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ORIGINAL PAPER

# Nitrogen sink strength of ectomycorrhizal morphotypes of *Quercus douglasii*, *Q. garryana*, and *Q. agrifolia* seedlings grown in a northern California oak woodland

**X. H. He · W. R. Horwath · R. J. Zasoski · Z. Aanderud · C. S. Bledsoe**

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**Abstract** Little information is known on what the magnitude of nitrogen (N) processed by ectomycorrhizal (ECM) fungal species in the field. In a common garden experiment performed in a northern California oak woodland, we investigated transfer of nitrogen applied as $^{15}NH_4$ or $^{15}NO_3$ from leaves to ectomycorrhizal roots of three oak species, *Quercus agrifolia*, *Q. douglasii*, and *Q. garryana*. Oak seedlings formed five common ectomycorrhizal morphotypes on root tips. Mycorrhizal tips were more enriched in $^{15}N$ than fine roots. N transfer was greater to the less common morphotypes than to the more common types. $^{15}N$ transfer from leaves to roots was greater when $^{15}NO_3^-$, not $^{15}NH_4^+$, was supplied. $^{15}N$ transfer to roots was greater in seedlings of *Q. agrifolia* than in *Q. douglasii* and *Q. garryana*. Differential N transfer to ectomycorrhizal root tips suggests that ectomycorrhizal morphotypes can influence flows of N from leaves to roots and that mycorrhizal diversity may influence the total N requirement of plants.

**Keywords** $^{15}N$-ammonium and $^{15}N$-nitrate · Ectomycorrhizal morphotypes · Nitrogen sink strength · Oak fine roots · *Quercus* species

X. H. He · W. R. Horwath · R. J. Zasoski · Z. Aanderud · C. S. Bledsoe (✉)
Department of Land, Air and Water Resources,
University of California,
One Shields Avenue,
Davis, CA 95616, USA
e-mail: csbledsoe@ucdavis.edu

X. H. He
Asian National Environmental Science Center,
University of Tokyo,
1-1-8 Midori-cho, Nishitokyo,
Tokyo 188-0002, Japan

## Introduction

Ectomycorrhizal communities in forests and woodlands are species rich (Gardes and Bruns 1996; Gehring et al. 1998; Baxter et al. 1999; Agerer 2006). Mycorrhizal diversity has been studied primarily in coniferous ecosystems (Gardes and Bruns 1996; Gehring et al. 1998; Kranabetter and Wylie 1998; Wurzburger and Bledsoe 2001; Izzo et al. 2005; Korkama et al. 2006; Taniguchi et al. 2007; Tedersoo et al. 2006; Kennedy et al. 2007). In oak woodlands, several studies used ectomycorrhizal morphotyping to study diversity (Berman and Bledsoe 1998; Baxter et al. 1999; Cheng and Bledsoe 2002; Lindahl 2002; Avis et al. 2003; Valentine et al. 2004; Moser et al. 2005). More recent studies of oak ectomycorrhizal diversity used molecular methods to identify fungal species (Baxter et al. 1999; Bergemann and Garbelotto 2006; Dickie et al. 2002; Dickie et al. 2004; Dickie and Reich 2005; Gebhardt et al. 2007; Jakucs et al. 2005; Kennedy et al. 2003, 2007; Kovacs et al. 2000; Richard et al. 2005; Smith et al. 2006a,b; Smith et al. 2007; Walker et al. 2005). All these studies document the high ectomycorrhizal diversity on oak trees in diverse environments.

Ectomycorrhizal species may occupy different ecological niches and have distinct functional roles, but functional diversity is not well understood (Cairney 1999; Cairney and Chambers 1999). Indirect evidence links functional diversity to morphological and genetic diversity (Read et al. 1985; Marschner and Dell 1994). Ectomycorrhizal enzyme activities (e.g., acid phosphatase and dehydrogenase) change with fungal species and with season (Buée et al. 2005). Ectomycorrhizal communities are altered by drought (Gehring et al. 1998). Ectomycorrhizal fungi differ in their natural abundance of carbon (C) and nitrogen (N) (Högberg et al. 1999; Hobbie and Colpaert 2003; Hobbie and Hobbie

2006). Ectomycorrhizal communities change in response to fertilization (Avis et al. 2003). Thus, research on diversity suggests that ectomycorrhizal species can function differently, particularly in N transfer and processing. One pathway for N transfer is from soil to mycorrhizas to plant, while another pathway is the internal cycling of N within a plant, from shoots to roots to ectomycorrhizas. In this study, we focused on the second pathway.

