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Using ^{13}C and ^{15}N Isotopes to Study Allocation Patterns in Oak Seedlings [1]

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Abstract

In California's oak woodlands, survival and growth of oaks may depend on a symbiosis between oak roots and fungi that form ectomycorrhizas. Ectomycorrhizal (ECM) fungi are major players in carbon (C) and nitrogen (N) utilization and cycling because they facilitate water and nutrient uptake from the soil into the plant. The ECM fungi also benefit because plants supply carbohydrates to their fungal partners. Little is known about the stoichiometry of N and C exchange within ECM plants. It is not known whether N uptake and transfer from ECM fungi to their plant host is related to C flow from host to ECM fungi.

We considered several questions. Do plants "reward" those ECM fungal species that supply more N to the plant by providing more C to these ECM species? Are N and C transfers linked? Are ECM roots that take up more N from soils greater sinks for C? What are the long-term and short-term transfers of N and C, as measured by natural abundance (long-term) and tracer studies (short-term)? The natural abundance (background levels) of ^{15}N and ^{13}C in oak seedlings sheds light on what are the N sources for oaks, and how oaks allocate C over the long term (years to decades). Tracer data shed light on short-term processes of N and C allocation in oaks.

In this study, we explored these questions by tracking C and N transfers within ECM blue oak seedlings of *Quercus douglasii* Hook&Arn. First, we determined the natural abundance (background) of N and C in control seedlings. N natural abundance was higher in ECM roots than in other tissues, while C natural abundance was higher in leaves than in other tissues. We traced C transfer from oak shoots into oak ECM roots, as well as N transfer from soil into oak ECM roots and into oak shoots. Our results suggest that both leaves and ECM tips were strong sinks for C and N. The stoichiometry of N and C into and out of ECM roots can help us understand how ECM fungi affect C allocation within oaks and how oaks respond to N supply from ECM roots.

Keywords: Blue oak, carbon allocation, nitrogen uptake, stable isotopes, stoichiometry.

Introduction

Blue oak (*Quercus douglasii* Hook&Arn.) is a broadly distributed tree species in California and occupies about 40 percent of California oak woodlands (Adams and others 1997). It is thought that oaks are highly dependent on ectomycorrhizal (ECM) fungi for growth and survival since ECM fungi play important roles by providing water and nutrients for the host plant, while the host provides photosynthates to its symbiotic partner. Our current understanding of this relationship suggests that in all types of mycorrhizal associations, except for those involving orchids or

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mycoheterotrophs, the C requirements of the symbiosis are met by transfer of photosynthates from the plant to the mycorrhizal fungi within plant roots (Smith and Read 1997).

The cost of maintaining the symbiosis has been estimated to range between 10 and 30 percent of photosynthate (carbohydrate) production. Thus, mycorrhizas may act as strong sinks for plant photosynthates, stimulating host photosynthetic rates, improving host nutrition, and influencing nutrient transfer from the soil. Mycorrhizas constitute checkpoints for exchange of N and C at the plant-soil interface (Martin and others 2001). However we do not understand how ECM fungi affect C and N dynamics. For example, do plants "reward" certain ECM fungi that take up more soil N by supplying these ECM roots with more C than other ECM fungi that take up less soil N? Are C and N transfers into ECM roots linked?

Nitrogen is often the limiting nutrient for forest growth. Thus, N cycling has been extensively studied in forest soils. Plants cannot directly access atmospheric N_2 and rely on soils for their N. Plants may compete with other soil organisms (bacteria, saprobic fungi, etc.) for soil N. In addition, soil N may be less available to plants when this element is in soil organic matter. ECM fungi are able to access soil N in inorganic forms (ammonium, nitrate) and also in organic forms, such as labile organic N, amino acids and proteins. Clearly, there are benefits to plants to support their ECM fungi, as well as benefits to the ECM fungi by associating with plant roots.

Movement of soil N is usually considered to be unidirectional, beginning in the soil, continuing to plant roots and finally to shoots (Marschner 1995). Carbon movement in the plant is controlled by gradients between production (source organs such as leaves) and consumption (sink organs such as non-green tissues). Thus, C movement can be either bidirectional (translocation within the plant) or unidirectional (from shoots to stems, to roots and then into the rhizosphere soil).

