Supplementary Information for the manuscript:

Ectomycorrhizal fungi mediate belowground carbon transfer between pines and oaks

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Supplementary Information

Supplementary Methods

Verification of ^{13}C in mycorrhizal DNA using UPLC- Tandem MS

To overcome the GC bias in DNA-SIP (see Discussion), we used a sensitive method[75] to quantify the ^{13}C enrichment of each nucleic acid. Briefly, after DNA-SIP fractionation, 20 µl of each fraction were sent to further analysis at the metabolomics unit (Targeted Metabolomics, Life Sciences core facilities, Weizmann Institute of Science, Rehovot, Israel). Each sample was treated with 95 µl of 88% formic acid to depolymerize the nucleic acids via hydrolysis. Next, samples were dried and suspended in 40 µl of a UPLC mobile phase solution. Standards were prepared for each nucleobase. The ^{13}C-enriched isotopic isomers were quantified by multiple reaction monitoring modes and were compared to the standards. An integral of the area of each enriched atom was calculated and converted into pg ng-1 using standards and normalizing to the DNA quantity in each sample, allowing for the quantitative comparison among samples of different DNA concentrations. DNA extracts (20 µl) were evaporated in a freeze-dryer, then treated with 100 ul of 88% formic acid at 70 °C for 2 hours to hydrolyze DNA into nucleobases as described by Wilheim et al. 2014. The obtained hydrosylates were dried entirely with nitrogen flow at 40 °C, then re-suspended in 100 ul of 0.05% formic acid and centrifuged (21,000 g, 5 min) to remove insoluble impurities. The soluble parts were placed in 250-µl glass inserts and analyzed by LC-MSMS.

LC-MS/MS

The instrument consisted of an Acquity I-class UPLC system (Waters) and Xevo TQ-S triple quadrupole mass spectrometer (Waters) equipped with an electrospray ion source and operated in positive ion mode was used for the analysis of nucleobases. MassLynx and TargetLynx software (version 4.1, Waters) were applied for the acquisition and analysis of data. The chromatographic separation was performed by using Cortecs T3 column (2.1 x 150 mm, 1.6 µm, Waters) at 30°C and gradient of mobile phase A (0.1% formic acid) and B (methanol) as following: 0-2 min, a linear increase from 0.5 to 10% B, 2-3 min, a linear increase from 10 to 70% B, 3-3.3 min, a linear increase from 70 to 100% B, 3.3-3.5 min held at 100% B, 3.5-4.0 min back to 0.5% B and equilibration at 0.5% B for 3 min. The flow rate was 0.3 ml/min, injection volume 2 ul. Standard curves were build using a mix of adenine, guanine, thymine, and cytosine (Sigma-Aldrich). MS parameters: capillary voltage 0.40 kV, cone voltage 15 V, collision gas 0.20 ml min^{-1}.

Supplementary Results

Soil respiration proxy in soil compartments

As root sampling is a destructive measurement that disturbs the soil and mycelium hypha, we measured the ratio between ^{13}C and ^{12}C in the soil compartment as a proxy for soil respiration (Fig. S7). Further, we tested whether an increase in the root tissue ^{13}C correlates to an increase in the soil ^{13}C signature. An increase in the donor ^{13}C in the soil compartments occurred during the 3rd day of labeling and peaked at the 4th day (18.2 ± 8.7%) then declining to values similar to unlabeled control treatment (1.38 ± 0.11% in donors, 1.10 ± 0.02% in unlabeled controls). In the recipient soil compartments, the peak occurred at day 5 (2.66 ±1.30%), gradually declining to unlabeled control levels at day 36 (1.15 ± 0.04%). A linear regression equation was established comparing these respiration proxy values for days where elevated ^{13}C was observed between donor and recipient compartments (days 3-7, $R^2 = 0.64$, $F_{(1,80)} = 147.5$, $p < 0.001$; Fig. S8).

Enriched DNA directly identified in the labelled fractions

The allocation of DNA across the density gradients reflects the influences of both isotope incorporation and Guanine plus Cytosine (GC) nucleic acids content since the density of DNA increases with its GC content [66]. Consequently, any comparison of density regions will reflect both the effects of (1) isotope incorporation (i.e., whether the organism exploited the labelled isotope substrate and incorporated it into its DNA) and (2) the organism's relative GC content. Therefore, we used an additional analysis employing a UPLC-MS/MS protocol on the same fractionated samples of the donor library that were sequenced to ensure the incorporation of ^{13}C atoms within the DNA of the sequenced organisms. In this analysis, each nucleobase: Adenine, Guanine, Cytosine and Thymine was examined separately (nucleobase). The enrichments of +0 ^{13}C atom were removed because the large quantity of signal masked the effect of the enriched atoms; On the other edge of the spectrum, enrichments of +5 ^{13}C atom were removed because peaks were unclear (See for Guanine: Fig. S11; for Adenine: Fig. S10). For Guanine and Adenine, an enrichment of +1 ^{13}C atom per nucleobase (5.9% natural variation) was observed in pre-and post-labeling (Fig. S10 and Fig. S11). However, enrichment of +2, +3, and +4 ^{13}C atoms was found only in post-labeling samples. Also, a more significant concentration of all the enrichment atoms was found in the heavier fractions.