Our goal was to determine whether different oak ectomycorrhizal morphotypes affected transfer of $^{15}N$ from leaves to ectomycorrhizal roots, thus providing evidence of functional diversity. In a field study, we grew seedlings of three oak species: deciduous *Quercus douglasii* Hook. and Arn. and *Q. garryana* Dougl. ex Hook, and evergreen *Q. agrifolia* Nee. We applied enriched $^{15}N$-nitrate and $^{15}N$-ammonium to oak leaves, and then measured transfer from leaves to stems, roots, and ectomycorrhizal root tips. We calculated N transfer to ectomycorrhizal root tips, N use efficiency (NUE) and N derived from source (NDFS). We asked the following questions: (1) Do oak species differ with respect to N transfer to ectomycorrhizal roots? (2) Does N form ($^{15}NH_4^+$ and $^{15}NO_3^-$) affect transfer to ectomycorrhizal roots? (3) Are some ectomycorrhizal morphotypes greater sinks for N than others?

## Materials and methods

### Site description

The University of California Sierra Foothill Research and Extension Center (SFREC) at 39°15′N, 121°17′W was the experimental site. Climate is characterized by hot, dry summers and mild, rainy winters (http://danrrec.ucdavis.edu/sierra_foothill/resources.html). Soils, developed from basic metavolcanic bedrock, are loamy in texture, 0.3−0.6m deep, well drained, pH6.4 (Cheng and Bledsoe 2002). Vegetation is dominated by *Q. douglasii* (100 to 500 trees $ha^{-1}$) and intermingled with *Q. wislizeni* A. DC., *Q. kelloggii* Newb., *Pinus sabiniana* Dougl. ex Dougl., *P. ponderosa* Lawson & C. Lawson, *Ceanothus cuneatus* (Hook.) Nutt., *Toxicodendron diversilobum* (Torr. & A. Gray) Greene, and understory annual and perennial grasses and forbs (Cheng and Bledsoe 2002).

### Experimental design

In fall 1999, we collected acorns from three native oak woodlands: *Q. douglasii* from SFREC, California, *Q. agrifolia* from Skinner–Shipley Reserve, California, and *Q. garryana* from Whetstone Savanna Preserve, Oregon. Surface-sterilized (10% bleach, 10min) acorns were germinated in pots (autoclaved field soil, coarse sand, fine sand, 1:1:1). Seedlings were grown in a greenhouse for 4months and were nonmycorrhizal when transplanted into the field site in March 2000. We planted 90 oak seedlings in three 50-m-diameter plots. Each plot contained ten groups of three seedlings, one of each oak species, planted 50cm apart in a triangular pattern. Seedlings were watered weekly during the first summer. After 2years, 54 seedlings survived.

### $^{15}N$ labeling, harvest, determination of mycorrhizal infection, and morphotyping

Two years after transplanting (April 2002), $^{15}N$ was applied in a 2-ml vial attached to a "donor" leaf of each seedling. Vials contained 1% N solution (9.0mg $^{15}N$/1.6ml); N was supplied as either $(NH_4)_2SO_4$ (54at.% $^{15}N$) or $KNO_3$ (57at.% $^{15}N$; Cambridge Isotope Laboratory, Andover, MA). We chose a 1% N solution because absorption through leaf surfaces can be limiting, and much of the surface application of $^{15}N$ will not be absorbed into the leaf tissues. Thus the 1% N solution was an appropriate concentration. After 9days, seedlings were harvested; nondonor leaves and stems were separated. Root systems were excavated to 30-cm depth, transferred to the laboratory on ice, and then washed. From each seedling, ectomycorrhizal root tips were removed, examined microscopically, and sorted by ectomycorrhizal morphotype based on color, morphology, and surface mantle characteristics such as presence of emanating hyphae, mantle patterns, and cystidia (Agerer 1987–1998; Goodman et al. 2002).

We collected a total of 4,727 ectomycorrhizal root tips and separated tips by oak species ($n = 3$), N source ($n = 2$), and morphotype ($n = 5$), for a total of 30 samples. Because it was necessary to have approximately 100μg N for stable isotope analysis, each sample was a composite of similar morphotypes from five to seven seedlings of the same oak species and same N treatment. Mycorrhizal infection (percent of fine root length colonized) was determined by the grid line intersect method (Brundrett et al. 1996). Mycorrhizal tips were freeze dried; other plant parts were oven dried (60°C). Dried plant samples were ground to a homogeneous fine powder for isotopic analyses.

