Advancing Understanding of the Role of Belowground Processes in Terrestrial Carbon Sinks through Ground-penetrating Radar: Final Report (DE-SC0008099)

PI: Frank P. Day, Old Dominion University

Executive Summary

Coarse roots play a significant role in belowground carbon cycling and will likely play an increasingly crucial role in belowground carbon sequestration as atmospheric CO_2 levels continue to rise, yet they are one of the most difficult ecosystem parameters to quantify. Despite promising results with ground-penetrating radar (GPR) as a nondestructive method of quantifying biomass of coarse roots, this application of GPR is in its infancy and neither the complete potential nor limitations of the technology have been fully evaluated. The primary goals and questions of this study fell into four groups: (1) GPR methods: Can GPR detect change in root biomass over time, differentiate live roots from dead roots, differentiate between coarse roots, fine roots bundled together, and a fine root mat, remain effective with varied soil moisture, and detect shadowed roots (roots hidden below larger roots); (2) CO_2 enrichment study at Kennedy Space Center in Brevard County, Florida: Are there post-fire legacy effects of CO_2 fertilization on plant carbon pools following the end of CO_2 application ? (3) Disney Wilderness Study: What is the overall coarse root biomass and potential for belowground carbon storage in a restored longleaf pine flatwoods system? Can GPR effectively quantify coarse roots in soils that are wetter than the previous sites and that have a high percentage of saw palmetto rhizomes present? (4) Can GPR accurately represent root architecture in a three-dimensional model? When the user is familiar with the equipment and software in a setting that minimizes unsuitable conditions, GPR is a relatively precise, non-destructive, useful tool for estimating coarse root biomass. However, there are a number of cautions and guidelines that should be followed to minimize inaccuracies or situations that are untenable for GPR use. GPR appears to be precise as it routinely predicts highly similar values for a given area across multiple scanning events; however, it appears to lack sufficient accuracy at small scales. Knowledge of soil conditions and their effects on GPR wave propagation and reception are paramount for the collection of useful data. Strong familiarity with the software and equipment is both important and necessary for GPR use in estimating coarse root biomass. GPR must be utilized at low soil moisture levels in

order to accurately represent existing coarse root structures. Our results from Disney Wilderness Preserve highlight the need for a strong understanding of the limitations of GPR, specifically knowledge of root structures (saw palmetto rhizomes) or environmental factors (low moisture content) that may hinder its application within a given system. The 3D modeling of course roots with GPR appears quite promising, as it has become more accurate and precise as the software has advanced and become more robust, but there is still a need for more precision before it will likely be able to model anything more than simple root systems comprised mostly of large diameter roots. Our results from Kennedy Space Center suggest that there are legacy effects from CO_2 fertilization in the form of more root mass providing a greater capacity for aboveground plant regrowth following fire, even 7 years after treatment ended.

Overview and Objectives

The temporal and spatial quantification of coarse roots is one of the most difficult aspects of belowground ecology. Coarse roots play a significant role in belowground carbon cycling and will likely play an increasingly crucial role in belowground carbon sequestration as atmospheric CO_2 levels continue to rise. Ground-penetrating radar (GPR) has been shown to be an effective, nondestructive method of quantifying biomass of coarse roots. GPR propagates electromagnetic waves into the soil, reflecting a portion of the energy back to the surface whenever the waves change speed as a result of contacting a buried object. Despite promising results, this application of GPR is in its infancy, and neither the complete potential nor limitations of the technology have been fully evaluated. Using a 1500 MHz antenna, we tested various scanning protocols and thresholds of application for GPR use across a variety of environmental conditions in the sandy soils of a sand-hill mixed oak community in Southeastern Virginia. Additionally, this site was used to test the potential for three-dimensional mapping of coarse roots using GPR. After adjusting GPR protocols based on the results of our experiments, we applied these techniques to measure coarse root biomass in plots from an 11-year CO_2 enrichment study in a scrub-oak community in Central Florida that ended in 2007 to determine if any evidence of previously high levels of CO_2 remained. These adjusted techniques were then applied to a palmetto and longleaf pine dominated flatwoods community in Central Florida as part of an ongoing carbon budget study. The two Florida systems provided an opportunity to determine if the techniques gleaned from the experimental study in Southeastern Virginia were site specific or if they could be applied to other ecosystem types.

