The Fate of Amino Acid in Soil Experiments: Bacteria, Roots and Fungi

Melissa Campbell
Clark University '11

Advisor: John Hobbie
The Ecosystem Center at the
Marine Biological Laboratory
in Woods Hole, MA

December 17, 2010

Abstract

Organic nitrogen (ON) is the most common form of nitrogen in the soil. If this form is the most abundant then many new questions must be addressed in order to fully understand the breakdown of ON such as what organisms are incorporating the nitrogen, the rate of uptake and the effect of concentration on uptake rate. Many measurements have been made regarding the uptake rate of amino acid in soil using radioactive isotopes, however many experiments use only one relatively large concentration. Concentration greatly affects the results and uptake rate by soil, microorganisms and plant roots, and organisms behave differently when different concentrations of free amino acids are present. In soil, bacteria are the most abundant at 0.7×10^9, and roots (largely with mycorrhizal fungal associations) comprise 1.2% of soil content. Five different concentrations (100nM, 1, 10, 100, 150 μM) of 14-C labeled leucine were tested to determine the uptake rate in roots with and without mycorrhizae, and roots from cucumber plant grown in the lab in sterile medium (coarse sands) to prevent endomycorrhizal symbiosis. Roots with mycorrhizal associations are able to take up the greatest amount of amino acid and therefore have an advantage over roots without mycorrhizal associations. Roots without mycorrhizae and cucumber roots took up much less amino acid than the roots with mycorrhizae at all concentrations. In nature, it is likely that most free amino acids in soils are taken up by microorganisms (including fungi) or become hidden in soil micropores, leaving plants starved of free amino acid. Concentrations must be chosen with great concern when assessing uptake rate of amino acid in soil, roots and microorganisms because different results will form if different concentrations are used.

Key Words and Phrases: 14-C leucine, amino acid, nitrogen, uptake rate, roots, bacteria

Introduction

Nitrogen is an important nutrient for all plants and the majority of nitrogen in the soil is in the organic form (Hawkes, 2007). This indicates that this aspect of the nitrogen cycle is important for further understanding of soil nutrient exchanges in addition to understanding which organism are able to take up ON. Furthermore, understanding of the molecular form of organic nitrogen, the rates of use and the effect of concentration on uptake rate are all ecological questions that arise when confronted with evolving nitrogen paradigms.

Dissolved organic nitrogen (N) and particulate organic nitrogen (N) are present as proteins within the soil. Proteins become broken down by enzymes into free amino acids that are available for uptake in the soil. Microorganisms and roots engage in strong competition over free amino acids within the soil (Jones, 2005a) and of which is able to incorporate the most free amino acid is ultimately in control of the nitrogen cycle as well as the carbon cycle (Hawkes, 2007). Furthermore, the depth of soil at which the roots and microorganisms interact

also greatly affects which obtains the greatest amount of nitrogen. Within the rhizosphere, roots have an affect on nutrient cycling (Ridder, 2005) as they remove nutrients from the soil and incorporate them into the shoots for growth (Hawkes, 2007). However, microbes are highly active and abundant at this depth of the soil (Ridder, 2005) and therefore are in complete control of N cycling within the soil (Hawkes, 2007). Researchers suggest that within the highly active rhizosphere, bacteria and soil microbes are equipped with the necessary breakdown tools to out-compete plant roots for the free amino acids (Ge, 2009). My experiment was performed on soil samples from the competitive rhizosphere soil layer to measure the uptake rate of amino acid in roots with and without mycorrhizal associations.

Research on nitrogen cycling has come a long way from the classical paradigm to the new paradigm (Schimel, 2004). The classical paradigm (Schimel, 2004) showed soil proteins depolymerized by enzymes to monomers, that is as amino acids, and taken up by microbes (Hobbie, 2010). Plants take up inorganic forms of nitrogen that are excreted or egested from microbes and are easily outcompeted by soil microbes for nitrogen (Hawkes, 2007). The idea of the new nitrogen cycle paradigm (Schimel, 2004), which shows that plants are able to directly incorporate organic nitrogen (ON) amino acid, has created awareness and stimulated research in the scientific community (Schimel, 2004). The new paradigm comes from experiments carried out in hydroponic culture with plant roots in nutrient solution or in laboratory flasks in which recently harvested roots of plants are incubated for short periods with isotope-labeled amino acids.

Many researchers have studied the uptake of amino acids into plants (Nasholm, 2008) but have tested at only one very large microMolar concentration (generally 100-150 μM). Obviously the concentration of amino acid in the experiment should be the same as the concentration available to the plants in the soil. Although the natural concentration available in soil is unknown, it is assumed that the concentration of amino acid available is several hundred microMolar. Studies conducted by Wright and Hobbie (1966) on phytoplankton and bacterial amino acid uptake competition indicate that concentration matters. The uptake process of bacteria became saturated with glucose at very low concentrations but the algae were able to continuously take up more and more glucose as concentrations increased. The idea is that biological membranes allow the diffusion of glucose through cells throughout increasing concentrations. At larger concentrations (2 mg Glucose/L) the bacteria were clearly outcompeted by the algae. However at much lower and more natural concentrations (0.1 mgGlucose/L) the bacteria out-compete the algae for monomer resources. The results that show concentration matters in aquatic experiments can be translated to the importance of measuring the uptake rate of amino acid in soil experiments.