The use of stable isotopes is a powerful approach to assess C and N interactions, especially belowground (Maillard and others 1994). Stable isotopes are a useful tool for analyzing the function of ECM fungi because variations in natural abundance of C and N, in both the plant host and the ECM fungi, may reflect the longer-term history of resource acquisition, loss, and internal cycling (Handley and Scrimgeour 1997). Ecological studies often use stable isotope data either at naturally occurring levels (natural abundance) or at levels outside the natural range (enrichment experiments). This enrichment technique involves an enrichment of the heavier isotope over background making the measurement of isotopic effects easy to detect, since the difference between natural abundance *vs.* enrichment is large. Moreover, variations in background natural abundance levels of most isotopes are very small, and stable isotope composition is usually reported as parts per thousand (‰). Changes in isotopic distributions of N and C across the fungal-host continuum can provide insights into the role of ECM fungi in N and C cycles.

Measurement of natural abundance of ^{13}C and ^{15}N isotopes in blue oak-ECM systems can give us a long-term (months to years and decades) view of C and N transfer into and out of both symbionts. In contrast, enrichment measurements give us a short-term view (hours to days), permitting us to follow the flows and rates of ^{13}C and ^{15}N without altering their natural behavior (Nadelhoffer and Fry, 1994).

In this study we asked four questions: First, what are the relative background (natural abundance) levels of the stable isotopes of C and N in different parts of blue oak seedlings? Second, how long does it take for foliar-applied C to move into roots and for soil-applied N to move into the plant? Third, will both C and N accumulate in fine and ECM roots? Fourth, are ECM roots stronger sinks for both C and N than other plant parts? These questions were investigated in a greenhouse experiment that measured transfers of the stable isotopes ^{15}N and ^{13}C in blue oak seedlings, applying ^{15}N to the soil and ^{13}C to the foliage.

Materials and Methods

Site Description and Experimental Design

Blue oak seedlings used in this greenhouse experiment were selected from a plot located within an oak-pine woodland at the University of California Sierra Field Research and Extension Center (SFREC) near Browns Valley, in northeastern California. SFREC is located in the Sierra Nevada foothills and has a typical Mediterranean climate. The plot was within an ungrazed site in the Koch Natural area (elevation ~600 m). Soils are classified as fine, mixed thermic Typic Haploxeralfs (Dahlgren and others 1997). Blue oaks are the dominant tree species; leaves emerge in late March; leaf drop usually occurs in October or November. The plot contained seedlings planted within nylon mesh cylinders to facilitate future removal of seedlings with intact roots. Seedlings were planted in 2002 and became mycorrhizal with ECM fungi native to the oak-pine woodland.

The greenhouse experiment included three treatments and four replicate seedlings for a total of 12 seedlings. Treatments were: (1) control (no ^{13}C or ^{15}N addition), (2) ^{13}C-glucose and ^{15}N-ammonium chloride (^{15}N-NH_4Cl) addition, and (3) ^{13}C-sodium bicarbonate (^{13}C-$NaHCO_3$) and ^{15}N-NH_4Cl addition. We chose glucose as a C source because it is rapidly transferred within the plant, although its absorption through leaves may be slow. We chose bicarbonate as a C source because it can be rapidly photosynthesized, although some CO_2 may be lost. Both ^{13}C treatments received ^{15}N-NH_4Cl application to the soil. We chose ammonium as a N source, since it does not leach from soils and would remain in the soil in the pots.

In March 2006, we dug up 12 blue oak seedlings within their mesh cylinders from our field plot, and we transferred the 4-year-old seedlings and associated soil into pots (10 cm x 10 cm x 30 cm). Seedlings had just begun to leaf out and had a few small leaves. Seedlings were allowed to acclimate to greenhouse conditions for two weeks while more seedling leaves emerged and expanded.

^{15}N and ^{13}C Labeling

Two weeks after seedlings were transplanted into pots, we added ^{15}N-NH_4Cl solution (27.5%N, 99 atom% ^{15}N) to the soil in all pots except the controls. In each of these pots, we injected 30 ml of isotopically enriched NH_4Cl solution in six locations around the seedling. We used an auto-refilling 18–gauge, 30 cm long syringe. The solution was released slowly as the needle was raised from the bottom to the top level of the pot over a distance of 25 cm. The amount of N injected into each pot was calculated to produce an average concentration of 2 $\mu g\ N\ g^{-1}$ soil.

After two additional weeks in which seedlings continued to grow and acquire ^{15}N from the soil, we added ^{13}C to five leaves on each plant, not including the four control plants. We applied ^{13}C as either sodium bicarbonate or as glucose. The four seedlings in Treatment 2 received 8 ml/seedling of ^{13}C-labeled glucose (20mM, 40%C, 99 atom% ^{13}C). The four seedlings in Treatment 3 received 8 ml of ^{13}C-labeled $NaHCO_3$ (1mM, 15.3%C, 99 atom% ^{13}C). Labeled solutions in 2 ml-microcentrifuge tubes were attached to the leaves (five tubes, one/leaf). Each leaf was inserted into a microcentrifuge tube containing 1.6 ml of the ^{13}C-solution. We cut the end of each leaf to facilitate the absorption of the solution.