### Determination of total N and $^{15}N$ content, statistical analyses

For %N and atom $^{15}N$ at.%, all samples were analyzed with a 20/20 Automated $^{15}N/^{13}C$ Analyzer-Mass Spectrometer (Europa Scientific, Crewe, UK) at the UC Davis Stable Isotope Facility. Calculations of $\delta^{15}N$ (‰) were based on Knowles and Blackburn (1993):

$$\delta^{15}N\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where $R$ is the ratio of $^{15}N/^{14}N$ (at.%) of the sample and standard. The value of $R_{standard}$ for atmospheric $N_2$ is 0.0036765. The Stable Isotope Facility used a standard $\delta^{15}N$ value of 1.33 ± 0.10‰ (mean ± SE, $n$ = 126, $(^{14}NH_4)_2SO_4$, Fisher Scientific International, USA). Background $^{15}N$ values for seedlings were determined by analyzing tissues from unlabeled seedlings collected at the field site. NUE was calculated as total seedling biomass/total seedling N content (Berendse and Aerts 1987). Calculation of %NDFS was modified from Shearer and Kohl (1993):

$$\%\mathrm{NDFS} = \left(\mathrm{atom}\%^{15}\mathrm{N excess\ sample}\right) / \left(\mathrm{atom}\%^{15}\mathrm{N excess\ labeling\ source}\right) \times 100$$

Data were analyzed by analysis of variance (ANOVA) procedures. Shapiro–Wilk and Kolomogrov–Smirnov tests were performed on all data, and transformations were made to meet assumptions of homoscedasticity before ANOVA (SAS/SAT User's Guide 1995). Differences in means were compared using Tukey's honestly significant difference at $P \leq 0.05$ level (Sokal and Rohlf 1995).

## Results

### Ectomycorrhizal colonization and morphotype abundance

For all characteristics in Table 1, there was no effect of $^{15}N$ source (nitrate or ammonium); data were combined. Oak seedling roots were heavily colonized by ectomycorrhizal fungi (75%, Table 1) and weakly colonized by arbuscular mycorrhizal fungi (14%, data not shown). Five common ectomycorrhizal morphotypes were observed (Fig. 1a–e): black with sparse, stiff hyphae, characteristic of *Cenococcum* (Fig. 1a); brown with inner mantle of net prosenchyma and outer mantle of regular synenchyma, characteristic of the Thelephoraceae (Fig. 1b); tan with mantle of interlocking irregular synenchyma, some cystidia, characteristic of members of the Pezizales, particularly *Tuber* spp. (Fig. 1c); white with mantle of felt prosenchyma, morphology characteristic

**Table 1** Ectomycorrhizal morphotype abundance and characteristics of 2-year-old field-grown seedlings of deciduous *Quercus douglasii* ($n$=12) and *Q. garryana* ($n$=14) and evergreen *Q. agrifolia* ($n$=10)

| Characteristic | *Q. douglasii* | *Q. garryana* | *Q. agrifolia* |
|---|---|---|---|
| Mycorrhizal infection (%) | 74±19a | 75±35a | 77±25a |
| Mycorrhizal tips, numbers/seedling | 124±40a | 63±14b | 112±27a |
| Morphotype abundance (%) | | | |
| Black | 35±7a, x | 44±6a, x | 38±4a, x |
| Brown | 44±9a, x | 31±6a, x | 35±6a, x |
| Hairy | 8±5a, y | 8±4a, y | 17±7a, y |
| Tan | 10±5a, y | 13±4a, y | 5±3a, y |
| White | 3±2a, y | 4±3a, y | 5±3a, y |
| Shoot height (cm) | 13±3b | 15±3b | 22±3a |
| Dry weight (g/seedling) | | | |
| Leaves | 1.2±0.1b | 1.4±0.2b | 2.4±0.5a |
| Stems | 1.3±0.1b | 1.5±0.2b | 2.6±0.5a |
| Roots (nonmycorrhizal) | 5.8±0.5b | 6.6±0.6b | 11.2±2.3a |
| Total | 8.3±0.7b | 9.5±0.9b | 16.2±3.1a |
| Root/shoot | 2.3a | 2.2a | 2.2a |
| Mycorrhizal root tips | $2.0\times10^{-3}$ | $2.2\times10^{-3}$ | $5.0\times10^{-3}$ |
| N concentration (%) | | | |
| Leaf | 2.6±0.2b | 3.2±0.5a | 2.2±0.3b |
| Stem | 0.9±0.2b | 1.1±0.3a | 0.8±0.1b |
| Roots (nonmycorrhizal) | 1.0±0.1b | 1.4±0.2a | 0.6±0.2c |
| Mycorrhizal root tips | 1.9b | 2.6a | 1.3c |
| N content (mg/plant part) | | | |
| Leaf | 31.2±3.6c | 44.5±7.3b | 50.1±7.5a |
| Stem | 12.0±2.0b | 15.6±3.1b | 22.0±5.8a |
| Roots (nonmycorrhizal) | 58.2±8.9b | 93.6±18.0a | 62.0±8.5b |
| Total | 101.4±9.6c | 153.7±19.9a | 134.1±15.7b |
| Root/shoot | 1.4a | 1.6a | 0.9b |
| Mycorrhizal root tips | $37.4\times10^{-3}$ | $52.1\times10^{-3}$ | $64.4\times10^{-3}$ |
| Seedling N index, biomass/N content | 82 | 62 | 120 |