My study focused on the effect of different experimental concentrations on amino acids for uptake into plants, fungi and bacteria. Further analysis was also conducted on uptake rate of roots from cucumber plants grown in a sterile medium (coarse sand) to prevent endomycorrhizal associations. The new 2010 nitrogen paradigm places emphasis on amino acids being incorporated more into bacteria and fungi and less directly into roots. The fungi that are associated with roots (mycorrhizal roots) allow roots to incorporate amino acids. In addition to the importance of concentrations is the difference between plant roots, plant roots with mycorrhizal association, and plant roots grown in a sterile medium.

It is understood that amino acids cross the membranes of cells in roots, but how the rate of uptake will vary with substrate concentration (i.e., leucine) is not well understood. Studies suggest that plants are able to take up amino acid more efficiently and sustainably than roots with mycorrhizal associations and bacteria (Neff, 2003). Hobbie and Hobbie (in press) debate this idea claiming that soil microbes and bacteria are in far greater abundance and more efficient at breaking down soil protein for this to be accurate. Furthermore, they state that leucine would most likely become incorporated into bacteria before plant roots could incorporate it.

Methods

Study Site

Soil samples were collected from the Woods Hole Oceanographic Institute (WHOI), School Street Woodlands (See image appendix) in Woods Hole, Massachusetts (41.31°N, 70.39°W). Soil samples were taken from the woodlands under ectomycorrhizal tree species such as black oak (*Quercus velutina* Lam.) and white oak (*Quercus alba* L.). I used a tulip soil cutter to collect sample from the top ten centimeters of the rhizosphere and for each experiment performed, I collected a fresh soil sample the same day. The study site was the closest woodlands to the laboratory in order to obtain the freshest soil and root samples for experiments. The average bulk density of the top ten centimeters of soil is 0.0023 g/cm^3. The top soil is comprised of 98% soil and 1.2% mycorrhizal roots.

Bacterial Count

One gram of soil, with roots separated, was suspended in a 20 mL scintillation vial with 15 mL of phosphate buffered saline and one mL of glutaraldehyde and then shaken (200 rpm) for 30 minutes. The sample was then sonicated for 10 minutes on low and then shaken for another 30 minutes (Ridder-Duine, 2005).

The solution was left to settle for 40 minutes before 100 µL of the suspended solution was transferred to 1.7 mL microcentrifuge tubes with 1.8 mL of phosphate buffered saline and 100

µL of 4',6-diamidino-2-phenylindole (DAPI). The tubes were incubated in the dark for 10 minutes before being filtered onto a 0.2 µm black polycarbonate filter (Millipore GS) with a 47 mm glass microfiber backing filter. The sides of the filter tower were washed with phosphate buffered saline to ensure that the entirety of the sample was filtered. The Millipore filter was placed on a microscope slide with a drop of immersion oil and covered with a cover slide that had a drop of immersion oil on both sides. Bacterial counts were performed under a Zeiss epifluorescence microscope at 100-x magnification.

14-C Labeled Leucine Root Uptake

Root uptake of 14-C labeled leucine was measured for five different µM concentrations (0.1, 1, 10, 100, 150) from three different root types with blanks killed at time zero and experiments run for one time point; 30 minute incubations.

Three different types of roots were used for the experiments and soil samples containing roots were collected and processed within 40 minutes of collection. The types of roots used were roots with mycorrhizae attached, roots with mycorrhizae removed and roots from cucumber plants grown in the lab in a sterile medium (See image appendix). Furthermore, the roots used were all approximately the same size in diameter and 2.5 cm in length.

Freshly collected soil samples were sifted under running DI water using a 500 µm sieve to obtain fine mycorrhizal roots. All roots were observed and measured under a Zeiss Stemi-2000 microscope. Roots that needed the mycorrhizae removed were also observed under the Stemi-2000 using fine bent-nose tweezers (Zeller, 2007).

The cucumber plants were grown in sand that was collected from Stony Beach, a beach in Woods Hole, Massachusetts owned by the Marine Biological Laboratory. The sand was sifted through a 5.6 mm sieve followed by a 2 mm sieve to obtain coarse sand. The sand was rinsed to remove salt, autoclaved for one hour and then rinsed again to further remove salt. Plants were watered with DI for one week until seedlings began to sprout. Hoagland's solution was then used for the duration of the experiment, five weeks, to keep the plants alive.

