Belowground carbon trade among tall trees in a temperate forest

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
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FOREST ECOLOGY

Belowground carbon trade among tall trees in a temperate forest

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Forest trees compete for light and soil resources, but photoassimilates, once produced in the foliage, are not considered to be exchanged between individuals. Applying stable carbon isotope labeling at the canopy scale, we show that carbon assimilated by 40-meter-tall spruce is traded over to neighboring beech, larch, and pine via overlapping root spheres. Isotope mixing signals indicate that the interspecific, bidirectional transfer, assisted by common ectomycorrhiza networks, accounted for 40% of the fine root carbon (about 280 kilograms per hectare per year tree-to-tree transfer). Although competition for resources is commonly considered as the dominant tree-to-tree interaction in forests, trees may interact in more complex ways, including substantial carbon exchange.

Stable carbon isotope labeling at the canopy scale is a powerful tool for tracing carbon allocation in forest ecosystems (*1*, *2*). In a dense forest, large quantities of photoassimilates may be exported to mycorrhiza and rhizosphere microbes (*3*–*11*), and hyphae of mycorrhizal fungi can form "underground highways" for carbon and nutrient exchange with and between plants (*9*). It has been suggested that because of the unpredictability of disturbance events and the divergence of responses among plant communities, mycorrhizal fungi and their host plant species are under selective pressure to evolve generality (*9*, *10*). The groups of plants that are interlinked through a common mycorrhizal network are hence termed "guilds" (*10*). The identity and ensemble of fungal species may affect plant community structure and ecosystem productivity (*12*, *13*), with mycorrhiza improving plant fitness by increasing phosphorus and nitrogen uptake (*14*). As a result, mycorrhizal networks are considered an integral part of the autotrophic system (*15*, *16*) and are essential components in ecosystem resilience to change. Yet, these benefits have traditionally been studied from a nutrient supply perspective, and the mycorrhiza "pipeline" was never shown to transfer considerable amounts (>1 g) of mobile carbon compounds among trees (*4*–*10*). In addition to mycorrhizal networks, carbon can be transferred through natural root grafts, which are anatomical fusions between two or more roots. Growth of interconnected trees in situ can be affected directly by the presence of root grafts—for example, by translocation of water and carbohydrates (*17*). Transport across root grafts has been demonstrated in numerous field studies using various methods, from dye injection to the use of radioactive tracers (*18*, *19*), but these grafts are restricted to trees of the same species or, at most, of phylogenetically closely related species (*17*–*20*).

Using a tall canopy crane (*1*, *2*), we continuously labeled five 40-m-tall Norway spruce trees (*Picea abies*) as part of a 5-year free-air CO_2 enrichment experiment (FACE) in a mixed forest in northwest Switzerland (*3*, *21*, *22*) (figs. S1 to S7). Five unlabeled *Picea* trees served as controls (fig. S8). We then measured $\delta^{13}C$ from "tip to toe," including canopy twigs, stems, and fine roots of labeled and unlabeled individuals of *Picea* and of neighboring trees belonging to different taxa (*Fagus sylvatica*, *Pinus sylvestris*, and *Larix decidua*). Except for the five labeled *Picea*, none of the trees were exposed to CO_2 labeling. Using industrial, ^{13}C-depleted CO_2 gas, our canopy labeling made the $\delta^{13}C$ signal of labeled trees more negative by 5.3 per mil (‰) compared to unlabeled control trees: Twig $\delta^{13}C$ was –31.4‰ in labeled and –26.1‰ in unlabeled *Picea* (Fig. 1). New fine roots of labeled *Picea*, isolated from 90 ingrowth cores (figs. S9 and S10) had 2.6‰ lower $\delta^{13}C$ values than the control trees growing in ambient air (no ^{13}C label) (Fig. 1). Almost the same isotopic signal was found among fine roots of similarly tall nonconspecific trees in the neighborhood that were unlabeled and contributed about half of the fine roots recovered from ingrowth cores (Fig. 2A). To validate that fine roots of the other taxa were not confused with those of *Picea*, we excavated roots from *Picea* (control and labeled) and neighboring tree species and traced them to the trunk of origin (figs. S12 to S14). Again, fine roots of these non-*Picea* taxa showed a ^{13}C signal similar to that of their neighboring *Picea* (either control or labeled) but jointly at a 2.6‰ less negative level when ^{13}C-labeled *Picea* was present (Fig. 2B). Hence, both the root-ingrowth-core data (with multiple individuals' input) and the data for intact root systems from three individuals belonging to three different tree genera yielded the same signals. Sapwood $\delta^{13}C$ of the 2010 to 2014 annual rings in stem cores taken at breast height from neighboring and nonneighboring non-*Picea* trees was –27.8 ± 0.1‰ and –26.9 ± 0.1‰, respectively—still a significant difference (P = 0.019).