The primary goals and questions of this study fell into four groups of objectives: (1) GPR methods: Is GPR sensitive enough to detect change in root biomass over time and at what scale? Can GPR differentiate live roots from dead roots? Can GPR differentiate between coarse roots, fine roots bundled together, and a fine root mat? What effect does varying soil moisture have on image quality and predictability? What are the thresholds of detection based on root size (diameter)? Is root shadowing (roots being hidden below larger roots) a concern when using GPR? What is the best method for producing accurate images with the GPR software? (2) CO_2 enrichment study at Kennedy Space Center in Brevard County, Florida: Are there legacy effects of CO_2 fertilization on plant carbon pools and system recovery from disturbance? (3) Disney Wilderness Study: What is the overall coarse root biomass and potential for belowground carbon storage in a restored longleaf pine flatwoods system? Can GPR effectively quantify coarse roots in soils that are wetter than the previous sites and that have a high percentage of saw palmetto rhizomes present? (4) Can GPR accurately represent root architecture in a three-dimensional model?

Approach

Biomass Estimation

In working with GPR, it is of paramount importance to accurately measure the local dielectric conductivity of the soil every day, for each field site, as it varies with regional climate differences and weather changes. A dielectric pit was constructed for each location where GPR was used. This entailed burying aluminum rods at increasing intervals of depth below undisturbed soil. The aluminum rods serve as markers for calibrating GPR to the dielectric conductivity of that site on a particular day. They are buried at known depths, thus when the GPR unit is adjusted for the rod depths, the dielectric constant is automatically adjusted.

The estimation of coarse root biomass, a critical role for GPR, was conducted at each of the three study sites. The method consisted of scanning 15 cm diameter cores. Excavating the cores to a depth of 60 cm, and sieving to determine the oven-dry mass of roots located within each core. The weights are then plotted against the number of root pixels determined by GPR to develop a regression for biomass estimation. Using these regressions, 0.25 m^2 plots were scanned at each site to develop an overall estimate for coarse root biomass in grams per square meter. The plots were excavated to 60 cm depth, roots were extracted and weighed, and comparisons were then made between GPR estimated and actual root biomass.

Methodological Capabilities

The methodological study was conducted using a grid of 15 experimental pits located at the Southeastern Virginia field site. The pits were scanned and excavated to a depth of 60 centimeters so that they could be used to ground truth the biomass estimation for this system. Once excavated, the removed soil was sieved to remove all coarse roots and backfilled into the pits. These pits were then used as the experimental units for the following experiments: 1) effect of increasing root density across three different size classes, 2) effect of shadowing created by large roots located directly over the top of smaller roots, 3) the ability to differentiate live and dead roots, 4) the ability to differentiate coarse roots compared to fine root bundles and a fine root mat, and 5) effect of soil moisture levels on GPR. All roots except for the “dead” roots used for these experiments were freshly excised from the study site, cut to approximately 15 cm lengths and the cut ends wrapped in parafilm to prevent moisture loss. The “dead” roots used in this study were acquired in similar fashion, but were oven-dried and reburied on site for a day so that they would acclimate to ambient soil moisture levels similar to roots that are no longer being sustained by living vascular tissues.

CO_2 Legacy

In the fall of 2013, twelve plots from the former long-term CO_2 enrichment experiment at Kennedy Space Center, Florida were located (6 ambient air plots and 6 elevated CO_2 plots). The CO_2 experiment ended in 2007 (Day et al. 2013). Within each experimental plot three 0.25 m^2 subplots were scanned using the protocols established in the Southeastern Virginia experiments. The aboveground vegetation was initially left intact and random transect sampling of each subplot was conducted to evaluate the necessity of aboveground plant removal in order to facilitate grid sampling methods. The aboveground vegetation within each 0.25 m^2 area was harvested and weighed. After the removal of the aboveground vegetation, the plots were rescanned in the established grid-sampling pattern. The plots were then marked so that they could be rescanned in the spring of 2014.

The thirty six subplots scanned in the fall of 2013 were rescanned in the spring of 2014 after any aboveground vegetation regrowth was removed. A randomly selected subplot in each plot was excavated to a depth of 60 cm and the roots were separated from the soil via sieving. The roots were bagged and brought back to Norfolk for analysis. All live aboveground vegetation was harvested from the entire original 12 experimental plots, weighed fresh in the field, and subsampled to obtain a fresh weight/oven dry weight conversion factor.