Tubes were attached to leaves on different branches, where possible, to create more uniform transfer into the entire plant. Tubes were attached vertically to branches and sealed with a small plug of glass wool to reduce evaporation. After three to four days, we re-filled the tubes with distilled water to enhance uptake of any remaining solution in the bottom of the tubes. During the day, seedlings were exposed to artificial light to increase absorption of ^{13}C-labeled solution. Plants were irrigated as needed, about once a week.

Harvest

In a sequential sampling with minimal disturbance to the soil, we removed a few ECM fine roots at four times: (1) before addition of the ^{15}N, day 0; (2) immediately before the addition of the ^{13}C, day 14; (3) the day after the addition of the ^{13}C, day 15; and (4) day 18. After 26 days, we harvested all plants, both roots and shoots. At each sampling time, except day 0, we collected three fine-ECM root samples from the upper root system (0-12 cm) and three samples from the lower root system (12-24 cm). These collections were done by opening the side of the plastic pot and carefully sampling several fine and ECM roots with minimal disturbance to the remaining roots and soil. Twelve days after application of ^{13}C (day 26), the tubes were removed and seedlings were harvested. We separated the plants into leaves, branches, stem and roots. Roots were further subdivided into four categories: taproot, coarse roots (> 2mm diameter), fine roots (0.5 < diameter > 2mm) and ECM roots (< 0.5mm diameter, bearing mycorrhizal tips). Height, stem diameter of the oaks and number of branches and leaves were recorded. Leaves to which tubes with ^{13}C-solutions were attached were not included in the isotopic analyses.

All plant materials were oven-dried (60°C, one week), weighed, ground to a fine powder and analyzed for total N, ^{15}N, total C and ^{13}C by a mass spectrophotometer at the University of California, Davis, Stable Isotope Facility. The four control seedlings, used to determine natural abundance of ^{15}N and ^{13}C, were harvested and analyzed similarly.

Calculations and Statistical Analyses

Two sets of samples were processed for isotope analyses, control seedlings for natural abundance determinations, and labeled seedlings for ^{15}N and ^{13}C enrichment. For natural abundance determinations, we harvested ECM root samples at day 0 and at day 26. For seedlings that received both ^{15}N and ^{13}C, we harvested fine ECM roots over time (day 14, 15, 18, and 26). This allowed us to determine the rate of transfer of both ^{15}N and ^{13}C into different parts of the seedlings.

We calculated $\delta^{15}N$ (‰) as follows (Shearer and Kohl 1993):

$$\delta^{15}N = [(^{15}N/^{14}N\text{sample} - {}^{15}N/^{14}N\text{standard})/ (^{15}N/^{14}N\text{standard})] * 1{,}000$$

Similar calculations were done for ^{13}C -enriched samples:

$$\delta^{13}C = [(^{13}C/^{12}C\text{sample} - {}^{13}C/^{12}C\text{standard})/ (^{13}C/^{12}C\text{standard})] * 1{,}000$$

Values were calculated relative to the international standard Vienna Dee Belemnite for ^{13}C (R=0.0112372) and for atmospheric N_2 for ^{15}N (R=0.0036765). For each individual sample, ^{15}N and ^{13}C % recovery was calculated as:

$\%^{15}N$ recovery = (amount ^{15}N excess sample/total ^{15}N added into one seedling pot)*100

$\%^{13}C$ recovery = (amount ^{13}C excess sample/total ^{13}C added into one seedling pot)*100

Where:

Amount isotope excess = (amount isotope/unit of tissue weight)*(atom% enriched sample - atom% control).

Data were analyzed using one-way ANOVA (^{15}N and ^{13}C parameters as main factors) or two-way ANOVA when isotopic composition per plant-part was compared (SPSS, Inc., Chicago, IL). Separation of means was performed using a Fisher's protected LSD at the 0.05 significance level.

Results

Seedling Biomass and Percent N

Since there were no differences among treatments in seedling biomass and percent N, data were averaged (n=12, *table 1*). Average seedling height was 24 cm and there were 2-5 branches/seedling and 15-45 leaves/seedling. Mean stem diameter was 7 mm, the mean length of the taproot was 24 cm. Seedling shoot to root ratio was 0.36±0.02. All seedlings were ectomycorrhizal.