Values (means±standard errors of the mean) in rows (a, b, c) or in columns (x, y) with different letters are significantly different ($P$=0.05).

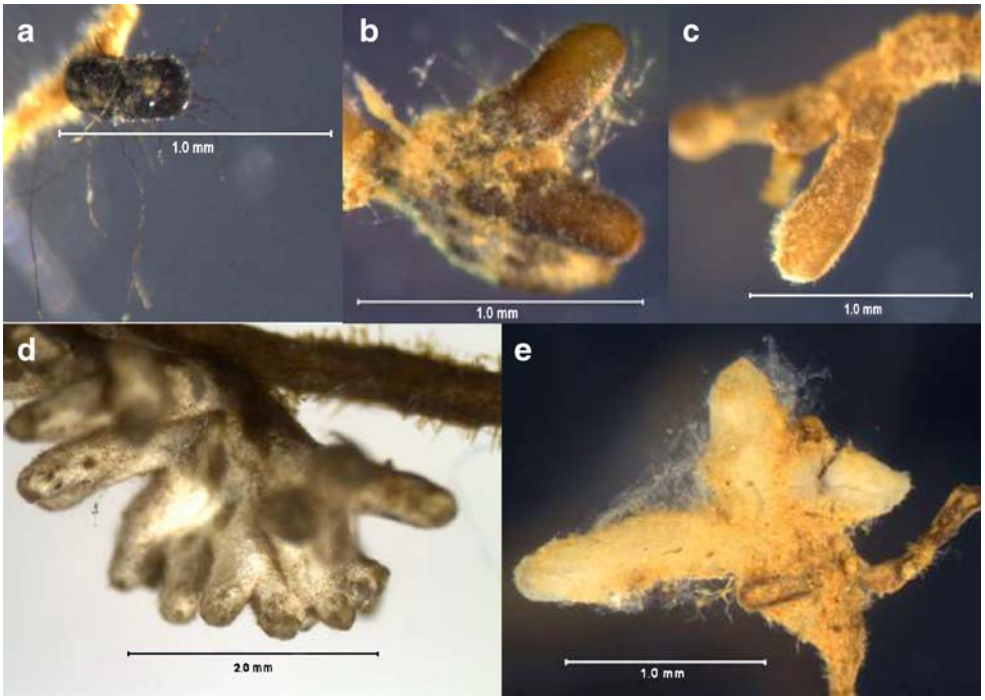


**Fig. 1** Five ectomycorrhizal morphotypes from oak seedling roots. **a** Black morphotype (*Cenococcum geophilum* complex); **b** brown morphotype, characteristic of Thelephoraceae; **c** tan morphotype, characteristic of Pezizales, including *Tuber* sp.; **d** white morphotype, characteristic of *Hebeloma* sp. and *Inocybe* sp.; **e** "Hairy" morphotype

of the genera *Hebeloma* and *Inocybe* as well as Boletales (e.g. *Boletus*, *Melanogaster*, *Octavianina*; Fig. 1d); tan with cottony emanating hyphae ("hairy," no known fungal type; Fig. 1e). Only a few roots were colonized by other rare morphotypes; these were not included in our analyses.