3D Architecture

A 4-m^2 plot (2 m x 2 m) was located within the Southeastern Virginia study site. Surface vegetation was clipped and the plot was scanned from the X and Y directions in a grid pattern during the summer of 2013. Each direction consisted of 100 parallel transects separated by 2 cm intervals for a total of 200 scans. The plot was then left for one year without disturbance. The plot was scanned again in the same pattern during the summer of 2014, after one year's growth. After the second scanning event, the soil was removed from the plot down to 40 cm using a wet/dry vacuum, leaving the coarse root system intact. Overhead images of the root architecture were taken using a digital camera and the actual root images were overlain with RADAN produced 3D images based on the GPR scans for comparison.

Findings

Biomass Estimation

Regressions developed for all three sites showed strong correlations between the number of pixels identified as roots by GPR and actual observed biomass ($R^2 = 0.80$ for Southeastern Virginia, 0.53 for the scrub-oak community at Kennedy Space Center, and 0.75 for the palmetto-longleaf pine flatwoods of the Disney Wilderness Preserve) (Fig. 1). In the Blackwater Preserve in Southeastern Virginia, our regression predicted 2578 g/m^2 of biomass compared to an average of 2637 g/m^2 actual root mass in 15 0.25 m^2 plots (Fig. 2). The Kennedy Space Center regression was less accurate for average biomass, predicting 6011 g/m^2 for the ambient plots and 6991 g/m^2 for the elevated plots, compared to 5060 g/m^2 and 5637 g/m^2 actual root mass extracted from 12 0.25 m^2 plots. The Disney Wilderness Preserve regression was also less accurate for average biomass, predicting 6076 g/m^2 of biomass compared to an average of 7472 g/m^2 actual root mass in 12 0.25 m^2 plots.

The Disney regression was the only one of the three regressions that significantly underestimated root mass, likely due to the presence of large palmetto rhizomes near the surface. As these structures were not a factor in Southeastern Virginia, and were avoided at Kennedy Space Center in keeping with previous protocols for the site, it had not yet been necessary to understand their influence on our methods. In order to understand how they would interact with GPR, we scanned and excavated three palmetto rhizome cores. When a regression of the 25 original cores for the site was applied to the scans of these cores, it underestimated root mass by an average of 25%, suggesting that GPR does not accurately see these structures. Four of our experimental plots had large palmetto rhizomes at or near the surface that did not appear to register any significant presence via GPR, resulting in an average underestimation of root mass of more than 1300 grams in each of these four plots, more than enough to cause the observed

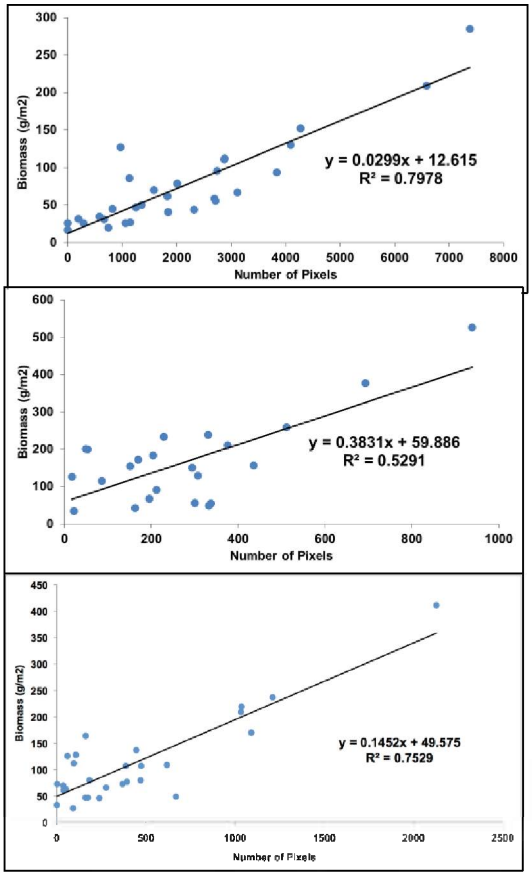

Figure 1. Regressions for Blackwater Preserve, Kennedy Space Center, and Disney Wilderness Preserve, respectively.