Carbon in the donor plant explains the amount of carbon transfer to the recipient plant

We used a set of linear regressions to test which physiological plant properties best explain the amount of ^{13}C signal found in the recipient trees (Fig. S9). For each donor and recipient pair, the $\delta^{13}C$ signal was calculated by summing the above-natural $\delta^{13}C$ values (>-24‰) across all sampling days for recipient and donor treatments and multiplying by the total root biomass of each plant. The

84 explaining variables tested were shoot height, diameter, and branching; leaf transpiration, leaf
85 photosynthetic assimilation, whole-plant carbon uptake, shoot biomass, root biomass, and total
86 biomass (the ratios of the shoot, root and total biomass of donor and recipient treatments were also
87 examined but are not shown). The highest regression value was found for $\delta^{13}C$ in the donor trees
88 (Fig. S9) ($R^2 = 0.80$, $F_{(1,6)} = 24.03$, $p = 0.002$). All other variables did not produce R^2 higher than 0.5.
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Supplementary Tables and Figures

Tables

Table S1. Soil tests result from three random samples after mixing 50% forest soil and 50% sand (v/v).

Lime (%)	Sand (%)	Silt (%)	Clay (%)	pH	Electric Conductivity (dS/m)	Na (mg/l)	Ca (mg/l)	Mg (mg/l)	N (mg/kg)	P (mg/kg)	SAR
11	84	11	5	7.7	0.8	1.8	138.0	16.2	5.4	3.4	0.9
11	83	8	9	7.7	0.8	1.7	129.3	15.2	5.4	3.0	0.9
11	82	10	8	7.7	0.8	1.8	137.3	15.2	5.9	3.0	0.9

Table S2. Height, diameter, and branching measurements were taken when transplanting the saplings (Ph and Qc n=150), and following the labeling (Ph and Qc n=15)

		Height (cm)	Diameter (mm)	Branching
Transplanting 24.12.20	Ph	40 ± 7	6 ± 1	23 ± 7
	Qc	43 ± 10	7 ± 1	4 ± 2
Labelling 27.7.20	Ph	79 ± 14	11 ± 1	25 ± 7
	Qc	75 ± 27	10 ± 1	14 ± 6

Table S3. Results of pairwise specific contrasts (using Mood's median test) for the summed $\delta^{13}C$ values in roots of the recipient and control treatments. The *p* values were corrected using an FDR correction (p. Adjust function, stats package). Significance codes: '**' 0.01, '*' 0.05.

	Days	**Median control**	**Median recipient**	**χ2**	**df**	***p* values**	**FDR adjusted *q* values**
	0	-27.5	-28.0	3.00	1	0.083	0.131
	4	-27.5	-25.5	0.25	1	0.617	0.679
	5	-27.5	-22.5	6.67	1	0.010**	0.036*
	7	-27.0	-22.0	4.06	1	0.044*	0.080
	9	-27.0	-20.0	4.06	1	0.044*	0.080
Roots	11	-27.0	-20.0	2.25	1	0.134	0.163
	14	-27.0	-19.5	6.67	1	0.010**	0.036*
	18	-26.0	-24.5	0.25	1	0.614	0.614
	22	-27.0	-24.0	4.65	1	0.031*	0.080
	28	-26.0	-24.5	6.67	1	0.010**	0.036*
	36	-26.0	-21.0	2.25	1	0.134	0.163
Stem	18	-26.5	-24.0	4.06	1	0.043*	

Table S4. Results of multiple t-tests for each compound, enrichments level and fractions comparing day 0 and day 9 (n=4). The *p* values were corrected using an FDR correction (p.adjust function, stats package) Significance codes: '**' 0.01, '*' 0.05.

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Compound	Enrichments	Fraction	*p* value
Adenine	1	10	0.0481*
Adenine	2	11	0.0083**
Adenine	2	12	0.0481*
Guanine	2	10	0.0481*
Guanine	3	10	0.0481*
Guanine	2	11	0.011**
Guanine	2	12	0.0469*
Guanine	3	12	0.0481*