Three sets for three 1.7 mL microcentrifuge tubes were prepared for each root type for each concentration experiment. The roots that were used as blanks were dried in a drying oven at $50^{\circ C}$ for 24 hours. The roots were then soaked in 100% cold , vortexed well, and incubated for one hour before performing the experiment. All roots were suspended and vortexed in microcentrifuge tubes containing 14-C labeled leucine, non-radioactive leucine, and Hoagland's solution. Roots were washed thoroughly with 50 mM potassium chloride (KCL) (Warren, 2009), transferred and crushed in 20 mL glass scintillation vials with Teflon lined caps (Fisher Scientific, Fair Lawn, NJ) containing 500 µL of 95% scintillation compatible ScintiGest tissue solubilizer (Fisher Scientific, Fair Lawn, NJ). Ten mL of ScintiSafe 30% scintillation cocktail (Fisher Scientific,

Fair Lawn, NJ) was added to the glass scintillation vials, vortexed and incubated for 30 minutes before the samples were counted on the Beckman-Coulter LS 6500 scintillation counter.

14-C labeled leucine Uptake in Bacteria

In order to effectively study the rate of uptake in soil by roots, fungi and bacteria, I made use of results of 14-C leucine uptake in bacteria from a paper by Rose Smith, a Semester in Environmental Student in 2008 at the Marine Biological Laboratory in Woods Hole. Her results are from her experiment conducted in the Oak Control soil warming plot at Harvard Forest in Petersham, Massachusetts. The experimental methods involved adding 14-C labeled leucine to the soil for 14 hours and then recording the 14-C in the soil and bacteria and the 14-C in the 14-CO_2.

Bacterial Production (Supplementary)

One gram of soil, with roots separated, was suspended in a 20mL scintillation vial with 15mL of deionized water (DI) and shaken for 30 minutes at 350 RPM. For each sub sample 500µL of the slurry was utilized and centrifuged on low for two minutes. Then 300µL of the suspended liquid from each sub sample was used for bacterial production.

The bacterial production experiment was performed with 150 µL concentrations. Blanks were killed with 100 uL 100% cold Trichloroacetic acid (TCA) prior to the addition of leucine. All samples were incubated with 14-C labeled leucine in addition to non radioactive leucine and Hoagland's solution (nutrient rich solution) for 30 minutes on ice. After the incubation, the samples were washed and centrifuged with 1 mL of 5% cold TCA then 1 mL of cold 80% ethanol. The supernatant was disposed of between washings. Samples were then left under the hood to dry for two to four hours to limit quenching due to ethanol residue. When the samples were dry, 1 mL of ScintiSafe scintillation cocktail was added to each tube and counted using the Beckman Coulter LS-6500 scintillation counter.

Results

Soil Content and Bacterial counts

The biological content of the soil is largely bacteria with a total count of 0.7×10^9 bacteria per cubic centimeter (cm^3). Total root content is almost all roots with mycorrhizal associations, with some root tip exposed. Furthermore, total root content make up approximately 1.2% of dry weight content of soil and 0.0027 g/cm^3 of soil dry weight in rhizosphere (Table 1). Soil (includes bacteria and microbes) and other contents such as twigs and rocks make up the rest, and soil accounts for 0.2191 g/cm^3 dry weight of the content within the rhizosphere (Table 1).

14-C Labeled Leucine Uptake in Roots

The cucumber roots, which were grown in a lab in a sterile medium, showed uptake at all concentrations and the roots were able to incorporate more leucine with increasing concentrations (Figure 1). At the greatest concentration, 150 µM, cucumber roots incorporated approximately 550 pmol 14-C labeled leucine (Leu)/hr/g dry root, and less than 100 pmol Leu/hr/g dry root at the lowest concentrations. Overall, the sample trend of leucine incorporation increases with increasing concentration. The killed blank cucumber root at the highest concentration did not take in as much leucine as the sample at the same concentration and therefore was effectively killed.

Roots with mycorrhizae attached incorporated tremendous amounts compared to the cucumber roots (Figure 2). All samples incorporated more leucine with increasing concentrations in a linear trend. At the greatest concentration of 150 µM the roots with mycorrhizae were able to take up approximately 1300 pmol Leu/hr/g dry root, and the lowest concentrations roots incorporated less than 200 pmol Leu/hr/g dry root. Roots with mycorrhizae at the lowest concentrations were able to incorporate more leucine than the cucumber roots at the same concentrations. The killed blanks for roots with mycorrhizae were effective and incorporated less than 100 pmol Leu/hr/g dry root except for the killed blank at 150 µM. The killed blank at 150 µM incorporated approximately 1250 pmol Leu/hr/g dry root, which was only approximately 50 pmol Leu/hr/g dry root less than the samples.

Roots with mycorrhizae removed showed the lowest incorporation of all root types (Figure 3). The trend stays consistent throughout all root types in which the root incorporates more leucine with increasing concentrations. At the greatest concentration of 150 µM, one sample was able to take up approximately 230 pmol Leu/hr/g dry root, however the uptake of samples at the greatest concentration was only slightly greater than the killed blank at that concentration. The killed blanks at all concentrations were never greater than the samples or the averages of the samples.

The trends of all experiments show that all root types are able to incorporate more leucine as concentrations increase (Figure 4). The y-axis label is measured in terms of pmol leu/hr/cm^3 dry weight in order to compare with dry weight soil; however, the trends do not change. Roots with mycorrhizae incorporate a considerably amount more than the other root types. Cucumber roots are able to take up more than roots without mycorrhizae, even though there is no endomycorrhizal association with the cucumber roots. Roots with mycorrhizae removed are roots from the sample site; they are unable to incorporate nearly as much as the same roots with the mycorrhizae attached.