Table 1—*Characteristics of Quercus douglasii seedlings. Values are means (n= 12) with standard errors.*

Plant part	Biomass (g)	Biomass allocation (%)	%N	N allocation (%)
Leaves	0.85±0.1	6.3±0.2	2.07±0.09	31.0±1
Branches	0.62±0.1	4.6±0.5	0.86±0.02	12.9±0.5
Stem	2.04±0.3	15±1.4	0.73±0.03	11.0±0.3
Taproot	8.20±1.4	62±1.7	0.45±0.03	6.7±0.4
Coarse roots	0.90±0.2	5.5±2	0.66±0.02	9.3±0.6
Fine roots	0.31±0.07	2.3±0.4	0.9±0.02	13.5±0.3
Ectomycorrhizal roots	0.58±0.05	4.3±0.3	1.03±0.05	15.6±0.9
	Total, 13.50		Mean, 0.96	

Natural Abundance

In control seedlings, natural abundance of $\delta^{13}C$ and $\delta^{15}N$ was higher in ECM roots than in other plant parts ($p<0.0001$), except leaves that had the highest $\delta^{13}C$ values ($p<0.0001$). Branches and stems had the lowest $\delta^{13}C$ values and together with coarse roots, had the lowest $\delta^{15}N$ values (*table 2*).

Table 2—*Natural abundance of ^{15}N and ^{13}C in Quercus douglasii seedlings. Values are means of seedlings from Treatment 2 only (n=4) with standard errors of the mean. Within columns, different letters denote significant differences (p<0.05).*

Plant part	δ15N(‰)	δ13C(‰)
Leaves	-3.79±0.46 b	-27.38±0.20 c
Branches	-4.87±0.29 a	-28.63±0.27 a
Stem	-4.37±0.34 b	-28.89±0.33 a
Taproot	-3.14±0.33 b	-27.90±0.25 b
Coarse roots	-4.59±0.16 a	-27.82±0.26 b
Fine roots	-3.47±0.21 b	-28.05±0.20 b
Ectomycorrhizal roots	-0.69±0.16 c	-27.89±0.11 b

Recovery of ^{15}N and ^{13}C in the Seedlings

The ^{15}N moved rapidly from soil into the plants; percent recovery was 1.7 percent. About half of the ^{15}N remained in the roots, half moved above ground, primarily into leaves (*fig. 1a*). Very little ^{13}C from the bicarbonate source was taken up by oak seedlings, thus, bicarbonate was not a satisfactory labeling method. However, in ^{13}C-glucose treated seedlings, ^{13}C moved into all plant parts. The percent recovery was 0.18 percent of the ^{13}C applied to the leaves. About 76 percent of the ^{13}C in the plant remained in leaves and branches and about 24 percent moved to the roots (mainly fine and ECM-roots; *fig. 1b*).