The black morphotype was the most abundant, found on 35–44% of roots on seedlings of all three oak species (Table 1). This morphotype was probably dominated by *Cenococcum geophilum*, based on the characteristic stiff bristly hyphae and surface patterns. The brown morphotype was also abundant in all oak species (31–44%). Other morphotypes were less common and not equally distributed among the oak species. On *Q. garryana* roots, in addition to black and brown morphotypes, the tan morphotype (13%) was present but rarely any other morphotypes. Similarly, *Q. douglasii* had both tan (7.9%) and hairy (8.2%) morphotypes, less of the white morphotype (2.3%). For *Q. agrifolia* roots, the less common morphotypes were comprised of hairy (17%), tan (5.1%), and white (4.7%).

## Seedling biomass, N concentration, and N content

*Q. agrifolia* seedlings were taller and had twice the biomass of *Q. garryana* or *Q. douglasii* seedlings (Table 1). For all three oak species, root/shoot ratios were similar with root biomass about twice that of shoot biomass. Leaf, stem, and root N concentrations were greater in *Q. garryana* than in *Q. douglasii* or *Q. agrifolia* (Table 1). *Q. douglasii* and *Q. agrifolia* had similar leaf and stem N concentrations, however, root %N in *Q. douglasii* was significantly higher than in *Q. agrifolia*. *Q. garryana* had greater total N (mg/plant) than *Q. douglasii* or *Q. agrifolia*, because of greater amounts of N in roots. Root-to-shoot N ratio was low (0.9) in *Q. agrifolia* and higher (1.5) in *Q. douglasii* and *Q. garryana*. Nitrogen use efficiency was about twofold greater for *Q agrifolia* (120) than for the other two species (mean = 72, Table 1).

## $^{15}$N transfer among oak leaves, stems, and roots

Natural abundance of $^{15}$N was similar for all three oak species but differed by plant part: leaves (0.36711 ± 0.00065at.%), roots (0.37145 ± 0.00025), and ectomycorrhizal root tips (0.36563 ± 0.00064). Leaves were highly $^{15}$N enriched after 9days labeling; $^{15}$N moved from the donor leaf throughout the seedling (Fig. 2). About 40% of NDFS remained in leaves of *Q. douglasii* and *Q. garryana*, somewhat less in *Q. agrifolia* (Fig. 2). As expected, more $^{15}$N moved to roots when $^{15}NO_3^-$ was supplied than when $^{15}NH_4^+$ was the N source, especially for *Q. agrifolia* (Fig. 2). Independent of $^{15}$N source, leaf $^{15}$N NDFS in *Q. douglasii* and *Q. garryana* was significantly greater than that in *Q. agrifolia*, but root NDFS was greater for *Q. agrifolia*.

## $^{15}$N transfer into ectomycorrhizal root tips

Percent N in ectomycorrhizal root tips (1.8%) was greater than in fine roots (1%; Tables 1 and 2). Percent N was low in *Q. agrifolia* root tips (1.3%) and higher in *Q. douglasii*