14-C Labeled Leucine Uptake in Bacteria

Results from experimental work done by Rose Smith, 2008 indicate that when leucine was added to the soil for 14 hours, most of the soil was incorporated by soil micropores or microorganisms (See figure appendix). The rest of the leucine, approximately 15%, was respired by microorganisms. Furthermore, my supplementary results of bacterial uptake of 14-C leucine at the greatest concentration (150 µM), as measured as protein formation indicate that bacteria are able to take up approximately 4.5 x 10^{13} pmol Leu/hr/ cm^3 dry weight soil, which is orders of magnitude more than roots with mycorrhizae attached (See figure index). Considering that soil (with microorganisms) make up majority of the soil content, and using Smith's (2008) results in addition to my supplementary bacterial uptake results, it is evident that the majority of the amino acid added to the soil is being taken up my microorganisms or becoming lost within the soil micropores.

Discussion

Soil Content and Bacterial counts

The rhizosphere is comprised mostly of soil with tremendous amounts of bacteria therefore giving bacteria the advantage of incorporating free amino acids over roots within the rhizosphere, which contains relatively low amounts of free amino acids (Jones, 2005a). Although roots make up a small percentage of soil content, roots affect the nitrogen and carbon cycle within the rhizosphere, creating a slight imbalance for microbes (Hawkes, 2007). Furthermore, microbial death also provides free amino acids that plants are able to take up. Most roots however are symbiotic with fungi creating another imbalance within the soil. Fungi are able to take up more free amino acids than plant roots and give processed nitrogen to the root in exchange for sugars although the carbon and nitrogen exchanged is processed and lower quality (Hawkes, 2007).

14-C Labeled Leucine Uptake in Roots

Cucumber plants are able to effectively incorporate greater amounts of leucine at increasing concentrations even though the cucumbers were grown in a lab in sterile medium (coarse sandy soils). This prevented the cucumber from forming any endomycorrhizal associations with fungi and therefore preventing what would have been the aiding of leucine uptake.

Roots with mycorrhizal associations have the advantage over roots without mycorrhizal associations because the symbiotic relationship of roots and fungi produces a large amount of very fine fungal hyphae. These hyphae have a tremendous surface area so uptake can be large. Also, hyphae give off enzymes for protein breakdown. Therefore it is advantageous to form plant roots to form symbiotic relationships with fungi in order to obtain more amino acids than

the plant root could incorporate on it's own due to the strong competition between plants and microbes in the soil (Warren, 2009).

Concentration matters in terms of amino acid uptake rate by roots and bacteria, even though the concentration found in nature is unknown (Warren, 2009). At low concentrations, plants are not as competitive for amino acid uptake and plant roots without mycorrhizal associations are the least competitive. However, the roots with mycorrhizal associations are able to be more competitive at the low concentrations due to the ability to incorporate monomers easier (Hawkes, 2007).

As concentrations increase in experimental conditions, so does the competition for free amino acids (Jones, 2005a). In Rose Smith's (2008) results, the ultimate fate of amino acid in soil is the incorporation by soil micropores or soil microbes. However the capacity for amino acid uptake by microbes becomes saturated as concentrations increase (Ge, 2009). This quick saturation causes plants with and without mycorrhizal associations to become more competitive for amino acids (Ge, 2009) and roots begin to show greater preference for certain types of nitrogen, primarily inorganic (nitrate and ammonium) (Warren, 2009).

Nitrogen paradigms are evolving as more research is conducted and scientists come to consensus on new findings. Scientists are well aware that bacteria have the advantage for amino acid uptake (Jones, 2005a) and are well aware that under certain conditions plants are able to short circuit the nitrogen cycle and directly take up monomers instead of waiting for inorganic form (Neff, 2003). However, scientists have published papers with results similar to the cucumber results obtained in this experiment and claim that plants are able to outcompete soil microbes for free amino acids (Schimel, 2004). The experimental methods performed by scientists generally use one large concentration of amino acid (Hobbie and Hobbie, in press) and perform the experiment in a laboratory setting (Ge, 2009). The concentrations used in the experiments, generally chemical measurements of 100 to 150 μM, are much too high and unnatural therefore saturating bacteria and roots that will continuously take up the free amino acid (Hobbie and Hobbie, in press). Furthermore, at such large concentrations the amino acid may be diffusing through the membrane into the organism due to the diffusion gradient rather than to active transport.

The most recent published papers regarding amino acid uptake by plants are largely limited by the use of one large concentration (Ge, 2009). My study on uptake rate with various 14-C leucine concentrations bring up interesting questions that must be answered in order to fully understand the dynamics of the nitrogen cycle. Root and microbe amino acid uptake varies greatly with concentration and sheds new light for scientists exploring this topic. In order to yield the most accurate and natural results, great care must be taken when determining concentrations for use in amino acid uptake in soil experiments.

Further questions that must be addressed when exploring the newest nitrogen paradigm (microbes take up more free amino acid than plant roots) are the pathways of use, the rates of use, and the effect of concentration on uptake rate.