Supplementary Figures

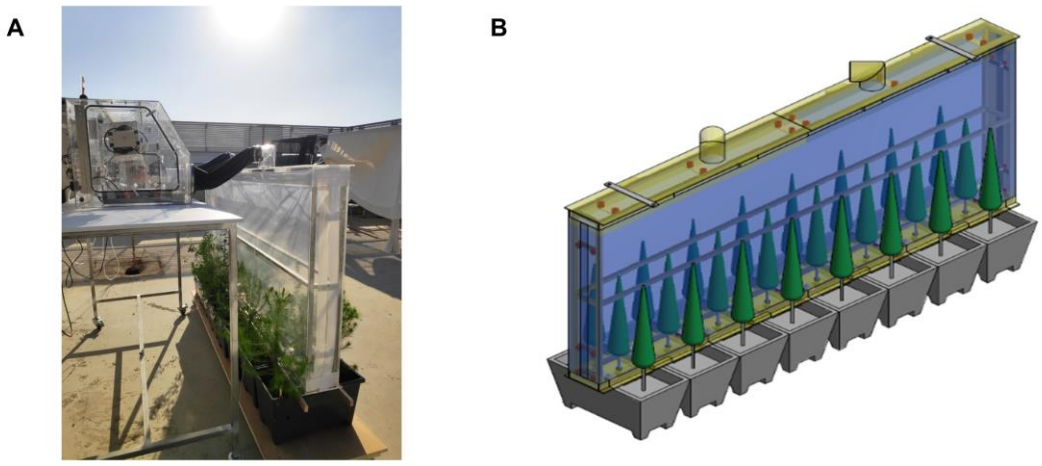


Figure S1. (a) the $^{13}CO_2$ plant labeling system; notice the glovebox connected to a custom-built enclosure around Donor crowns. (b) a scheme of the enclosure box.

Figure S2. The presence of extensive mycelia in the root systems of study trees. In this example, white mycelia are observed around roots of a Recipient *Pinus halepensis* sapling from the experiment, facing the Donor compartment.

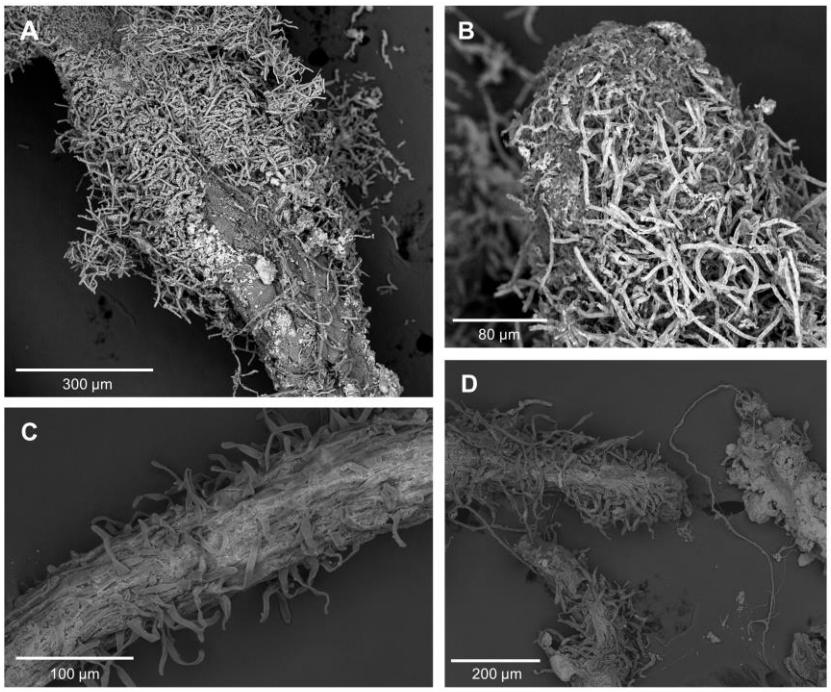


Figure S3. SEM images of fine root tips that were chosen for the DNA-SIP fractionation and sequencing. (a, b) pine root tips; (c, d) oak root tips