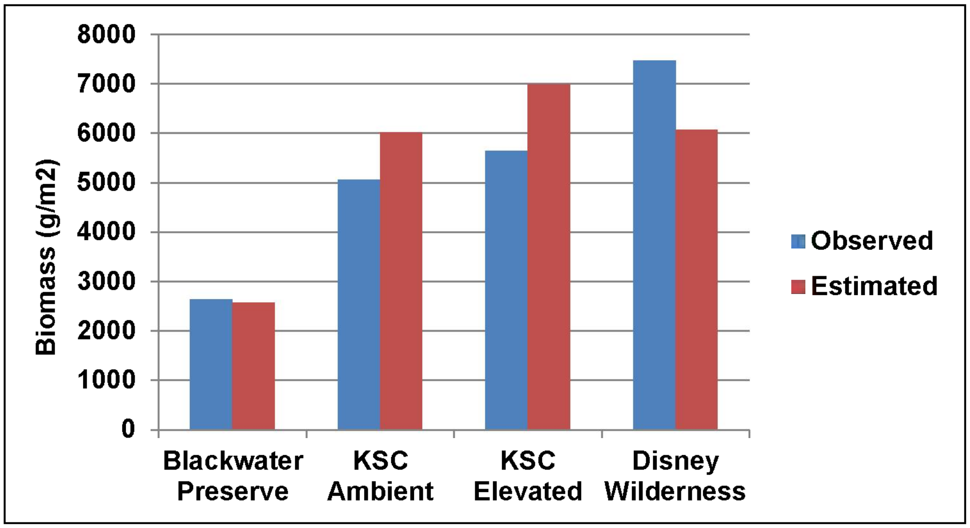

Figure 2. Observed versus GPR estimated biomass for each study site.

disparity.

These results suggest that GPR is a suitable method for coarse root biomass estimation across different systems, particularly those consisting largely of sandy soils, as it is able to predict biomass values along a continuum of increasing biomass. However, there do appear to be root structures (i.e. saw palmetto rhizomes and large structures flush with the soil surface) that are problematic for biomass estimation via GPR.

It is important to consider changing soil conditions during scanning events, as these changes can result in differences in the intensity of reflection that need to be corrected during processing in order to ensure that all data are comparable. Due to variability in weather conditions during scanning of the two Florida sites, it was necessary to use multiple pixel intensity thresholds to reconcile data collected on different days under different conditions.

Both the Virginia and Kennedy Space Center regressions were accurate across the full sample area, but were less accurate in predicting individual plot biomass, with average differences between observed and predicted of 483.5 grams in Virginia, 511 and 510 grams in the ambient and elevated plots of Kennedy Space Center. The Disney Wilderness Preserve regression produced plot biomass estimates that were significantly different ($P < 0.05$) from the observed plot biomass values as well as having a larger average difference between observed and predicted, 827 grams per plot. This may be the result of a higher density of palmetto on site and it will be problematic until GPR can be tuned to see palmetto rhizomes as well as other root structures.

Freeform scanning at the Kennedy Space Center site with the aboveground vegetation still intact produced a biomass estimate that was significantly higher than the observed biomass ($P < 0.05$). Both efforts of vegetation free grid scanning at the same site provided estimates that were not significantly different from the observed, suggesting that grid scanning is necessary for accurate results. Additionally, grid scanning at the KSC site was quite precise, varying only 4% in predicted biomass between November 2013 and March 2014.

Multi-directional scanning (0^{o}, 45^{o}, 90^{o} and 135^{o}) proved to be most effective as the number of pixels can vary between different scan directions, likely due to the variance in the angle of approach on the roots in situ. In a few plots, this manifested itself in the form of one of the four directions being significantly different than the other three, thus multi-directional scanning mitigates the variance due to direction of scanning. Additionally, subsampling multiple

15 cm core sections from all transects of a given plot to develop an average number of pixels per core for each plot was most effective. This again is likely due to the need to balance high variability in pixel counts across different core sections.

Our results suggest that all scanning for a dataset should be completed as quickly as possible, within a window of consistent weather conditions in order to minimize the effects of changing soil conditions on GPR sensitivity. These effects can often be corrected within the GPR software, but should be avoided if at all possible as this requires more subjective investigator manipulation than is ideal.