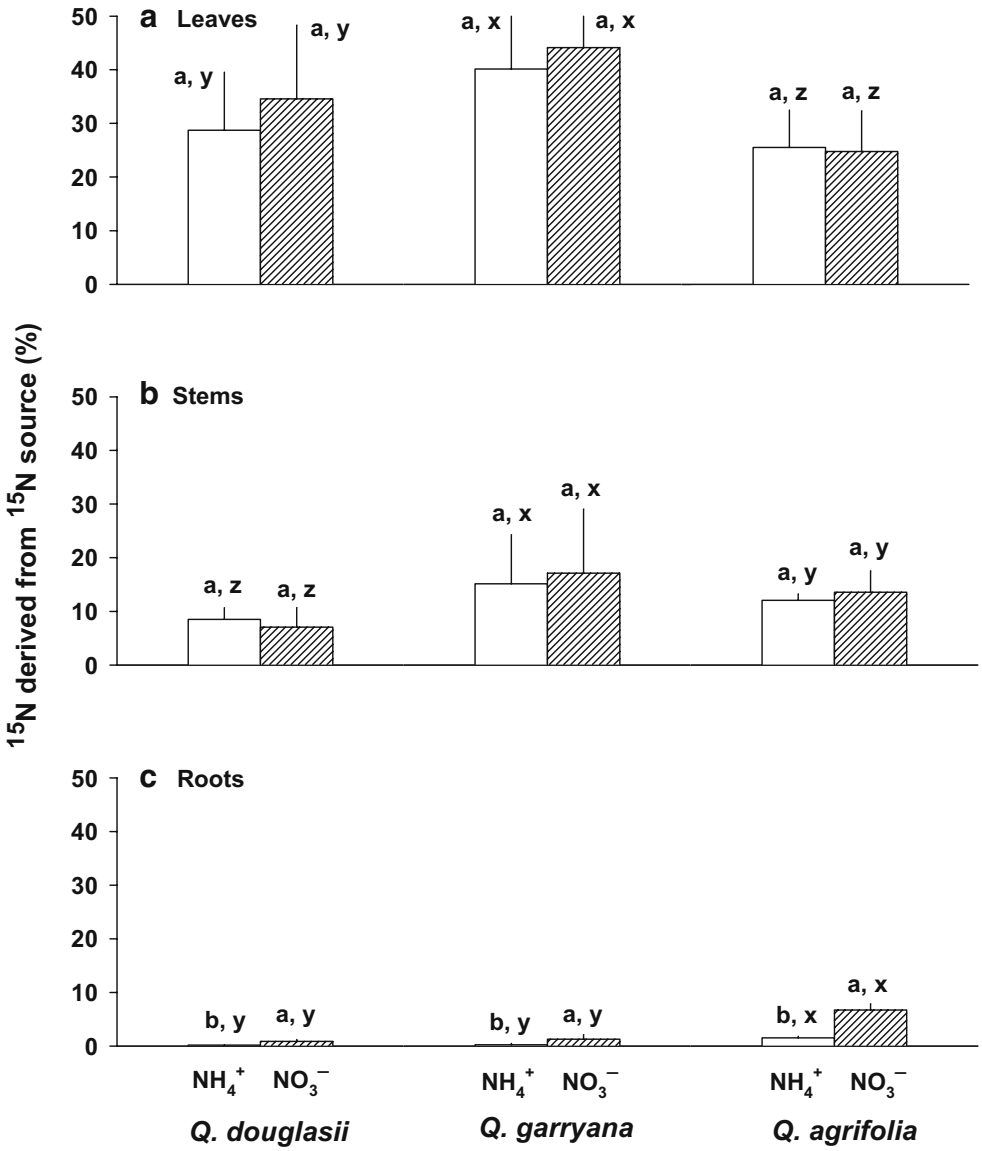


**Fig. 2** Effect of N source ($^{15}NH_4^+$ or $^{15}NO_3^-$) on $^{15}N$ accumulation in oak leaves, stems, and roots. Values of NDFS (%$^{15}N$ derived from $^{15}N$ source, mean±SE, $n$=5–7) are significantly different ($P$=0.05) if followed by different letters between $^{15}N$ source (a, b) and between oak species (x, y, z)

(1.9%) and *Q. garryana* (2.3%) root tips (Table 2). Based on a fine-root carbon content of 40% (data not shown), we calculated C/N for ectomycorrhizal root tips. Ratios were similar among morphotypes but differed by oak species: *Q. agrifolia*, 31, *Q. douglasii*, 22, and *Q. garryana*, 17.

Because of the small sample size of root tips and the minimum total N required for mass spectrometric analyses, it was necessary to combine ectomycorrhizal root tips of three morphotypes (hairy, tan, and white) into a sample labeled as "other." Thus, we were unable to determine the $^{15}N$ in each of the three morphotypes but only for the combined sample. The $^{15}N$ at.% and $^{15}N$ content of ectomycorrhizal root tips were not different between the two N sources; data were combined (Table 2). Patterns were similar for all three oak species (Table 2). Root tips of the "other" category were more $^{15}N$ enriched than were root tips of either brown or black morphotypes, irrespective of $^{15}N$ source or oak species (Table 2). Three fourths of the mycorrhizal root tips were colonized by the black and brown morphotypes, but these root tips acquired less $^{15}N$ than the white, tan, and hairy root tips (Tables 1 and 2). The $^{15}N$ content was greatest in ectomycorrhizal root tips of *Q. agrifolia* (Table 3).

## Discussion

### Cycling of $^{15}N$ within oak seedlings

Plants acquire N via mycorrhizal roots from soils via mycorrhizal networks connecting plants belowground (Newman 1988; He et al. 2003; Simard and Durall 2004) However, plants also circulate N within the plant via the phloem as nitrate and low-molecular-weight organic N