Conclusions

The fate of amino acid in soil is to be largely absorbed by soil micropores or soil microorganisms. In terms of roots, roots with mycorrhizae attached are able to take up the most leucine at any of the concentrations observed. Due to the unknown amount of natural leucine in soil, I was unable to answer my original question of the real fate of amino acid in soils, however my results cast doubts on conclusions reached by many researchers that test uptake at only one large concentration. Overall, my experiments shed light on an important limitation that many published results have and that is the use of only one concentration when assessing uptake of amino acid in soil. Many more experiments regarding the concentration of amino acids that are available to microbes in soils must be developed in order to truly understand and answer pressing questions about the ever changing nitrogen paradigm.

Acknowledgments

Thank you first and foremost to Ken Foreman for directing such a rewarding program. Additional thanks for Zoe Cardon and Suzanne Thomas for advice and expertise on plant growth, Will Daniels for his patience in assisting with experiments and calculations. And unconditional thanks to Dr. John Hobbie for his patience, enthusiasm and support.

References

Ge, Tida., Song, S., Roberts, P., Jones, D.L., Huang, D., Iwasaki, K. 2009. Amino acids as a nitrogen source for tomato seedlings: The use of dual-labeled (^{13}C, ^{15}N) glycine to test for direct uptake by tomato seedlings. Environmental and Experimental Botany; 66: 357-361

Hawkes, C.V. 2007. The Rhizosphere; Ch.1 Root interactions with soil microbial communities and processes. Elsevier Publishing, USA.

Hobbie, J.E., Hobbie, E.A. 2010. Amino acid cycling in plankton and soil microbes studied with radioisotopes: measured amino acids in soil do not reflect bioavailability. Biogeochemistry

Jones D.L., Shannon, D., Thippaya, J.F., Farrar, J.F. 2005a. Plant capture of free amino acids is maximized under high soil amino acid concentration. Soil Biology and Biochemistry; 37: 179-181

Jones D.L., Kemmitt, S.J., Wright, D., Cuttle, S.P., Bol, R., Edwards, A.C. 2005b. Rapid intrinsic rates of amino acid biodegradation in soils and unaffected by agricultural management strategy. Soil Biology and Biochemistry; 37: 1267-1275

Neff, J., Chapin, S.F. III., Vitousek, P.M. 2003. Breaks in the Cycle: Dissolved Organic Nitrogen in Terrestrial Ecosystems. Frontiers in Ecology and the Environment; 1(4):205-211

Nasholm, T., Ekblad A., Nordin A., Giesler, R., Hogberg, M., Hogberg, P. 1998. Boreal Forest Plants Take up Organic Nitrogen. Journal Nature; Vol. 392:30

Nasholm, T. kielland, K., Ganeteg, U. 2009. Uptake of organic nitrogen by plants. New Phytologist; 182: 31-48

Ridder-Duine, A. , Kowalchuk G., Gunnewiek P., Smant W., Veen J., Boer W. 2005. Rhizosphere bacterial community composition in natural stands of *Carex arenaria* (sand sedge) is determined by bulk soil community composition. Soil Biology and Biochemistry 37: 349-357

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology; 85:591–602
Schimel, J.P., Chapin, S.F. 1996. Tundra plants uptake amino and NH4+ nitrogen in Situ: Plants compete well for amino acid N. Ecology; 77(7): 2142-2147

Warren, C.R. 2009. Does Nitrogen Concentration Affect Relative Uptake Rates of Nitrate, Ammonium, and Glycine. Journal of Plant Nutrition and Soil Science; 172: 224-229

Wright R., Hobbie J.E. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology; 47(3): 447-464

Zeller, B., Brechet, B., Maurice, J.P., Letacon, F. 2007. ^{13}C and ^{15}N isotopic fractionation in trees, soils and fungi in a natural forest stand and a Norway spruce plantation. Annals of Forest Science; 64(4): 419-429