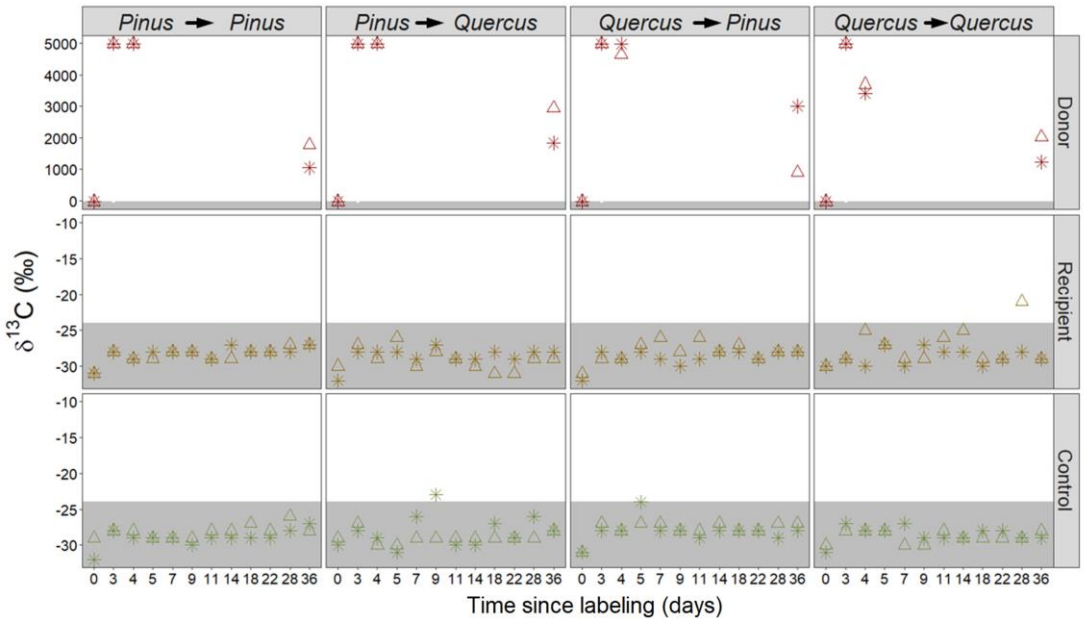


Figure S4. Leaf $\delta^{13}C$ of three treatments: Donor, Recipient and Control. Triangles and asterixis denote different biological repeats (n=2) of each pair combination. Panels represent pair combinations (Donor and Recipient). Grey area marks the $\delta^{13}C$ natural variation of -24‰ and below.

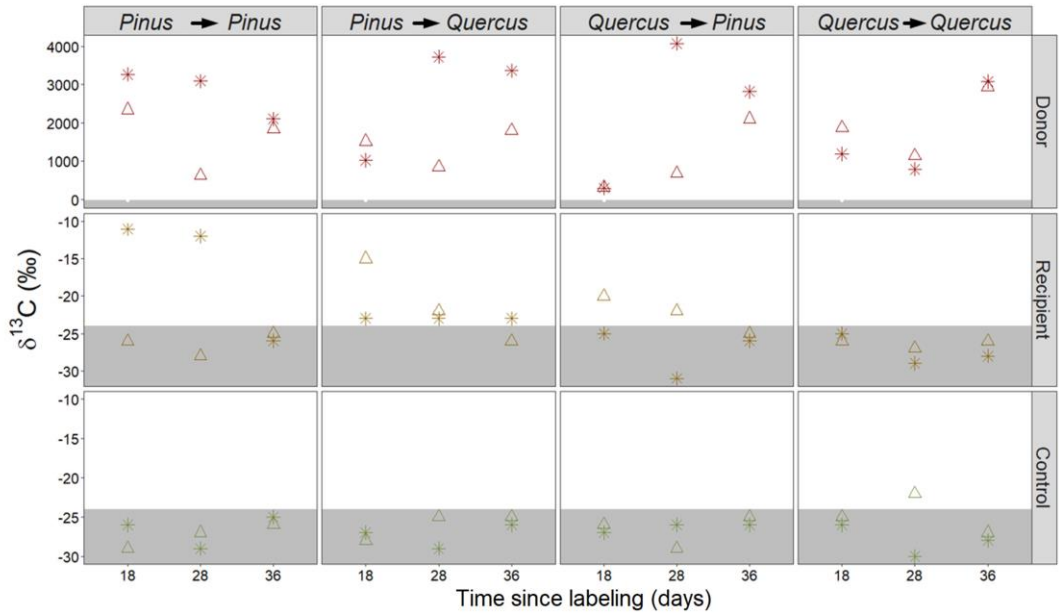
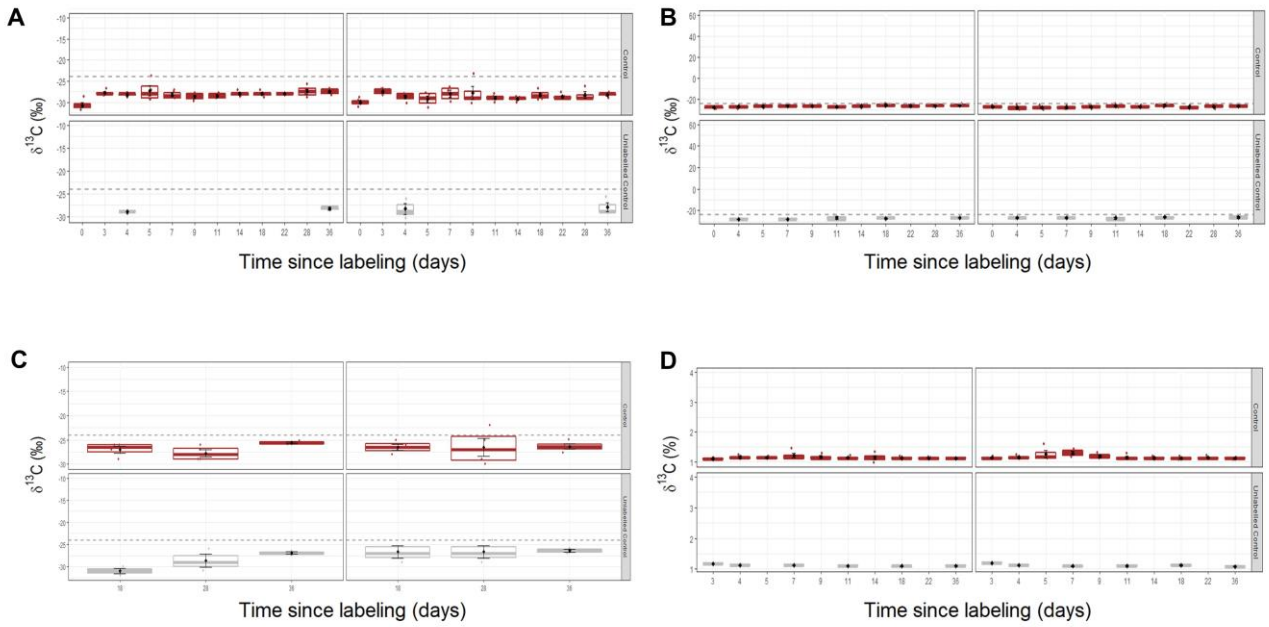