**Table 2** Biomass and N characteristics (%N, $^{15}N$ atom % excess and $^{15}N$ content) of ectomycorrhizal root tips sorted into morphotypes for three oak species

| Parameter | Oak species | Morphotype | | |
|---|---|---|---|---|
| | | Black | Brown | Other[a] |
| Biomass, μg/root tip | *Q. douglasii* | 16.9 a, x | 10.2 a, x | 27.4 a, x |
| | *Q. garryana* | 18.4 a, x | 17.0 a, x | 21.4 a, x |
| | *Q. agrifolia* | 36.4 a, y | 40.6 a, y | 36.1 a, y |
| Nitrogen, % | *Q. douglasii* | 1.88 a, y | 1.84 a, y | 1.83 a, y |
| | *Q. garryana* | 2.35 a, x | 2.36 a, x | 2.24 a, x |
| | *Q. agrifolia* | 1.27 a, z | 1.28 a, z | 1.31 a, z |
| $^{15}N$ at.% excess$\times 10^4$ | *Q. douglasii* | 48 b, z | 94 b, z | 1070 a, y |
| | *Q. garryana* | 85 b, y | 122 b, y | 1080 a, y |
| | *Q. agrifolia* | 104 b, x | 187 b, x | 2012 a, x |
| $^{15}N$ content, μg/tip$\times 10^6$ | *Q. douglasii* | 13.0 b, z | 15.1 b, z | 466 a, y |
| | *Q. garryana* | 30.7 b, y | 44.0 b, y | 621 a, y |
| | *Q. agrifolia* | 45.2 b, x | 104 b, x | 941 a, x |
| | Mean | 29.6 | 54.4 | 676 |

For each parameter, values in rows (a, b) or columns (x, y, z) followed by the same letter are not significantly different ($P$=0.05).
[a] To obtain sufficient material for mass spectrometric analyses, it was necessary to combine samples of three morphotypes (hairy+tan+white) into the "other" category.

compounds (Marschner 1995; Silla and Escudero 2003; Cooke and Weih 2005). In our study, root $^{15}N$ content was greater when $^{15}NO_3^-$ was supplied than when $^{15}NH_4^+$ was supplied, demonstrating the greater mobility of nitrate compared to ammonium. More $^{15}N$ was transferred to roots of evergreen *Q. agrifolia* than to roots of the other two deciduous oak species. During our experiment in April, *Q. agrifolia* was fully leafed out, while *Q. douglasii* and *Q. garryana* were in early stages of leaf expansion. Differences in phenology may have contributed to increased N transfer to roots of *Q. agrifolia*. Mycorrhizal root tips were significantly enriched in $^{15}N$ concentration and N content compared to fine roots, but there were no effects of N source suggesting that the mobility of nitrate did not affect transfer from roots to ectomycorrhizal root tips.

## Differential $^{15}N$ transfer into ectomycorrhizas

In our study, there were five common ectomycorrhizal morphotypes on oak seedlings. Other researchers found

**Table 3** Two-way ANOVA for ectomycorrhizal root tips for four parameters for main effects (oak species, ectomycorrhizal morphotype); main effects were significant, interactions were not ($P$=0.05)

| Parameter | *df* | SS | *F* value | *P*>*F* |
|---|---|---|---|---|
| Biomass, mg/seedling | | | | |
| Oak spp. | 2 | 1,468 | 8.54 | 0.004 |
| Morphotypes | 2 | 106 | 0.619 | 0.553 |
| Oak spp. × morphotypes | | | | |
| Nitrogen, % | | | | |
| Oak spp. | 2 | 3.159 | 591.5 | <0.001 |
| Morphotype | 2 | 0.005 | 0.857 | 0.447 |
| Oak spp.×morphotypes | | | | |
| $^{15}N$ at.% excess$\times 10^4$ | | | | |
| Oak spp. | 2 | 494,888 | 2.490 | 0.121 |
| Morphotypes | 2 | 6,571,432 | 33.080 | <0.001 |
| Oak spp.×morphotypes | | | | |
| $^{15}N$ content, μg/tip$\times 10^6$ | | | | |
| Oak spp. | 2 | 122,804 | 1.330 | 0.298 |
| Morphotypes | 2 | 1,609,306 | 17.400 | 0.0002 |
| Oak spp.×morphotypes | | | | |

$^{15}N$ source was not significant, data were combined.

similar diversity. In a riparian oak site in California's Central Valley, nine morphotypes were observed on 4-year-old *Q. lobata* seedlings (Berman and Bledsoe 1998). In a New Jersey oak woodland, Baxter et al. (1999) found nine ectomycorrhizal types on seedlings of *Q. rubra*. In southern Oregon, Valentine et al. (2004) found nine morphotypes on seedlings and many more (39) ectomycorrhizal morphotypes on mature trees of *Q. garryana*. In a California coastal live oak woodland, about 40 ectomycorrhizal morphotypes were found on *Q. agrifolia* seedling roots (Egerton-Warburton and Allen 2001; Lindahl 2002).