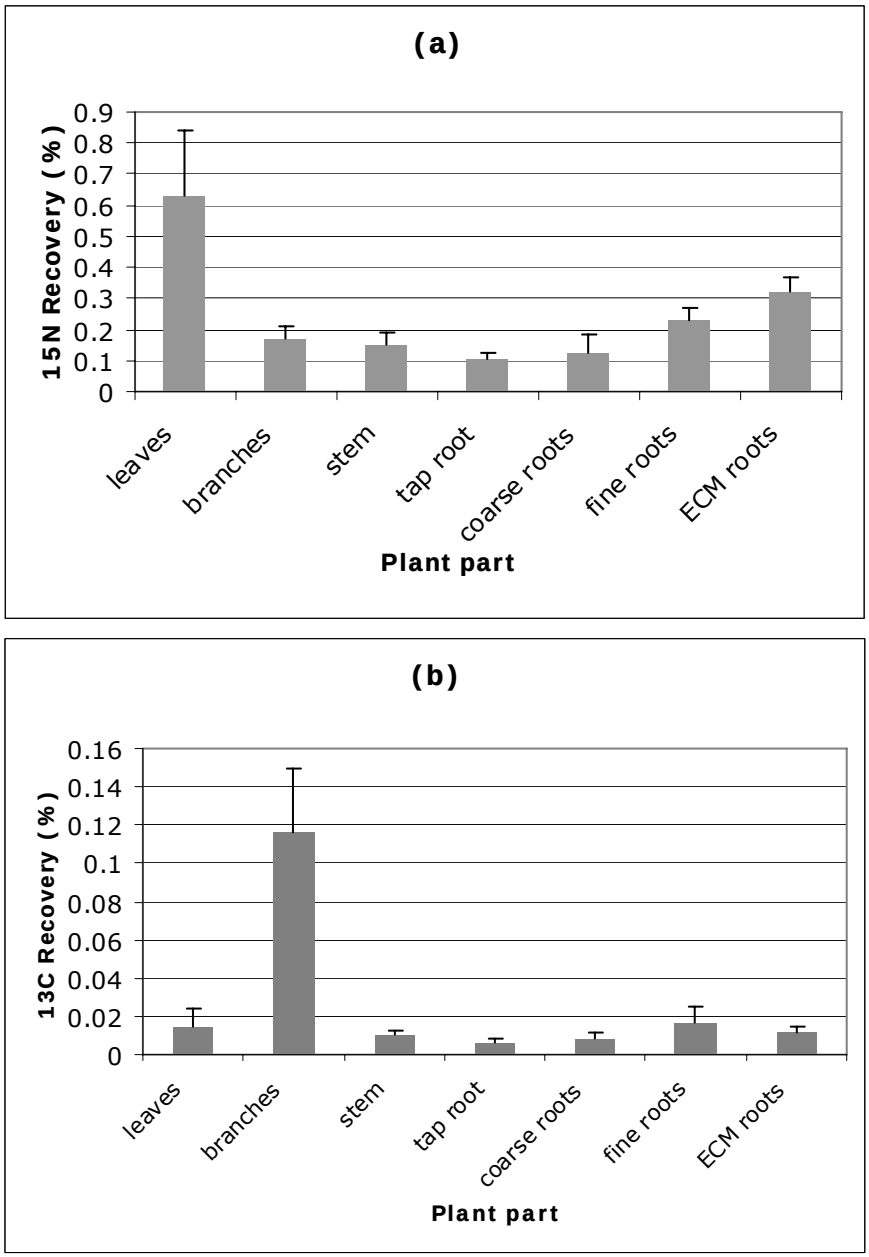


Figure 1—Percent recovery after 26 days: (a) ^{15}N and (b) ^{13}C. ^{15}N-ammonium was applied to the soil, ^{13}C-glucose was applied to oak leaves. Values are means from Treatment 2 seedlings (n = 4) with standard errors shown by vertical bars.

^{15}N and ^{13}C Uptake into Fine-ECM Roots Over Time

We found ^{15}N in the ECM and fine roots of seedlings within 14 days, at which point we applied ^{13}C-glucose to four seedlings and ^{13}C-bicarbonate to four seedlings. We sampled a few ECM roots from each seedling from day 0 (^{15}N addition) to day 14 (^{13}C source addition) and to days 15, 18, and 26 (final harvest). Values of δ^{15}N increased from 4 to 26 days after ^{15}N application (*fig. 2a*). We detected ^{13}C from the labeled glucose one day after application (day 15); δ^{13}C values continued to increase over time. We could not detect ^{13}C from sodium bicarbonate even 12 days after its application (day 26) (*fig. 2b*).