Methodological Capabilities

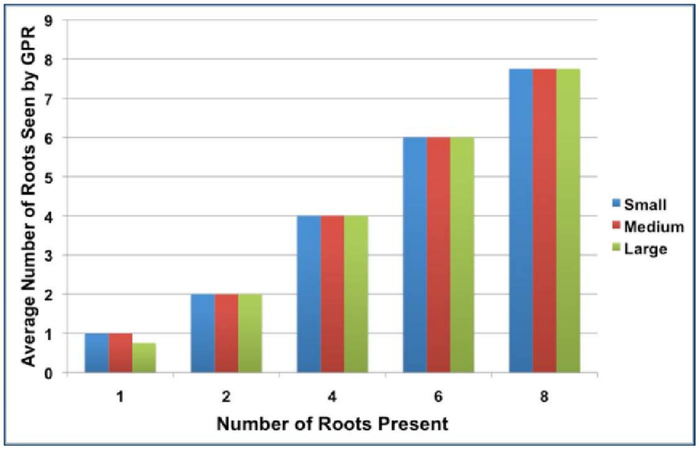

Figure 3. Mean number of GPR observed roots in three size classes in experimental pits.

Our experiments showed that GPR was able to quantify root growth simulated by increasing root density, but struggled to accurately differentiate roots of different cross-sectional diameters. Three size classes of roots (10 mm, 20 mm, and 30 mm diameter) were scanned at increasing density of roots within each experimental plot. GPR was able to quantify increases in root density across all three size classes with a high degree of accuracy (Fig. 3). GPR was also able to accurately represent the medium size roots as larger structures than the small roots, seeing an average of nearly twice as many pixels as roots. However, large roots were identified as having an average number of pixels approximately half that of small roots. This result is surprising given that medium diameter roots appeared larger than small diameter roots and that all large roots were identified by GPR. After processing, the large-root structures appeared as small, concentrated structures rather than larger blobs. This could be explained by any number of factors, such as lower moisture content of the cortex in large roots or stronger collapse of the hyperbolic reflectors during image processing, but the cause is currently unknown.

Our experiments also showed that root shadowing does not appear to exert a strong negative influence on GPR representation of roots (Fig. 4) as there was not a significant difference between the number of shadowed and unshadowed roots seen by GPR. Shadowed roots had more than double the average deviation from the mean when compared to unshadowed roots, suggesting there is some signal disruption, but the average numbers of pixels for shadowed and unshadowed roots were not significantly different.

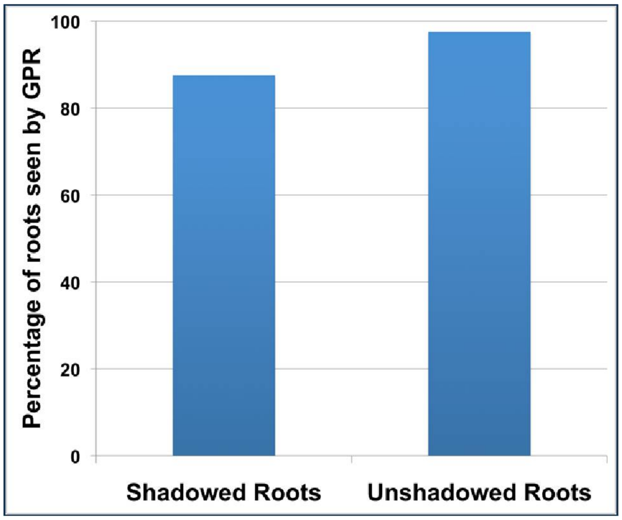

Figure 4. Percentage of roots observed by GPR when roots are shadowed or not by larger roots.

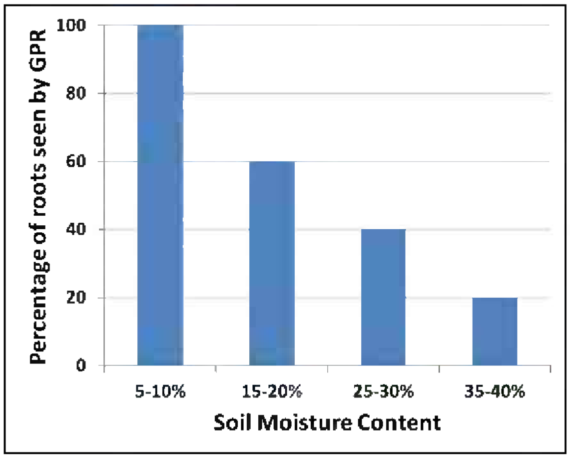

Figure 5. Effects of increasing soil moisture on GPR reliability.

