295. https://doi.org/10.1086/673757

Friesen ML (2012) Widespread fitness alignment in the legume-rhizobium symbiosis. New Phytol 194:1096–1111. https://doi.org/10.1111/j.1469-8137.2012.04099.x

Fukami T (2015) Historical contingency in community assembly: integrating niches, species pools, and priority effects. Annu Rev Ecol Evol Syst 46:1–23. https://doi.org/10.1146/annurev-ecolsys-110411-160340

Garcia K, Delaux P, Cope KR, Ané J-M (2015) Molecular signals required for the establishment and maintenance of ectomycorrhizal symbioses. New Phytol 208:79–87. https://doi.org/10.1111/nph.13423

Hasselquist NJ, Metcalfe DB, Inselsbacher E, Stangl Z, Oren R, Näsholm T, Högberg P (2016) Greater carbon allocation to mycorrhizal fungi reduces tree nitrogen uptake in a boreal forest. Ecology 97:1012–1022. https://doi.org/10.1890/15-1222.1

He X, Critchley C, Ng H, Bledsoe C (2005) Nodulated N2-fixing *Casuarina cunninghamiana* is the sink for net N transfer from non-N2-fixing Eucalyptus maculata via an ectomycorrhizal fungus Pisolithus sp. using 15NH4+ or 15NO3− supplied as ammonium nitrate. New Phytol 167:897–912. https://doi.org/10.1111/j.1469-8137.2005.01437.x

Herman DJ, Firestone MK, Nuccio E, Hodge A (2012) Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. FEMS Microbiol Ecol 80:236–247. https://doi.org/10.1111/j.1574-6941.2011.01292.x

Hoeksema JD, Kummel M (2003) Ecological persistence of the plant-mycorrhizal mutualism: a hypothesis from species coexistence theory. Am Nat 162:S40–S50

Hortal S, Plett KL, Plett JM, Cresswell T, Johansen M, Pendall E, Anderson IC (2017) Role of plant–fungal nutrient trading and host

control in determining the competitive success of ectomycorrhizal fungi. ISME J 11:2666–2676. https://doi.org/10.1038/ismej.2017.116

Ji B, Bever JD (2016) Plant preferential allocation and fungal reward decline with soil phosphorus: implications for mycorrhizal mutualism. Ecosphere 7:e01256. https://doi.org/10.1002/ecs2.1256

Johnstone RA, Bshary R (2008) Mutualism, market effects and partner control. J Evol Biol 21:879–888. https://doi.org/10.1111/j.1420-9101.2008.01505.x

Jones MD, Durall DM, Cairney JWG (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytol 157:399–422. https://doi.org/10.1046/j.1469-8137.2003.00698.x

Kennedy PG (2010) Ectomycorrhizal fungi and interspecific competition: species interactions, community structure, coexistence mechanisms, and future research directions. New Phytol 187:895–910. https://doi.org/10.1111/j.1469-8137.2010.03399.x

Kennedy PG, Bruns TD (2005) Priority effects determine the outcome of ectomycorrhizal competition between two *Rhizopogon* species colonizing *Pinus muricata* seedlings. New Phytol 166:631–638

Kennedy PG, Higgins LM, Rogers RH, Weber MG (2011) Colonization-competition tradeoffs as a mechanism driving successional dynamics in ectomycorrhizal fungal communities. PLoS One 6:e25126. https://doi.org/10.1371/journal.pone.0025126

Kennedy PG, Mielke LA, Nguyen NH (2018) Ecological responses to forest age, habitat, and host vary by mycorrhizal type in boreal peatlands. Mycorrhiza 28:315–328. https://doi.org/10.1007/s00572-018-0821-4

Kiers ET, Denison RF (2008) Sanctions, cooperation, and the stability of plant-rhizosphere mutualisms. Annu Rev Ecol Evol Syst 39:215–236. https://doi.org/10.1146/annurev.ecolsys.39.110707.173423

Kiers ET, Rousseau RA, West SA, Denison RF (2003) Host sanctions and the legume-rhizobium mutualism. Nature 425:78–81. https://doi.org/10.1038/nature01931

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bucking H (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science (80-) 333:880–882. https://doi.org/10.1126/science.1208473

Kornfeld A, Horton TW, Yakir D, Turnbull MH (2012) Correcting for nonlinearity effects of continuous flow isotope ratio mass spectrometry across a wide dynamic range. Rapid Commun Mass Spectrom 26:460–468. https://doi.org/10.1002/rcm.6120

Leski T, Rudawska M (2012) Ectomycorrhizal fungal community of naturally regenerated European larch (Larix decidua) seedlings. Symbiosis 56:45–53. https://doi.org/10.1007/s13199-012-0164-4

Liao H-L, Chen Y, Vilgalys R (2016) Metatranscriptomic study of common and host-specific patterns of gene expression between pines and their symbiotic ectomycorrhizal fungi in the genus *Suillus*. PLoS Genet 12:e1006348. https://doi.org/10.1371/journal.pgen.1006348