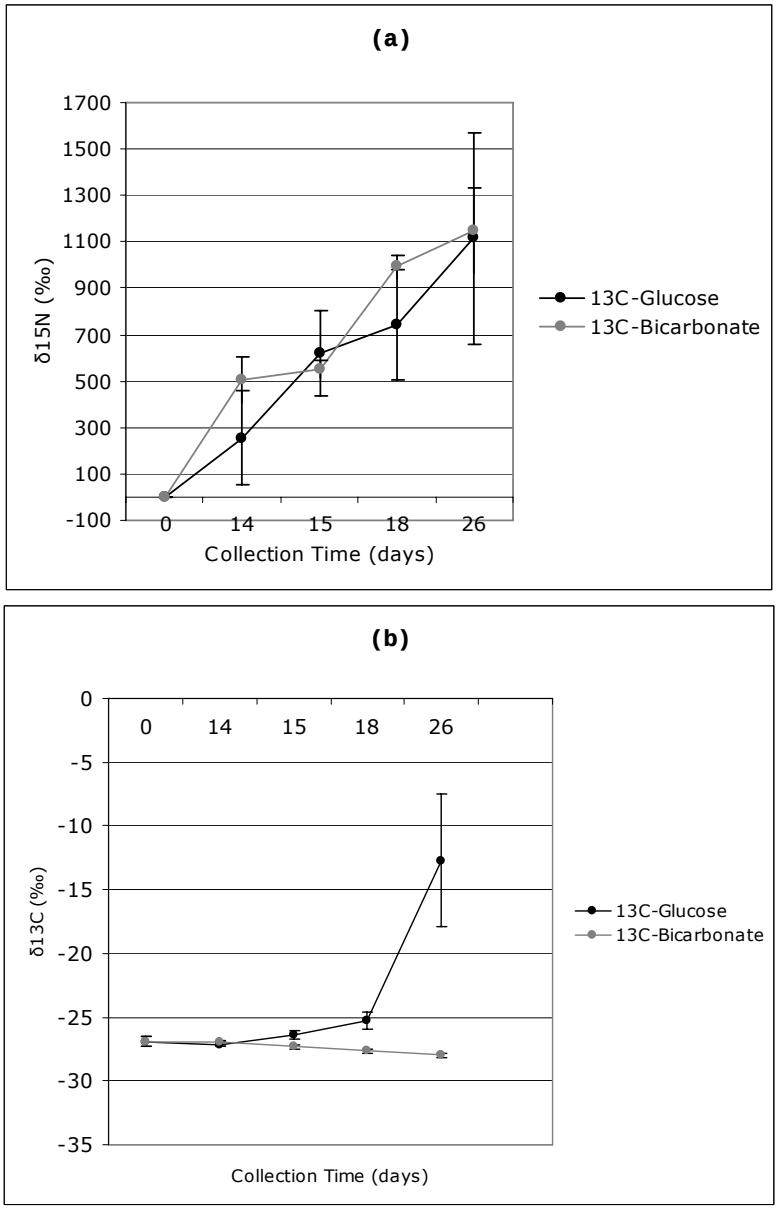
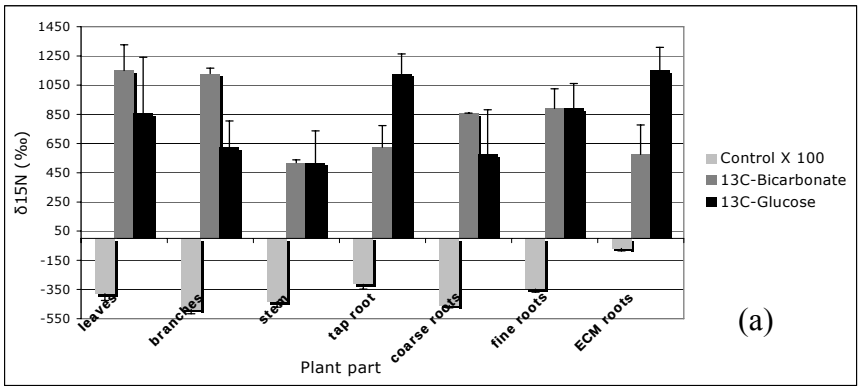


Figure 2—Transfer of ^{15}N and ^{13}C into fine-ECM blue oak seedling roots, 26 days after ^{15}N application to soil in day 0, and 12 days after ^{13}C application to leaves (day 26); (a) δ^{15}N (‰) and (b) δ^{13}C (‰). Values are means of Treatments 2 and 3 (n=4 for each) with standard errors of the mean shown by the vertical bars.

^{15}N and ^{13}C Allocation in the Seedlings

^{15}Nitrogen and ^{13}C from glucose were both readily detectable in all parts of the seedlings. Since we did not find differences in $\delta^{13}C$ and $\delta^{15}N$ values between the upper and the lower part of the oak roots, data were averaged. There were no significant differences in $\delta^{15}N$ in seedlings treated with either ^{13}C source (*fig. 3a*). Similarly, there were no significant differences in $\delta^{13}C$ in control seedlings and seedlings treated with ^{13}C-bicarbonate (*fig. 3b*).



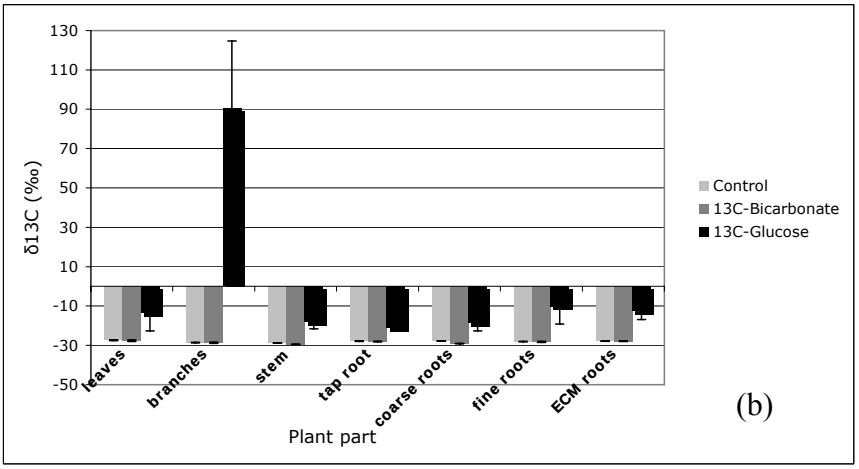


Figure 3—Allocation of ^{15}N and ^{13}C in oak seedlings after 26 days. (a) $\delta^{15}N$ (‰) and (b) $\delta^{13}C$ (‰). Light grey bars: control; dark grey bars: ^{13}C-bicarbonate treatment; black bars: ^{13}C-glucose treatment. Note: $\delta^{15}N$ values for control seedlings are multiplied by 100 for visibility.