Figure S5. Stem $\delta^{13}C$ of three treatments: Donor, Recipient and Control. Triangles and asterixis denote different biological repeats (n=2) of each pair combination. Panels represent pair combinations (Donor and Recipient). Grey area marks the $\delta^{13}C$ natural variation of -24‰ and below.



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119 Figure S6**.** (a) Leaf (b) root (c) stem and (d) ^{13}C in the soil compartments of two treatments: control
120 and unlabeled control. Bold circles denote the average of biological repeats, (n=2) unlabeled control,
121 and (n=4) control. Panels in each plot represent the specie tested (*Pinus*, right; *Quercus*, left). Dashed
122 line marks the $\delta^{13}C$ natural variation of -24‰.

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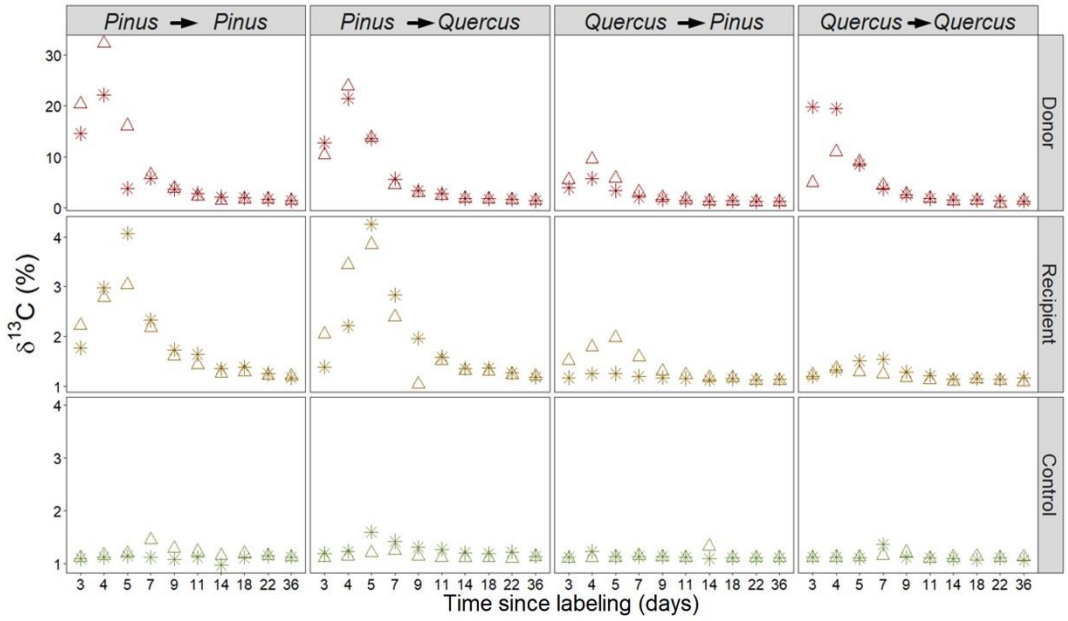


Figure S7. Soil respiration $\delta^{13}C$ of three treatments: Donor, Recipient and Control. Triangles and asterixis denote different biological repeats (n=2) of each pair combination. Panels represent pair combinations (Donor and Recipient).