Moisture, on the other hand, does appear to play a significant limiting role in the use of GPR. Soil moisture content was increased in the experimental pits from the ambient 5-10% up to 15-20%, 25-30%, and a maximum of 35-40%. As soil moisture increased, GPR positively recognized significantly fewer large roots ($P < 0.01$) (Fig. 5). Even an increase of only 5-10% resulted in a significant decrease in the effectiveness of GPR to recognize large roots. Additionally, the same incremental increase in soil moisture resulted in a significant decrease in the number of pixels determined to be roots, suggesting that those roots that were correctly identified by GPR were producing significantly weaker reflections.

Root moisture content also appears to play a significant role in GPR effectiveness. “Dead” roots were simulated by placing oven-dried root segments in the ground on site so that they could acclimate to the ambient soil moisture levels in a similar fashion to a real dead root. When these acclimated “dead” roots were placed in the experimental pits and scanned, GPR was significantly less likely to identify them compared to live roots of similar size, correctly identifying only 15% of the “dead” roots ($P < 0.01$) (Fig. 6). This suggests that root moisture content plays a significant role in the effectiveness of GPR to identify coarse roots, specifically that there needs to be a substantial difference between root moisture content and the moisture content of the surrounding soil in order to produce a strong enough change in the speed of propagation of the GPR wave to identify a root.

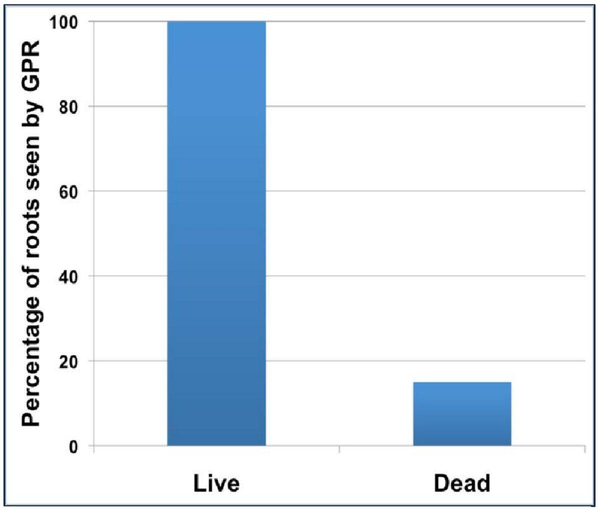

Figure 6. Percentage of live versus dead roots observed by GPR.

GPR was also unable to reliably identify either fine root bundles or fine root mats when compared to coarse roots of similar diameter and size ($P < 0.01$). As these fine root bundles and root mats were the same size as easily identifiable coarse roots, this is most likely due to an insufficient difference between moisture content of the fine roots and root mat fragments and the surrounding soil.

CO_2 Legacy

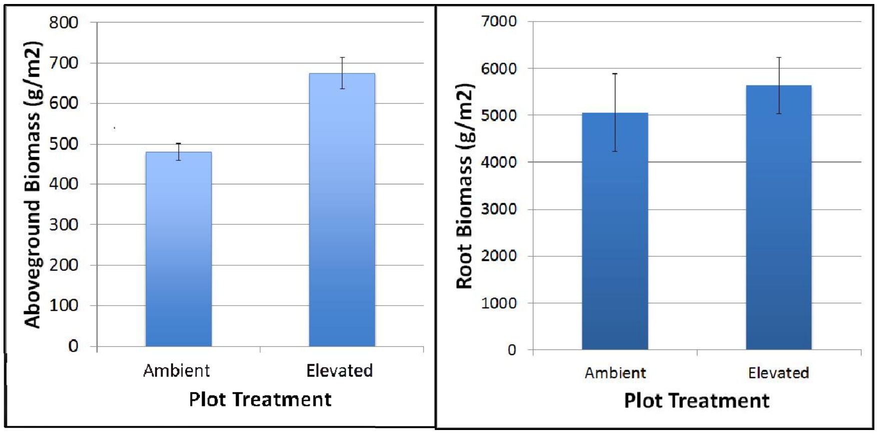

Figure 7. Aboveground and belowground biomass in 2014 for ambient and elevated CO_2 plots used in the previous long-term open-top chamber CO_2 enrichment study.