Amount of ^{15}N and ^{13}C Transferred into Plant Parts

The greatest amount of ^{15}N was found in leaves (87 ng ^{15}N/mg dry weight) followed by ECM-roots (44 ng ^{15}N/mg dry weight) and fine roots (~32 ng ^{15}N /mg dry weight; *table 3*). Although much of the ^{13}C remained in the branches (54.5 µg ^{13}C/mg), some ^{13}C was transferred into fine and ECM roots.

Table 3—*Transfer of ^{15}N and ^{13}C within oak seedlings after foliar application of ^{13}C-glucose and soil application of ^{15}N-NH_4Cl. Values are means of Treatment 2 seedlings (n=4) with standard errors. Within columns, different letters denote significant differences ($p<0.05$).*

Plant part	^{15}N ng/mg tissue	^{13}C µg/mg tissue
Leaves	87.1±29.1a	6.70±4.7b
Branches	23.4±0.5b	54.5±15.7a
Stem	20.8±0.6b	4.70±1.4b
Taproot	14.6±0.3b	3.10±0.6b
Coarse root	17.0±0.8b	3.80±1.6b
Fine roots	31.8±0.5b	7.90±3.9b
Ectomycorrhizal roots	44.5±0.7b	5.50±1.2b

Discussion

Seedling Biomass and Percent N

The goal of this experiment was to follow transfer of the isotopes through the oak seedlings. Treatment with ^{15}N and ^{13}C did not affect seedling nutrient status, and control untreated seedlings did not differ in biomass and percent N from the seedlings treated with ^{15}N and ^{13}C. Leaves and fine and ECM roots were only 11 percent of the total plant biomass, but contained 60 percent of the total plant N. In contrast, the taproot represented 60 percent of the seedling biomass, but contained only 7 percent of the total plant N. We expected to find high amounts of N in fine and ECM roots since N assimilation in trees occurs almost entirely in root tips (Martin and Botton 1993). The high percent N in leaves might be due to the high N requirement for protein synthesis during production of foliage. In our experiment, foliar percent N was significantly greater than root percent N, as was shown for *Q. douglasii* by He and others (2006). In this study, the oak shoot-to-root ratios were similar to those reported for the same species by Cheng and Bledsoe (2005).

Natural Abundance

Natural abundance of stable isotopes presents a time-integrated long-term picture of functional processes that are usually difficult to measure directly (Robinson 2001). In contrast, enrichment studies present a short-term view of the movement and storage of these isotopes. In our experiment, natural abundance of both ^{15}N and ^{13}C isotopes was measured in control untreated seedlings. ECM roots from these control plants had higher $\delta^{15}N$ values than any other plant parts, indicating their active role in nitrogen uptake. Fungal $\delta^{15}N$ values increased with the proportion of N taken up and transferred to the plant, leading to a greater difference between fungus and host. The higher values of $\delta^{15}N$ in ECM roots are mainly due to fractionation against ^{15}N during the transfer of N from fungus to the host (Taylor and others 2003). The ^{15}N

abundance in plants is closely related with the presence and type of mycorrhizae (Michelsen and others 1998, Emmerton and others 2001). The sporocarps are enriched in ^{15}N compared to plants (Gebauer and Dietrich 1993, Taylor and others 1997, Hobbie and others 1999, Trudell and others 2004). Even the sheaths of ECM fungi are enriched, compared to their host plants (Högberg and others 1996).

Delta^{13}C values were higher in leaves, as expected, since leaves are actively photosynthesizing carbon into carbohydrates and thus, are a strong sink for C. Delta^{13}C values were also high in roots that were also a strong sink for C. Enrichment in ^{13}C by ECM roots could be due to demand by the fungi for ^{13}C-enriched carbohydrates (Henn and Chapela 2001). Further studies are in progress to determine if different ECM types (morphotypes or genetically different genera and species) are greater sinks for N and C than other species.

Recovery of ^{15}N and ^{13}C in the Seedlings

After 26 days, recovery of both ^{15}N and ^{13}C was low, suggesting that longer times might be required for the isotopes to move throughout the plant. There are other possible causes of low recovery. For example, low ^{15}N recovery might be due to slow root growth relative to shoot growth, since the young seedlings had rapidly expanding leaves. In addition, greenhouse conditions may increase nitrification processes, during which, isotope discrimination can be as great as a 40‰ of fractionation factor. ECM fungi generally prefer ammonium over nitrate as an N source. However, under limiting supplies of ammonium, nitrate (depleted after mineralization) would represent an alternative N source. Thus, seedlings could have taken up naturally occurring un-labeled nitrate instead of ammonium, leading to a low recovery of the ^{15}N. Recovery of ^{13}C was low, perhaps due to difficulties in absorption of the glucose by the somewhat thick cuticle on the oak leaves. Perhaps transplant shock in moving seedlings from the field to the greenhouse may have contributed to the low recovery.