Because our FACE system operated in the canopy only (20 to 40 m aboveground), tank CO_2, and thus the ^{13}C label, were not present in the understory. This was ascertained first by ^{13}C signals in understory plants, which are exclusively vesicular-arbuscular mycorrhizal: *Paris quadrifolia*, *Mercurialis perennis*, and *Rubus fruticosus*. $\delta^{13}C$ values in rhizomes/root stocks from these three species growing under both unlabeled and labeled *Picea* showed the typical, very negative signals for deep shade plants (from –30.2 to –34.5‰) (fig. S15). Besides differences among species, however, there was absolutely no signal difference between samples collected under unlabeled and labeled *Picea* and no difference between years. Second, we checked the canopy crowns of the trees neighboring the labeled *Picea* individuals for traces

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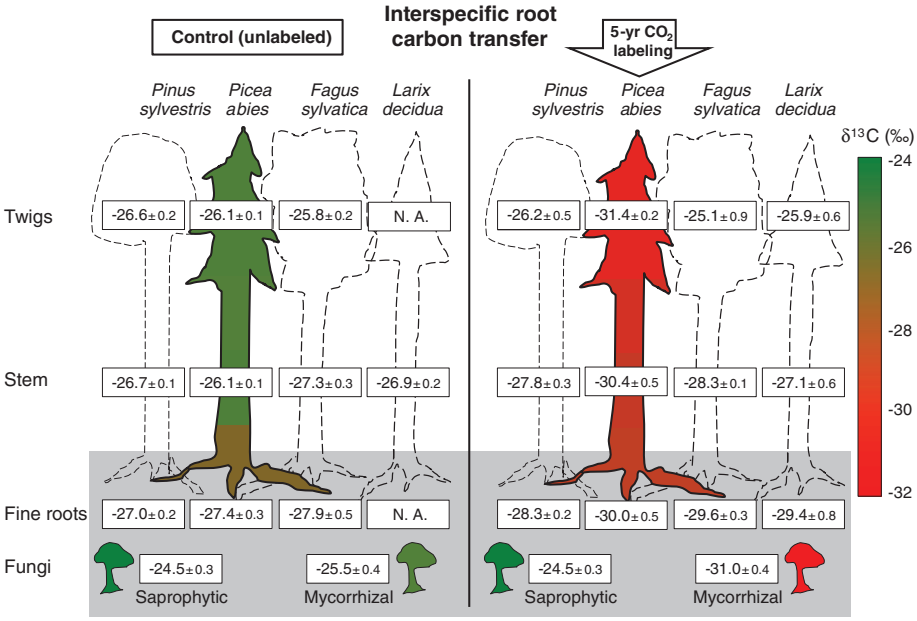

Fig. 1. Transfer of carbon from labeled spruce to fine roots of neighboring nonspruce trees. $\delta^{13}C$ gradients (means ± SE; N = 4 to 5 samples from 1 to 5 trees) within tree compartments and fungal sporocarps in the studied mixed forest stand near Basel, Switzerland. The use of industrial, ^{13}C-depleted CO_2 gas for the FACE allowed for identifying carbon allocation in and from spruce trees exposed to labeled CO_2 ($\delta^{13}C \leq -30.0‰$), compared with other carbon in wood and fungi ($\delta^{13}C > -27.5‰$). Linear gradients were assumed between measurement points.

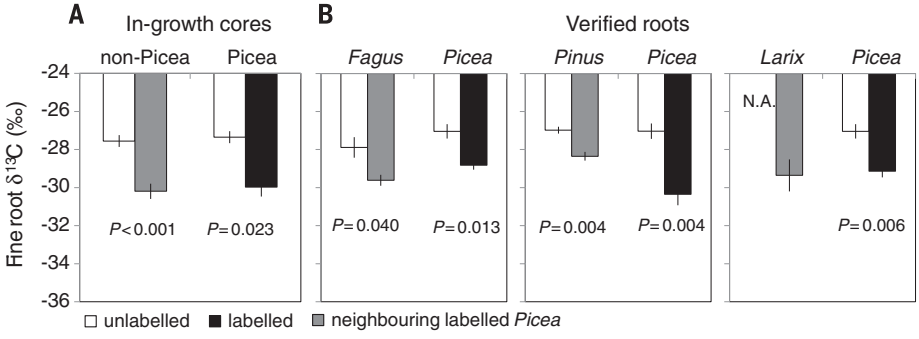

Fig. 2. Fine roots of unlabeled beech, pine, and larch trees carry the isotopic signal of labeled spruce. $\delta^{13}C$ in fine roots from ingrowth cores located in overlapping root spheres of spruce and neighboring tree species (**A**) and from three undisturbed soil volumes circumjacent to roots of spruce trees under labeled CO_2 and neighboring trees of other species (where species were absolutely verified) (**B**). Roots of labeled spruce individuals and of neighboring nonspruce trees had significantly lower $\delta^{13}C$ than trees growing in the same stand that were not exposed to labeled CO_2. Each bar is a mean ± SE of five trees, each with three core triplets (ingrowth cores) and of four root samples from one individual tree (verified roots). *P* values are from analysis of variance. All available *Larix* trees were neighboring the labeled *Picea*, and none were neighboring the unlabeled *Picea* (fig. S8).