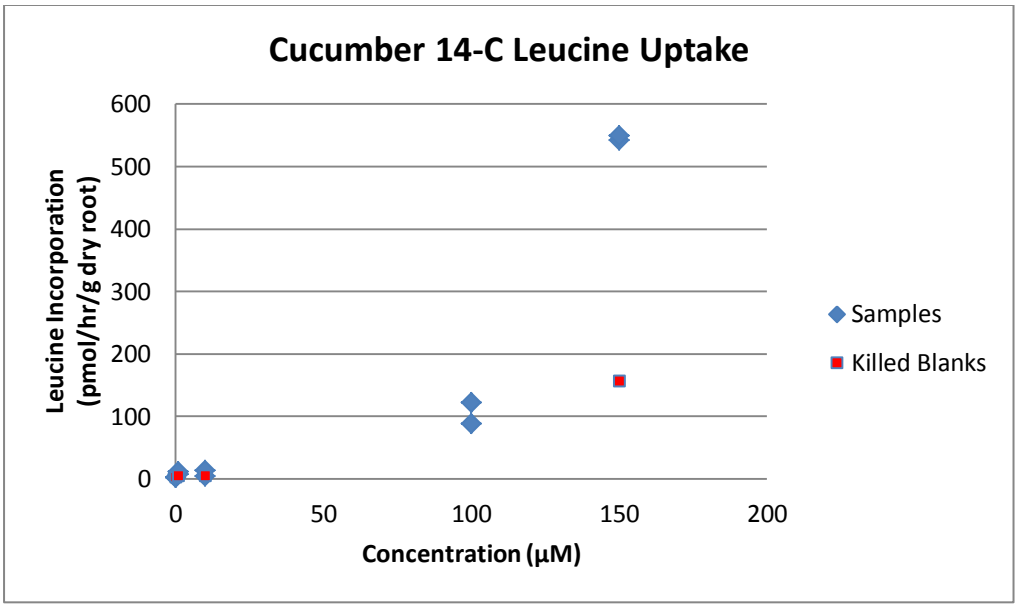


Figure 1. 14-C labeled uptake into cucumber roots from cucumber plants grown in lab in sterile medium, figure includes cucumber root killed blanks.

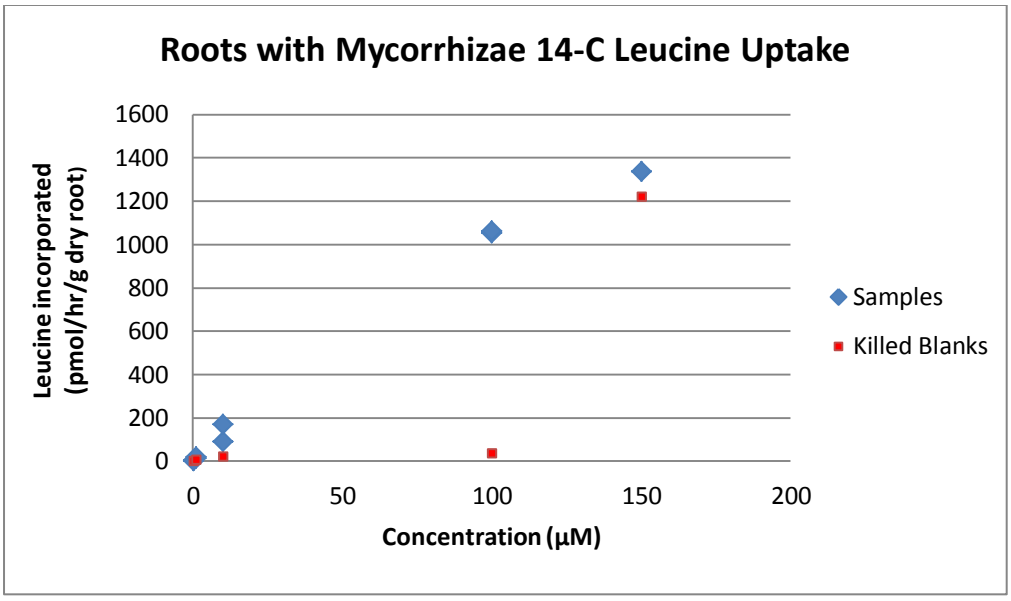


Figure 2. 14-C labeled leucine into oak roots with mycorrhizae attached. Soil samples from the WHOI School Street woodlands study site.

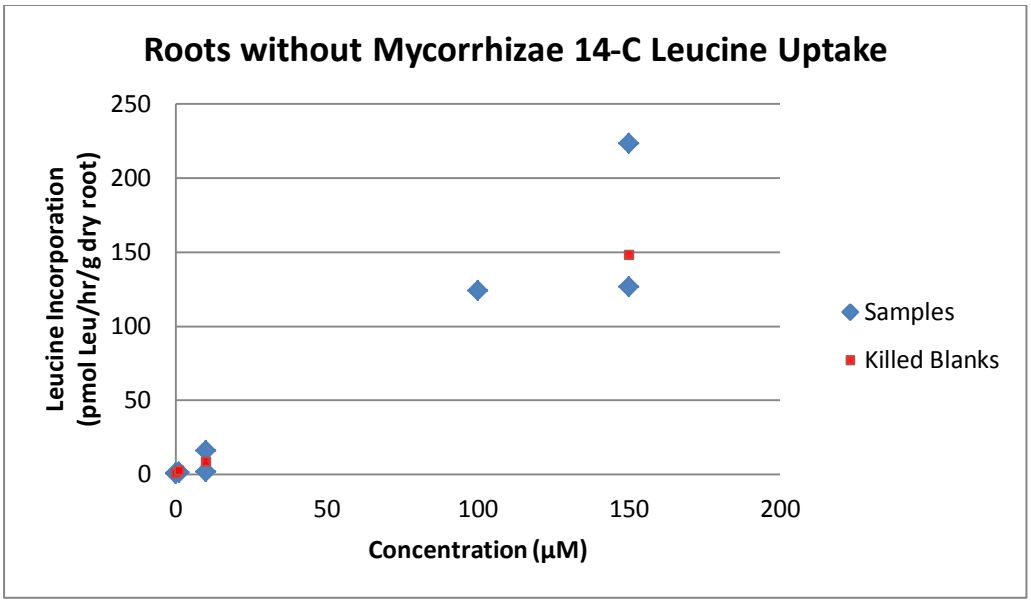


Figure 3. 14-C labeled leucine uptake into roots with mycorrhizae removed collected from soil samples from the WHOI School Street woodlands study site.