We expected to find ^{13}C primarily in the unlabeled leaves, but were surprised to find 76 percent in the leaves, stem and branches, but mainly in the branches. We suspect that enrichment was lower in leaves, due to dilution effects from photosynthesis of unlabeled carbon dioxide. The incorporation of new unlabelled carbon ($^{12}CO_2$) from the atmosphere, used for photosynthesis in the leaves, would dilute the ^{13}C pool and decrease the $^{13}C/^{12}C$ ratios in the leaves. We suspect that much of the enrichment in the stems was due to incorporation of ^{13}C into structural materials (cellulose, etc.).

^{15}N and ^{13}C Uptake and Allocation

The ^{15}N from the NH_4Cl was detected in fine roots at our first sampling time, 14 days following its application into soil and continued to increase up to our final harvest at 26 days. Cheng and Bledsoe (2005) and He and others (2006) reported similar rapid transfer of ^{15}N in oak seedlings and saplings. Transfer of ^{15}N within the plant occurs from ECM roots to leaves. It is assumed that a fraction of the N taken up from the roots passes upwards in the xylem to the shoot, where it is transferred to the phloem. The remaining fraction is transferred directly to the root phloem (Dewar 1993). Leaves might act as N sinks since we recovered a significantly greater percentage of ^{15}N in leaves than in the rest of the plant.

We could not detect ^{13}C from the bicarbonate, indicating no or very low absorption of this ^{13}C source by the leaves. However, the movement of glucose-derived -^{13}C from the leaves to the fine and ECM roots was rapid; we detected ^{13}C one day after its application. Other authors have also reported similar rapid transfer of labeled-C to the roots (Taylor and others 2004). We detected high amounts of ^{13}C in the branches so they may act as storage organs of C.

Conclusions

In this study we asked four questions:

(1) What are the background (natural abundance) levels of the stable isotopes of C and N in the different parts of blue oak seedlings? – We found that ECM roots had significantly higher $\delta^{15}N$ values than other plant parts, while leaves had significantly higher $\delta^{13}C$ values. Branches and stems had the lowest $\delta^{13}C$ values and, together with coarse roots, had the lowest $\delta^{15}N$ values.

(2) How long does it take for foliar-applied C to move into roots and for soil-applied N to move into the plant? – We tested the suitability of ^{13}C-labeled glucose and bicarbonate as ^{13}C sources applied to the leaves of the seedlings. We found that bicarbonate was not suitable as a ^{13}C source applied into the leaves. However, feeding leaves with ^{13}C-glucose, under controlled conditions, was efficient for labeling above- and below-ground biomass of blue oak seedlings. We discovered that it takes one day for ^{13}C to get into the roots. It probably takes less than 14 days for the ^{15}N to get into the roots and less than 26 to get into the leaves. Translocation of N and C through the plant is fast, even when applying the ^{15}N in soil, where there are other organisms that utilize this element. It would be necessary to sample roots earlier than 14 days to know how rapidly N moves to the leaves.

(3) Will both C and N accumulate in fine and ECM roots? – Yes, N accumulated in fine and ECM-roots and C was found in fine and ECM roots in higher amounts per mg of tissue than in the taproot and coarse roots, although taproots are more directly linked to the C transfer pathway from leaves than are fine roots.

(4) Are ECM roots stronger sinks for both C and N than other plant parts? – Yes, ECM roots acted as strong sinks of C and N but both elements were translocated to other parts of the plants also. In addition to ECM roots, leaves were also strong sinks for C and N, probably due to demand for protein synthesis for leaf development.

In further studies, this tracer methodology combined with morphotyping and molecular identification of the ECM tips, might allow us to know the individual $\delta^{15}N$ and $\delta^{13}C$ values of each fungal species on plant roots. Using these values, we could determine which ECM species supply more N to their host plant. Species with higher $\delta^{15}N$ values may transport a greater proportion of their N to their plant host (Hobbie and others 1999). In response, the plant may reward the fungus and transfer more C to these fungal species. This information about C and N cycling in oak seedlings can be extended to California oak woodlands, and further research on this topic will provide information that could be implemented in designing more efficient management practices for oak ecosystem conservation.

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