of ^{13}C label in 2-year old high-canopy twigs from all four compass directions in each crown. No influence of the ^{13}C label could be found. $\delta^{13}C$ in twig xylem of *Picea* was –26.1 ± 0.1‰ and –31.4 ± 1.1‰ in unlabeled and labeled trees, respectively. In twig xylems of the three neighboring tree species (*Fagus*, *Pinus*, and *Larix*), $\delta^{13}C$ was –25.1 ± 0.9, –26.2 ± 0.5, and –25.9 ± 0.6‰, respectively, as previously observed in these trees (*23*). Only in twigs sampled in the small crown fraction immediately next to the crowns of the labeled *Picea* (<20% of the crown circumference), $\delta^{13}C$ was slightly lowered compared with the aforementioned values (–28.1, –29.2, and –28.8 in twigs of *Fagus*, *Pinus*, and *Larix*, respectively). The remainder of the crown periphery and the crown center showed no label, and hence the overall crown volume that was slightly influenced by the isotope label was <10%. Moreover, the $\delta^{13}C$ values in those proximal twigs were still 2.2 to 3.3‰ above those in the labeled *Picea* twigs.

The tree-to-tree transfer of labeled carbon was so strong in this study that roots of different tree taxa (of which only *Picea* was labeled) shared an almost similar isotopic signature: –30.0‰ and –29.1‰ in labeled and neighbor trees, respectively (Fig. 1). The decrease in $\delta^{13}C$ of unlabeled neighbor roots, and the parallel increase in $\delta^{13}C$ of labeled roots relative to their source tissues (Fig. 1), indicate a bidirectional carbon exchange. To estimate the direction and magnitude of the carbon exchange, we compared the aforementioned $\delta^{13}C$ values with those prevailing without any labeled carbon transfer ("baseline" signatures). We then applied a simple carbon isotope mixing calculation between roots of labeled and neighbor trees using the equation $a \times n + (100 - a) \times m = p$, where a is the contribution of one of two sources to a mixture (in %), n is its isotopic signature, m is the isotopic signature of the other source, and p that of the mixed product. In the neighbor roots, the $\delta^{13}C$ value of –29.1‰ reflects a decrease by 1.7‰ from a mean baseline value of –27.4‰ observed in roots of the same tree species growing around unlabeled *Picea* (Fig. 1). However, a baseline signature in the labeled *Picea* roots is harder to estimate, because we had no reference observation of labeled *Picea* that did not exchange carbon with non-*Picea* neighbors. To this mixing, one must add an intrinsic dilution by the contribution of older, unlabeled carbon to current fine root growth (*3*, *24*). We do know that in control *Picea*, $\delta^{13}C$ of roots was 1.3‰ more negative than that of the canopy twigs (Fig. 1) (a common observation), and hence a premixed baseline for the labeled *Picea* root would be –32.7‰ (–31.4 minus 1.3‰). Thus, our isotope mixing calculation had to solve for a mixing ratio that would satisfy (i) a 2.7‰ increase in the labeled *Picea* root signal (–30.0 minus –32.7‰); (ii) a 1.7‰ decrease in the neighbor root signal; and account for (iii) the intrinsic dilution ratio with old stored carbon. We found that a 20% contribution of older, unlabeled carbon to current fine-root growth of labeled *Picea*, and an isotope-mixing ratio of 60% self and 40% exchanged carbon between fine roots of labeled and unlabeled trees, satisfied the ^{13}C signal changes at both sides of the transfer (Fig. *3*).

The magnitude of the exchange can be estimated: *Picea* fine-root biomass production estimated from our ingrowth cores (*11*) was 60 g m^{-2} a^{-1}