The Kennedy Space Center site, which was the location of an 11-year CO_2 enrichment study, experienced a prescribed burn in 2012, between the end of the study in 2007 and our current study. We were able to evaluate response to the fire disturbance and determine if there were any legacy effects of the previously elevated levels of CO_2. Aboveground biomass was significantly higher in the six previously elevated plots when compared to the ambient plots, despite having returned to ambient atmospheric CO_2 levels for nearly seven years ($P < 0.01$) (Figure 7). There was not a significant difference in the belowground biomass, but similar to the original study, the elevated plots averaged 12% higher root biomass when compared to the ambient plots, suggesting that there may be an effect there, but that this experiment was not powerful enough to detect it with only six replicates. This trend was also seen in the GPR biomass estimates, as GPR predicted 10% more biomass in the elevated plots in November of 2013 and 16% more biomass in March of 2014.

3D Architecture

The 4 m^2 root architecture plot was found to have a number of large roots, primarily concentrated on one half of the plot area. GPR was consistent, predicting 19,230 pixels in 2013 and 18,906 in 2014. Additionally, GPR appears to be accurate in identifying the vertical positions of these large roots, accurately identifying the major roots in the correct depth order.

While our methodological experiments showed that coarse roots of 10 mm diameter were accurately observed, this scanning effort suggests that roots smaller than 20 mm diameter were not always observed by GPR. Additionally, the differences in pixels identified between 2013 and 2014 suggests that this technique is not precise enough to measure seasonal growth, as it is not possible to determine if new pixels are growth or the result of different soil conditions between sampling events. When the GPR generated pixel overlays based on different depth slices were placed on top of the photograph of the actual roots, most of the larger roots were accurately mapped (Fig. 8).