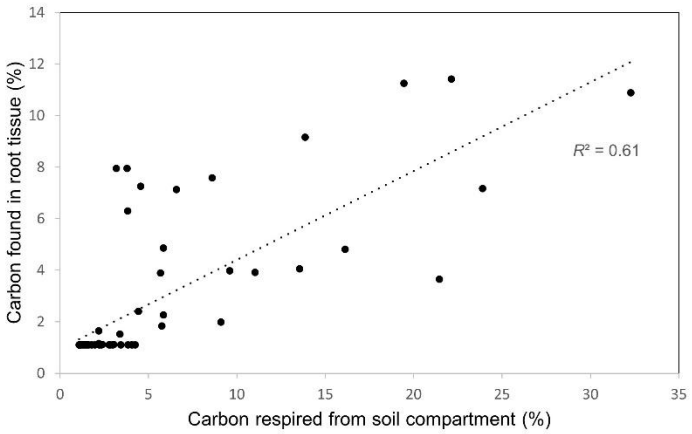


Figure S8. Carbon found in recipient root tissue in days 4, 5 and 7 as a function of respiration proxy. Days 4, 5 and 7 were chosen because of elevated ^{13}C was observed in these specific days. Both variables were measured by CRDS. The regression line represented by the dashed line, the R^2 and p values of the model appear in the graph.

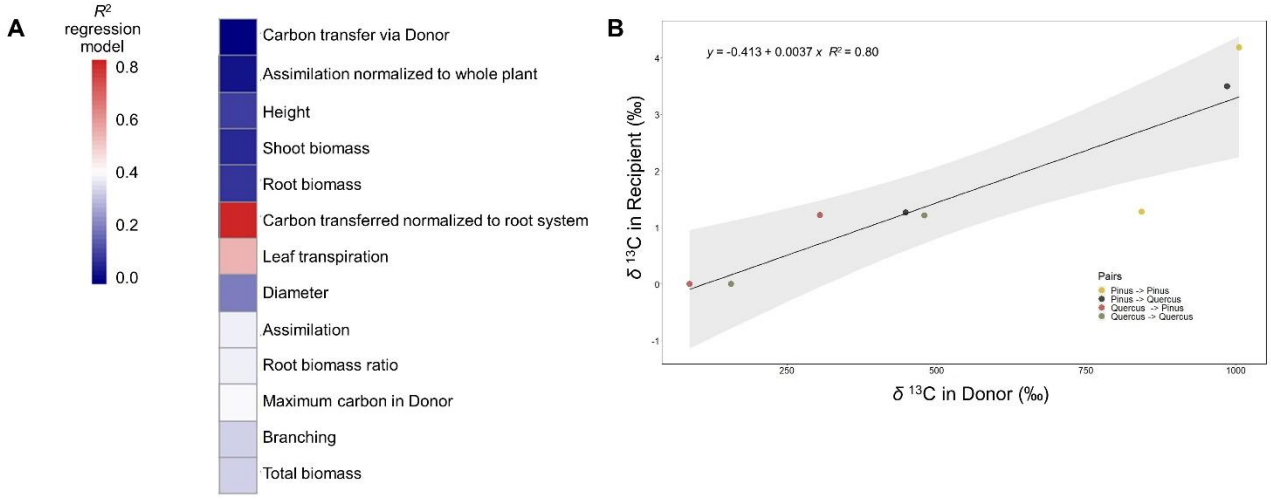


Figure S9. (a) Heat map summarizing R^2 of linear regressions between recipient root ^{13}C signal and multiple attributes measured. (b) The amount of carbon integrated up scaled to the root system size; the graphic result of the highest R^2 value. It is calculated by summing the values that are above natural $\delta^{13}C$ across all sampling days for recipient and donor treatments and multiplying by the total root biomass of each plant.