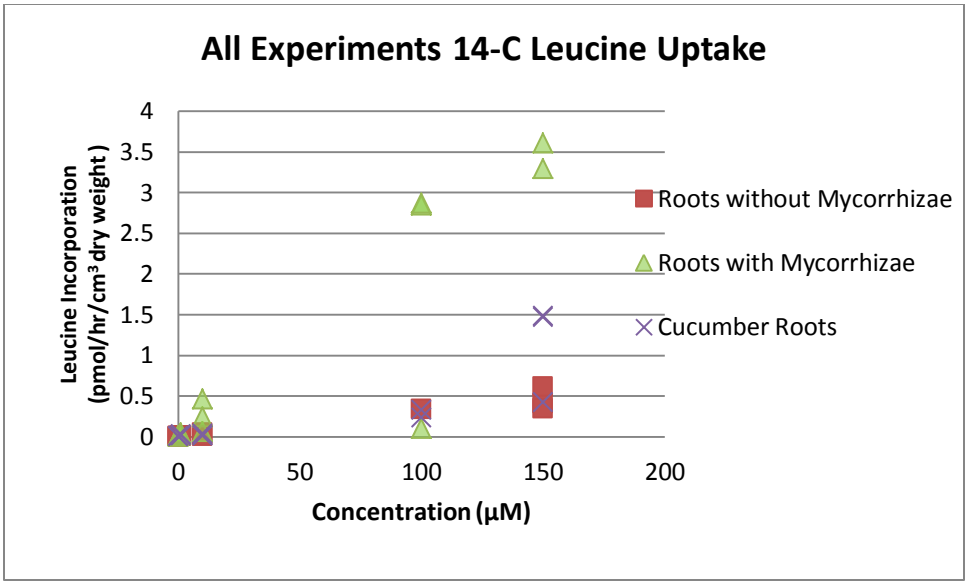
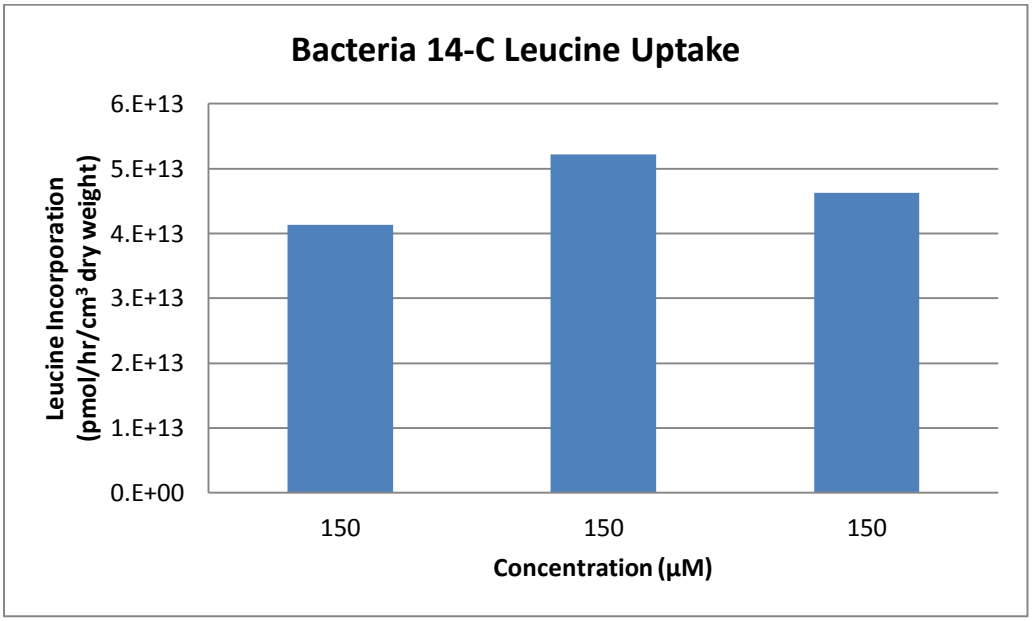


Figure 4. 14-C labeled leucine uptake results in all experiments; leucine incorporation units are in pmol/hr/cm^3 dry weight.

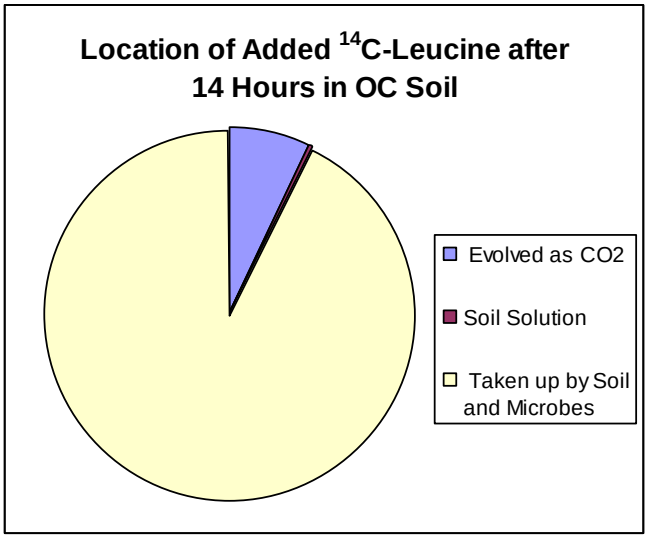
Table 1. Soil content of soil samples obtained from the WHOI School Street woodlands study site.