Lofgren L, Nguyen NH, Kennedy PG (2018) Ectomycorrhizal host specificity in a changing world: can legacy effects explain anomalous current associations? New Phytol 220:1273–1284. https://doi.org/10.1111/nph.15008

Marx D (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153–163

Massicotte HB, Molina RJ, Luoma DL, Smith JE (1994) Biology of the ectomycorrhizal genus, *Rhizopogon*: II. Patterns of host-fungus specificity following spore inoculation of diverse hosts grown in monoculture and dual culture. New Phytol 126:677–690

Moeller HV, Neubert MG (2016) Multiple friends with benefits: an optimal mutualist management strategy? Am Nat 187:E1–E12. https://doi.org/10.1086/684103

Molina R, Horton TR (2015) Mycorrhiza specificity: its role in the development and function of common mycelial networks. In: Horton TR (ed) Mycorrhizal Networks, 1st edn. Springer Netherlands, Dordrecht, pp 1–39

Nara K, Hogetsu T (2004) Ectomycorrhizal fungi on established shrubs facilitate subsequent seedling establishment of successional plant species. Ecology 85:1700–1707. https://doi.org/10.1890/03-0373

Näsholm T, Högberg P, Franklin O, Metcalfe D, Keel SG, Campbell C, Hurry V, Linder S, Högberg MN (2013) Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? New Phytol 198:214–221. https://doi.org/10.1111/nph.12139

Nylund J, Wallander H (1989) Effects of ectomycorrhiza on host growth and carbon balance in a semi-hydroponic cultivation system. New Phytol 112:389–398. https://doi.org/10.1111/j.1469-8137.1989.tb00328.x

Oldroyd GED, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume-rhizobial symbiosis. Annu Rev Genet 45:119–144. https://doi.org/10.1146/annurev-genet-110410-132549

Peay KG (2016) The mutualistic niche: mycorrhizal symbiosis and community dynamics. Annu Rev Ecol Evol Syst 47:143–164. https://doi.org/10.1146/annurev-ecolsys-121415-032100

Peay KG (2018) Timing of mutualist arrival has a greater effect on *Pinus muricata* seedling growth than interspecific competition. J Ecol 106:514–523. https://doi.org/10.1111/1365-2745.12915

Plett JM, Kemppainen M, Kale SD, Kohler A, Legué V, Brun A, Tyler BM, Pardo AG, Martin F (2011) A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. Curr Biol 21:1197–1203. https://doi.org/10.1016/j.cub.2011.05.033

Plett JM, Daguerre Y, Wittulsky S, Vayssieres A, Deveau A, Melton SJ, Kohler A, Morrell-Falvey JL, Brun A, Veneault-Fourrey C, Martin F (2014) Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. Proc Natl Acad Sci U S A 111:8299–8304. https://doi.org/10.1073/pnas.1322671111

Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H (2009) Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. Proc R Soc B 276:4237–4245. https://doi.org/10.1098/rspb.2009.1015

Pringle EG (2016) Integrating plant carbon dynamics with mutualism ecology. New Phytol 210:71–75. https://doi.org/10.1111/nph.13679

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from https://www.R-project.org/

Rineau F, Stas J, Nguyen NH, Kuyper TW, Carleer R, Vangronsveld J, Colpaert JV, Kennedy PG (2016) Ectomycorrhizal fungal protein degradation ability predicted by soil organic nitrogen availability. Appl Environ Microbiol 82:1391–1400. https://doi.org/10.1128/AEM.03191-15

Schmitz AM, Harrison MJ (2014) Signaling events during initiation of arbuscular mycorrhizal symbiosis. J Integr Plant Biol 56:250–261. https://doi.org/10.1111/jipb.12155

Slater C, Preston T, Weaver LT (2001) Stable isotopes and the international system of units. Rapid Commun Mass Spectrom 15:1270–1273. https://doi.org/10.1002/rcm.328

Smith SE, Read DJ (2008) Mycorrhizal Symbiosis, 3rd edn. Academic Press, San Diego

Talbot JM, Bruns TD, Smith DP, Branco S, Glassman SI, Erlandson S, Vilgalys R, Peay KG (2013) Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. Soil Biol Biochem 57:282–291. https://doi.org/10.1016/j.soilbio.2012.10.004

Tedersoo L, Smith ME (2013) Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. Fungal Biol Rev 27:83–99. https://doi.org/10.1016/j.fbr.2013.09.001

Valtanen K, Eissfeller V, Beyer F, Hertel D, Scheu S, Polle A (2014) Carbon and nitrogen fluxes between beech and their ectomycorrhizal assemblage. Mycorrrhiza 24:645–650. https://doi.org/10.1007/s00572-014-0581-8

Van Wees SC, Van der Ent S, Pieterse CM (2008) Plant immune responses triggered by beneficial microbes. Curr Opin Plant Biol 11:443–448. https://doi.org/10.1016/j.pbi.2008.05.005

Walder F, Niemann H, Natarajan M, Lehmann MF, Boller T, Wiemken A (2012) Mycorrhizal networks: common goods of plants shared under unequal terms of trade. Plant Physiol 159:789–797. https://doi.org/10.1104/pp.112.195727