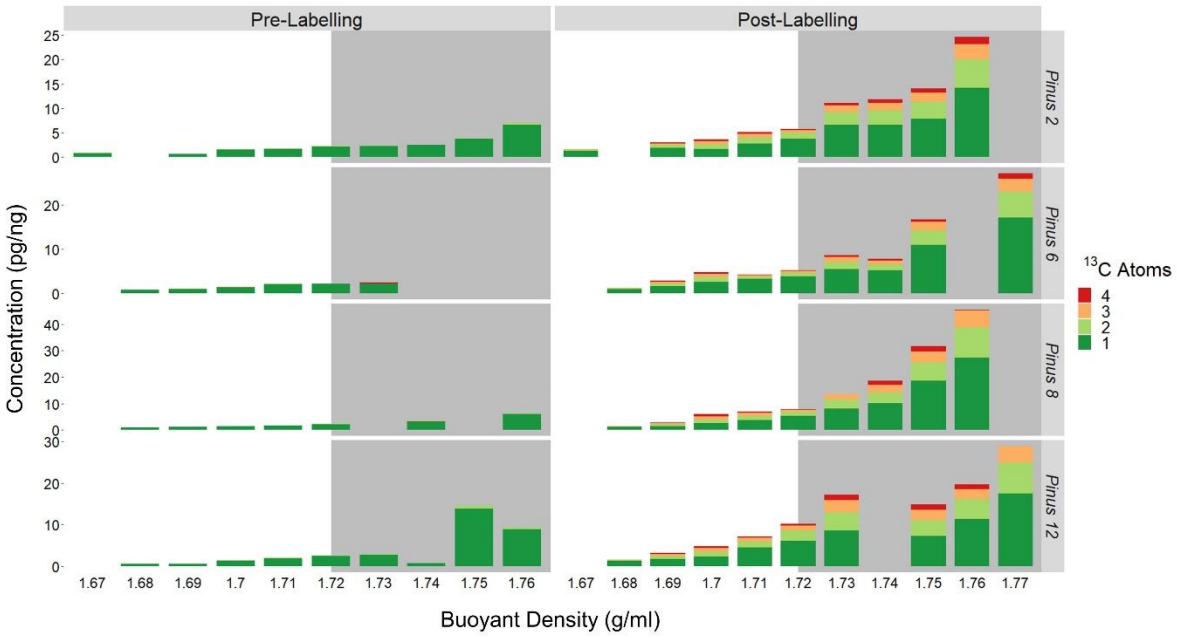


Figure S10. Concentration of ^{13}C atom enrichment for Guanine as a function of buoyant density measured for each fraction. Each panel is shows SIP gradient fractions pre- and post-labelling, from the same pine saplings that were sequenced (n=4). Grey area highlights which fractions ^{13}C-DNA drift to after ultracentrifugation (buoyant density between 1.72-1.77 g/ml), whilst white area denotes where ^{12}C-DNA drift to (1.67-1.72 g/ml). The gradient color represents the number of ^{13}C atoms found in Guanine nucleobases

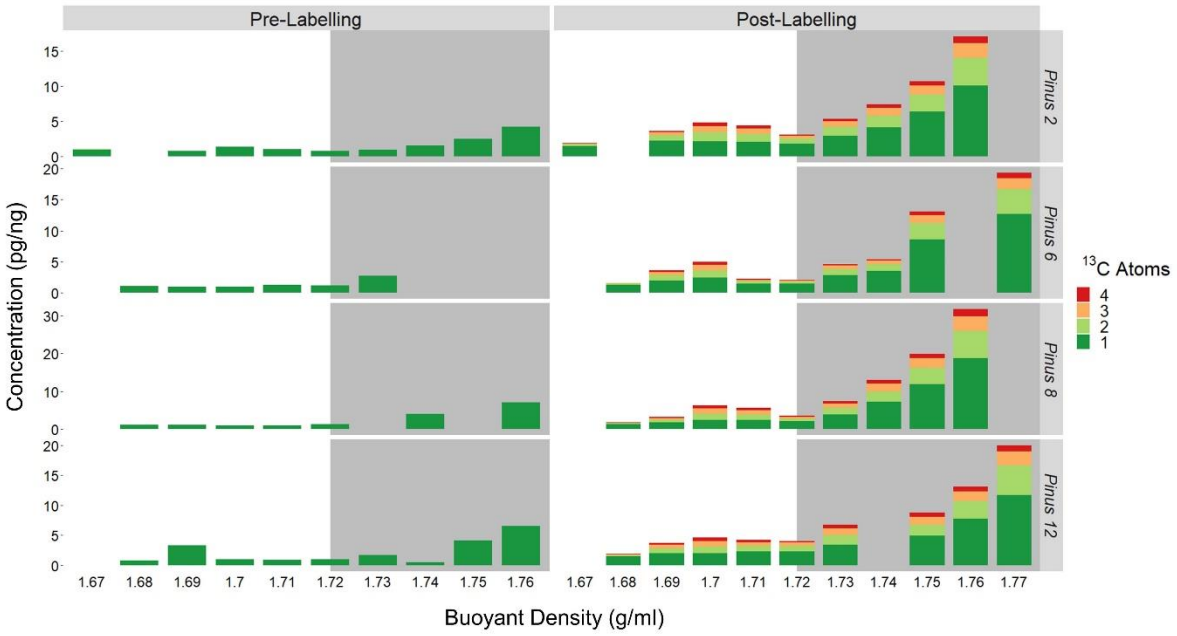
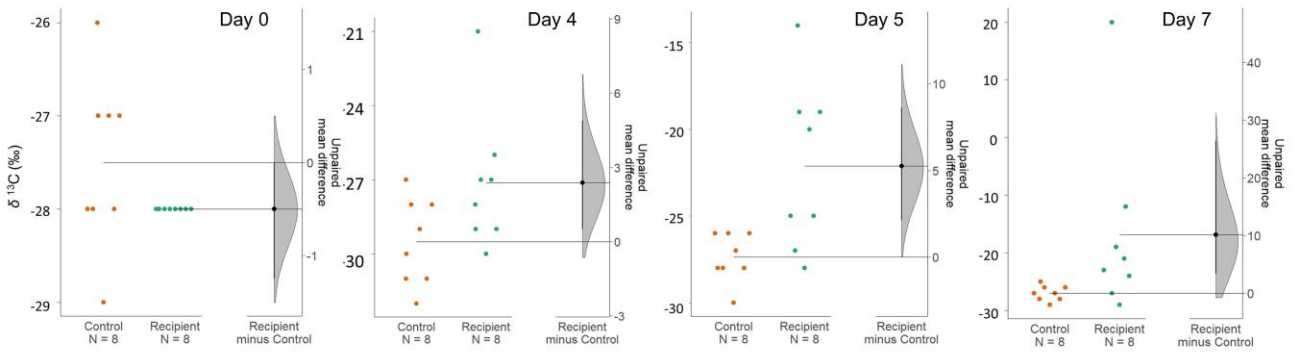