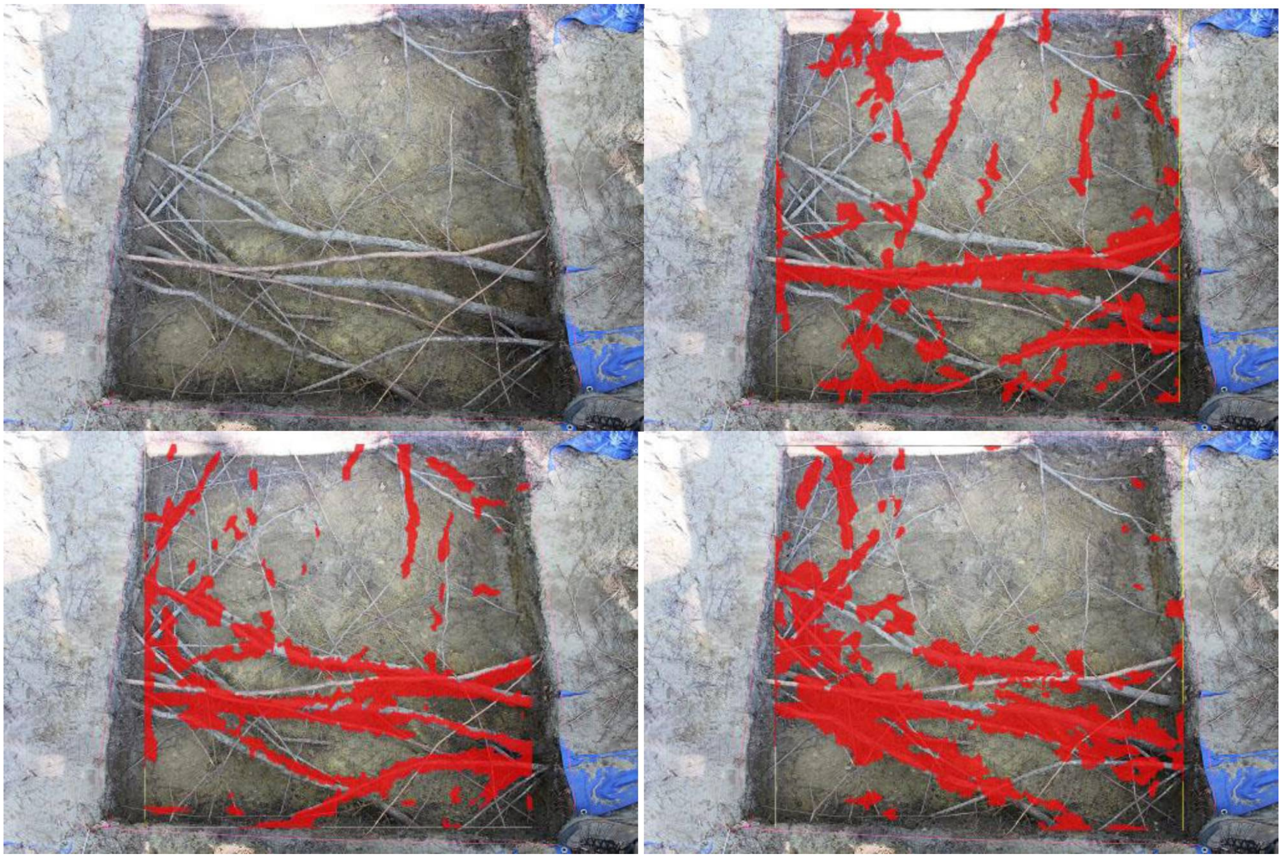

Figure 8. Excavated root architecture plot with pixel overlays from 2014 GPR scans (red). The three overlays represent scan slices at different depths, Moving from upper right (closest to the soil surface) clockwise through the lower left (the deepest slice).

Conclusions

When the user is familiar with the equipment and software in a setting that minimizes unsuitable conditions, GPR is a relatively precise, non-destructive, useful tool for estimating coarse root biomass. However, there are a number of cautions and guidelines that should be followed to minimize inaccuracies or situations that are untenable for GPR use. Our results from Disney Wilderness Preserve highlight the need for a strong understanding of the limitations of GPR, specifically knowledge of root structures (saw palmetto rhizomes) or environmental factors (low moisture content) that may hinder its application within a given system. The 3D modelling of course roots with GPR appears quite promising, as it has become more accurate and precise as the software has advanced and become more robust, but there is still a need for more precision before it will likely be able to model anything more than simple root systems comprised mostly of large diameter roots. Our results from Kennedy Space Center suggest that there are legacy effects from CO_2 fertilization in the form of more root mass providing a greater capacity for aboveground plant regrowth following fire, even 7 years after treatment ended.

Recommendations on Technology

- GPR appears to be precise as it routinely predicts highly similar values for a given area across multiple scanning events; however, it appears to lack sufficient accuracy at small scales.
- Knowledge of soil conditions and their effects on GPR wave propagation and reception are paramount for the collection of useful data.
- Strong familiarity with the software and equipment is both important and necessary for GPR use in estimating coarse root biomass.
- GPR must be utilized at low soil moisture levels in order to accurately represent existing coarse root structures.

Next Steps

- The use of GPR should be extended to more diverse systems in order to fully understand its potential for coarse root observation.
- Further, more comprehensive studies of the potential for 3D modelling of root systems by GPR are necessary before this technique might be widely applied.

Presentations

Bain, J.C., F.P. Day, and J.R. Butnor. 2013. Optimizing ground-penetrating radar for measurement of coarse root dynamics. DOE TES Meeting. Potomac, MD.

Bain, J.C., F.P. Day, and J.R. Butnor. 2013. Optimizing ground-penetrating radar for measurement of coarse root dynamics. INTECOL Meeting. London, England.

Bain, J.C., F.P. Day, and J.R. Butnor. 2014. Optimizing ground-penetrating radar for measurement of coarse root biomass and its application in determining elevated CO_2 legacy effects in an 11-year Florida experiment. DOE-TES Meeting. Potomac, MD.

Bain, J.C., F.P. Day, and J.R. Butnor. 2014. Optimizing ground-penetrating radar for measurement of coarse root biomass and its application in determining elevated CO_2 legacy effects in an 11-year Florida experiment. Ecological Society of America Meeting.

Sacramento, CA.

Manuscripts in Preparation

Bain, J.C., F.P. Day, and J.R. Butnor. Testing the limitations of ground-penetrating radar imaging of roots. Plant and Soil.

Bain, J.C., F.P. Day, and J.R. Butnor. CO_2 legacy 7 years following a long-term CO_2 enrichment study. Ecology.

Bain, J.C., F.P. Day, and J.R. Butnor. Ground-penetrating radar estimates of coarse root biomass in Virginia and Florida. New Phytologist.

Bain, J.C., F.P. Day, and J.R. Butnor. 3-D imaging of root systems by ground-penetrating radar. Plant and Soil.