	Dry Weight (g/cm3)	Percent present in dry weight soil (%)
Soil (g)	0.2191	98.6
Roots (g)	0.0027	1.2

Figure Appendix

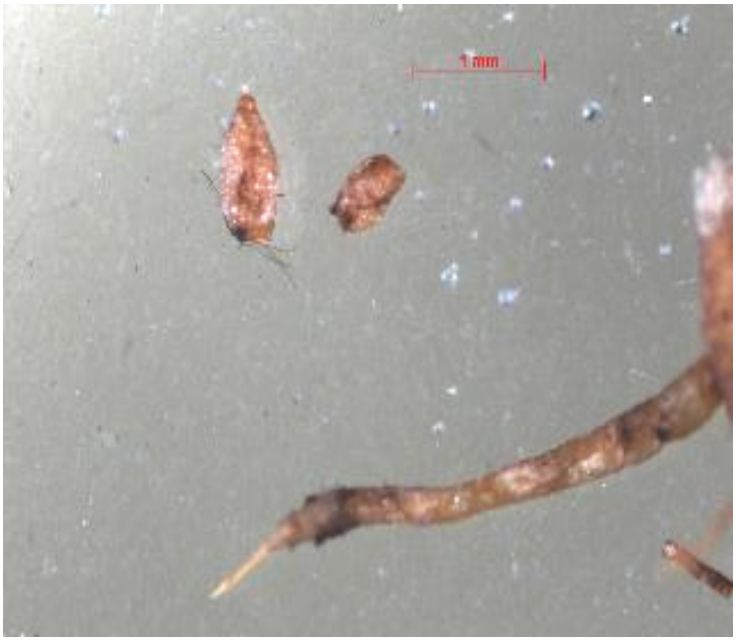


14-C labeled leucine uptake in bacteria collected from soil samples from the WHOI School Street woodlands study site.

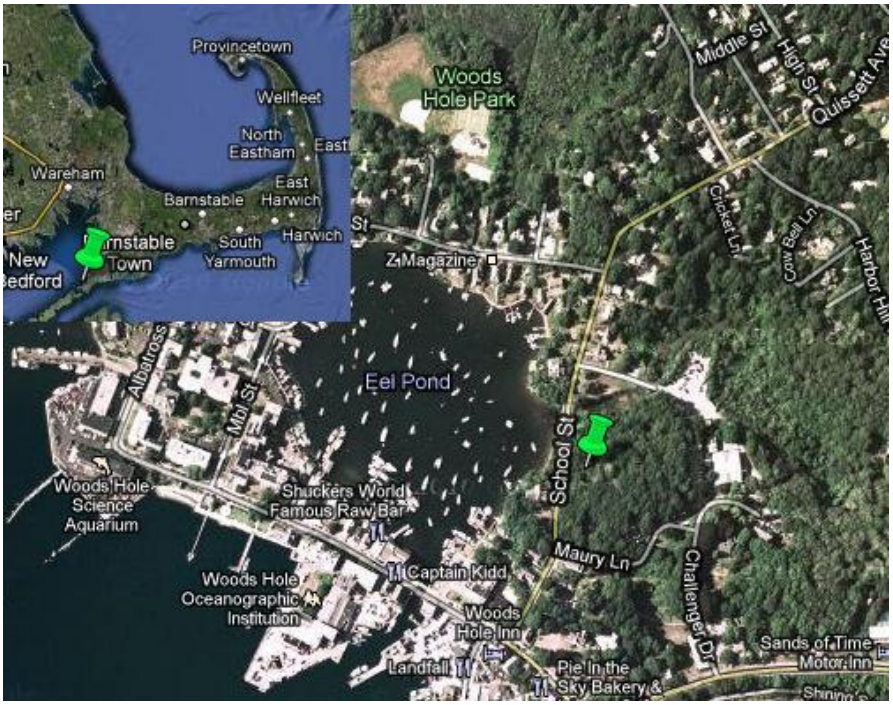


14-C labeled leucine incorporation after incubation in soil for 14 hours at the Oak Control plot in Harvard Forest, Massachusetts.
(Figure courtesy of Rose Smith SES, 2008)

Image Appendix



Roots with mycorrhizae attached in addition to a root tip with fungal sheath removed
(Photo property of Bianca Kissel SES, 2006)



Study Site located at the Woods Hole Oceanographic Institute School St. woodlands in Woods Hole, Massachusetts

(Photo from Google Maps, google.com)

Cucumber plants grown in the lab in sterile medium consisting of coarse sands