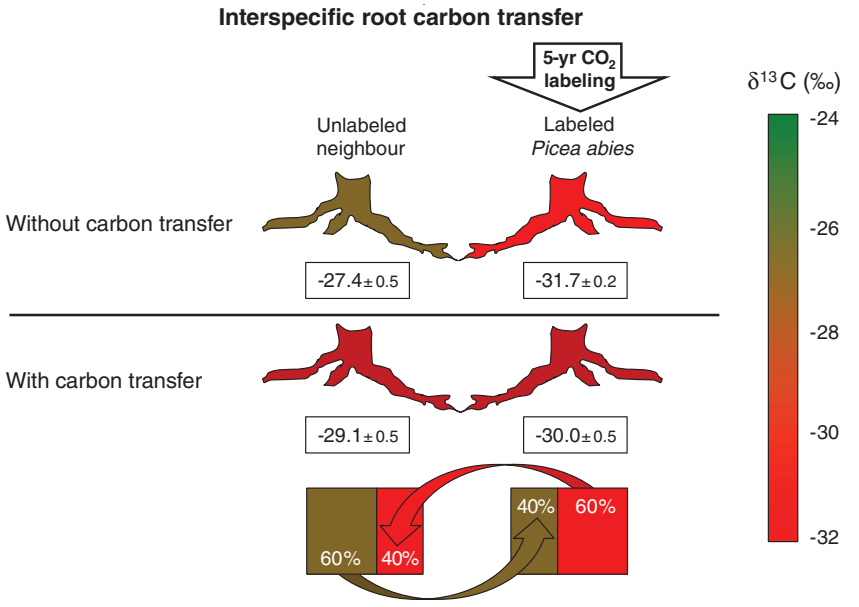

Fig. 3. Bidirectional root carbon transfer between mature forest trees. Estimation of the magnitude of the interspecific root carbon exchange in the studied mixed forest stand based on the observed $\delta^{13}C$ values. An isotope mixing ratio of 60% self and 40% exchanged carbon between fine roots of labeled and unlabeled trees satisfies the ^{13}C signals in both.

at 1- to 12-cm depth, upscaled linearly to 150 g m^{-2} a^{-1} for the entire 30-cm soil profile. Assuming an average root carbon concentration of 46%, this corresponds to a fine-root production of 69 g carbon m^{-2} a^{-1}. If 40% of this fine-root carbon came from an exchange via mycorrhiza, this carbon transfer flux equals 28 g carbon m^{-2} a^{-1}—i.e., 280 kg ha^{-1} a^{-1}, which is equivalent to 4% of the forest net carbon uptake (net primary production).

The carbon transfer that we observed most likely occurred through common ectomycorrhiza networks, which are very abundant at this site (table S1), and also exhibited the labeled carbon in their "fruit" bodies near labeled *Picea* (*3*) and are a substantial carbon sink in Norway spruce forests (*16*). Host specificity is a known trait among ectomycorrhiza taxa, yet common networks and the formation of trophic guilds play a crucial role in forest dynamics (*25*). For example, in a mixed Central European forest, 75 ectomycorrhiza taxa were identified on *Fagus sylvatica* roots (*26*); 29% and 10% of the ectomycorrhiza species were shared with one or two other tree species, respectively; however, it is noteworthy that the 61% host-specific ectomycorrhiza species colonized only 20% of the root tips (*24*). The ectomycorrhiza species *Russula ochroleuca* (Pers.) has been previously identified on roots of all four tree species studied here (*27*), and a *Russula* species was identified in our forest site (table S1). A taxonomic search in the ectomycorrhiza database (www.deemy.de) (*28*) revealed three other genera found at our site that are common symbionts to our four study tree species—namely, *Cortinarius*, *Lactarius*, and *Tricholoma*.

Our earlier study on this site (*3*) also showed zero $\delta^{13}C$ labeling in saprophytic fungi (Fig. 1) and decreasing mycorrhizal $\delta^{13}C$ with decreasing distance from the labeled *Picea*. Our results indicate a bidirectional carbon exchange (Fig. 3) rather than a one-way transfer (*17*), which is not along a demand-supply gradient as previously reported (*10*, *17*). Considering that all studied trees were dominant, healthy, and tall individuals, growing without obvious carbon limitation, no a priori source-sink gradients might be expected here (*29*). It has been suggested that carbon transfer between trees via mycorrhiza is rather regulated to satisfy the needs of the mycorrhiza itself (*7*). In our case, it is still possible that labeled *Picea* transferred excess carbon belowground (*3*) and, in turn, enhanced mycorrhizal activity and proliferation.

The mild, yet significant increase in sapwood $\delta^{13}C$ at the base of trees neighboring the labeled *Picea* (Fig. 1) indicates slight aboveground allocation of imported carbon. So far, root carbon uptake was shown in "green-to-ground" corn and in willow cuttings using labeled carbonate ($NaH^{14}CO_3$ and $H^{13}CO_3^-$) (*31*), as well as in pine seedlings (*30*), but not in mature trees in the field.

Finally, the observed interspecific carbon transfer among tall trees in our study can become increasingly important for forests under stress conditions (e.g., drought or spring frost) or after disturbance such as wildfire, when divergence in species' responses come into play (*5*–*10*, *14*, *20*, *23*). The magnitude, direction, and control of these transfer fluxes and their importance are yet to be resolved, and they add a new dimension and level of complexity to known ecosystem processes.

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SUPPLEMENTARY MATERIALS

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