For stable isotopic analyses, there was not sufficient material of the three less common types, and they were combined into an "other" category (tan, white, hairy) for analyses. Thus, we cannot determine $^{15}N$ enrichment of each of the three types in "other." However, the three morphotypes—black, brown, and "other"—differed in $^{15}N$ enrichment with greatest enrichment in the "other" samples. This pattern was repeated for all three oak species showing that ectomycorrhizal morphotypes differed in N sink strength, independent of host species or N source. This differential $^{15}N$ enrichment among ectomycorrhizal morphotypes is evidence for ectomycorrhizal functional diversity in nitrogen transfer. Thus, a combined set of morphotypes ("other") gained more N than the other two well-defined morphotypes, black and brown. Another study (Treseder et al. 2004) demonstrated ectomycorrhizal functional diversity in carbon acquisition, showing species-specific patterns for carbon.

## Ectomycorrhizal diversity

In our study, three quarters of the oak seedling roots were ectomycorrhizal. Similar levels in oak fine roots have been reported (Egerton-Warburton and Allen 2001; Lindahl 2002; Cheng and Bledsoe 2002; He et al. 2006). We collected five common ectomycorrhizal morphotypes. These morphotypes may not reflect individual fungal species but several species. The black morphotype, found on roots of all three oak species, was probably *Cenococcum*, which produces distinctive morphological structures. *Cenococcum* may be subdivided into three (Douhan and Rizzo 2005) or five (Smith et al. 2004) lineages. However, our study did not use molecular methods necessary to further subdivide *Cenococcum* into different lineages.

There are differences in ectomycorrhizal exploration types (Agerer 2001) that may relate to resource demand (i.e., nitrogen). The black morphotype was probably *Cenococcum*, which has surface thick black hyphae of limited extension and may be considered as a "contact type." *Cenococcum* is not expected to invest in emanating hyphae and therefore would not be expected to be as much of a sink for N. In contrast, our "hairy" type has extensive hyphal development and might be expected to be a greater sink for N.

In other studies near our field site, molecular methods were used to characterize ectomycorrhizal diversity on mature trees of *Q. douglasii* (Smith et al. 2007) and *Q. wislizeni* (Morris, personal communication). The most common ectomycorrhizal taxa on these two oak species were *Cenococcum*, *Inocybe*, *Laccaria*, *Lactarius*, Sebacinaceae, Thelephoraceae, and Pezizales (including *Tuber*). Their molecular data were supported by a wide diversity of ectomycorrhizal sporocarps associated with *Quercus* spp. at the site (Smith et al. 2007). Based on this molecular data from oak tree roots and our observations of seedling roots (Bledsoe and Southworth, personal communication), the brown morphotype probably included members of the Thelephoraceae, the tan morphotype *Tuber* sp. and related Pezizales (*Genea*, *Helvella*), and the white morphotype *Hebeloma*, *Inocybe*, and Sebacinaceae.

## Differential root production of oak species

*Q. agrifolia* seedlings were taller than *Q. douglasii* and *Q. garryana* seedlings, and their biomass was greater. The ability of the evergreen oak *Q. agrifolia* to photosynthesize year around may have resulted in increased growth. For all oak species, root/shoot ratios were similar, with below-ground biomass double that of aboveground. This emphasis on root production is to be expected in plants growing in a Mediterranean climate with summer drought (Gordon and Rice 1993; Millikin and Bledsoe 1999; Cheng and Bledsoe 2002; Aanderud et al. 2003). Although seedling biomass was greatest in *Q. agrifolia*, total N content was greatest in *Q. garryana*, lower in *Q. agrifolia*, and least in *Q. douglasii*. Compared to deciduous oaks, root %N was lower in *Q. agrifolia*. Low root %N in *Q. agrifolia* correlated with significant $^{15}N$ transfer from leaves to roots. *Q. agrifolia* seedlings used N efficiently, allocating additional N to the roots as it became available in shoots.

In summary, foliarly applied $^{15}N$ was transferred from oak leaves to ectomycorrhizal root tips. Our results document differential $^{15}N$ enrichment in different mycorrhizal morphotypes. In all three oak species, one morphotype group (tan, hairy, and white) accumulated about 20 times more $^{15}N$ than the more abundant black and brown morphotypes, although ectomycorrhizal root tip N concentrations were similar among morphotypes. Our results demonstrate that not all ectomycorrhizas are "created equal" and that N transfer to roots can be influenced by ectomycorrhizal morphotypes.

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