Figure S11. Concentration of ^{13}C atom enrichment for Adenine as a function of buoyant density measured for each fraction. Each panel is shows SIP gradient fractions pre- and post-labelling, from the same pine saplings that were sequenced (n=4). The grey area highlights the ^{13}C-DNA labelled fractions 1.72-1.77 (g/ml), while the white area represents the ^{12}C-DNA unlabeled fractions 1.67-1.72 (g/ml). The gradient color represents the number of ^{13}C atoms found in Guanine nucleobases


Day 0
Day 4
Day 5
Day 7
δ 13C (‰)
Unpaired mean difference
Control N = 8
Recipient N = 8
Recipient minus Control

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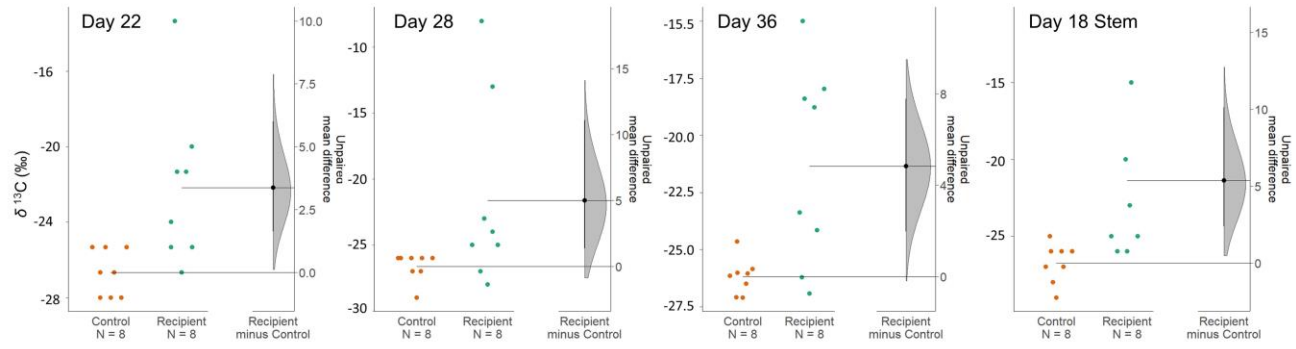
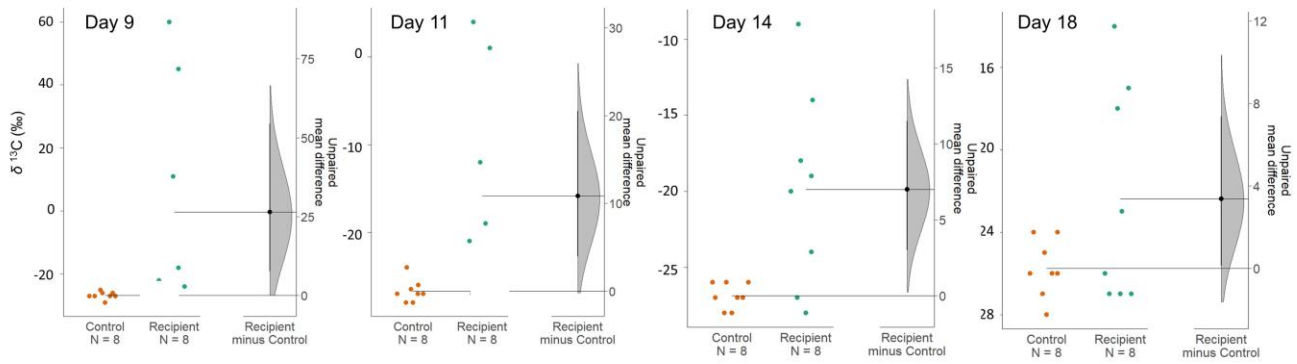

Day 22
Day 28
Day 36
Day 18 Stem
δ 13C (‰)
Unpaired mean difference
Control N = 8
Recipient N = 8
Recipient minus Control

158



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160 Figure S12. Estimation plots for specific contrasts between the root tissues $\delta^{13}C$ of the control and
161 recipient compartments throughout all measurement days. A specific contrast for stem tissues in day
162 18 is presented in the bottom right panel. The mean difference (the effect size; produced using the R
163 package dabestr) and its 95% confidence interval (based on a BCa bootstrap) is displayed as a point
164 estimate and vertical bar respectively, on a separate but aligned axes.