Kearney Foundation Fellowship
Final Report Summary

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Project Title: Minimally Invasive Monitoring of Grassland Carbon Inputs in a Long Term Nitrogen Addition Experiment

Mentor Professors Name: Dr. Susan Trumbore and David LeBauer

Introduction:
Nitrogen (N) pollution affects many of California's ecosystems and the amount of N that is delivered to these ecosystems will continue to increase. Plant growth is strongly dependent on nitrogen availability. Many previous studies have seen this, yet most of these studies have focused on the response of plants under experimental conditions to high rates of N over a few years [1]. This research investigates plant response to a range of deposition rates after ten years of N addition. The goal of this research was two-fold: to better understand long term affects of low rates of N deposition in California on carbon (C) inputs, as well as to investigate non-destructive methods to estimate NPP from greenness in these long term plots.

Methods:

At the University of California Sedgwick Natural Reserve there has been a continuous input of four levels of N addition: 0 and 10 g N m^{-2} y^{-1} have been applied since September 1999 and 1 and 4 g N m^{-2} y^{-1} have been applied since March of 2000. At the two research sites—located on a valley and hillside—there were six replicate 2 x 4m plots at each level of N addition for a total of 48 plots.

I used multiple methods to compare the biomass of grass using destructive sampling with estimates of growth based on less destructive methods. I tested two visual estimates of aboveground biomass: percent greenness by eye and percent greenness calculated from a digital camera CCD sensor. First, D. LeBauer estimated greenness by eye (looking at the amount of aboveground live biomass), then he took a picture of each plot with an Olympus S4 digital camera, and then harvested 1.5 m x 0.1 m strips of above ground plant material on June 8th, 2008. I sorted live and dead above ground biomass and then I dried the samples at 60° C for 48 hours. Then, I determined the mass of live and dead plant material.

To evaluate plant growth, using a non-invasive method, I measured the greenness of each plot using a regular digital camera. I cropped pictures of each plot to 0.5 m by 1 m in the same coordinates for each picture to regulate the light intensities and then used the RGB split tool in Image J software to determine the average intensity of light recorded by the red, green, and blue sensors in each of the plots. Then, I determined the relative intensity of green compared to the red and blue lights reflected by the plot. To calculate the relative intensity of greenness from the CCD tool on the camera I used the following formula: Calculated Channel %= Channel DN/ (Total RGB DN).

I also estimated below ground biomass using root screens. Root screens can be used to estimate root length, and I sought to test if root length correlated with root biomass in these plots. In November 2006, D. LeBauer placed 10 cm by 15 cm pieces of window screen at 45° angles in the soil, and he collected these screens at six week intervals. Here I report the correlation between root intersections and root biomass from the final harvest when root biomass data were collected. Using the gridline-intercept method, a 2-d estimation of length, I counted every time the roots crossed the plane of the screen to get a sense of root length for the April and June Harvests. I also identified which roots were live and which ones were dead.

Results

Aboveground biomass was positively correlated to observed greenness across all plots (Figure 1). On the other hand, greenness derived from digital camera images was negatively correlated to aboveground biomass (Figure 2), and observed versus camera greenness were negatively correlated to each other (Figure 3). Consistent with previous years, biomass was not positively correlated to the level of N addition (Figure 4). In the graphs below, the P values show that there was an effect of N on the live aboveground biomass. We can see that plant biomass declines at N addition rates above 0 at hill and above 1 gN m^{-2} y^{-1} at valley. I also looked at the affect of using different parts of the images when I cropped them in gimp to calculate greenness and found that the part of the pictures that were cropped significantly changed the value of the channel percent (data not shown). Changing the area cropped in the the images varied the light intensity, possibly because of the spatial heterogeneity of the plots. Therefore, it was important to keep the area cropped consistent between images.

1. Correlation between inferred greenness and aboveground biomass was strong

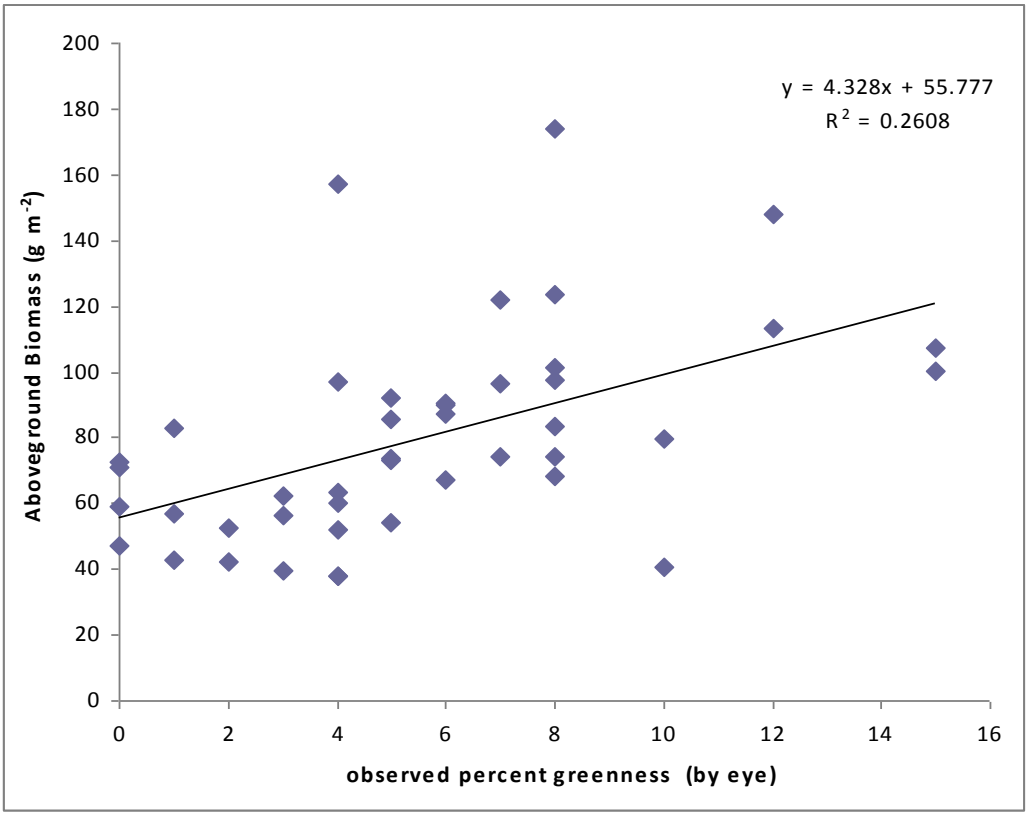


Figure 1: Observed Percent Greenness (Eye) versus the Weight of Aboveground Biomass

2. The correlation between the CCD greenness and biomass was significant, but negative

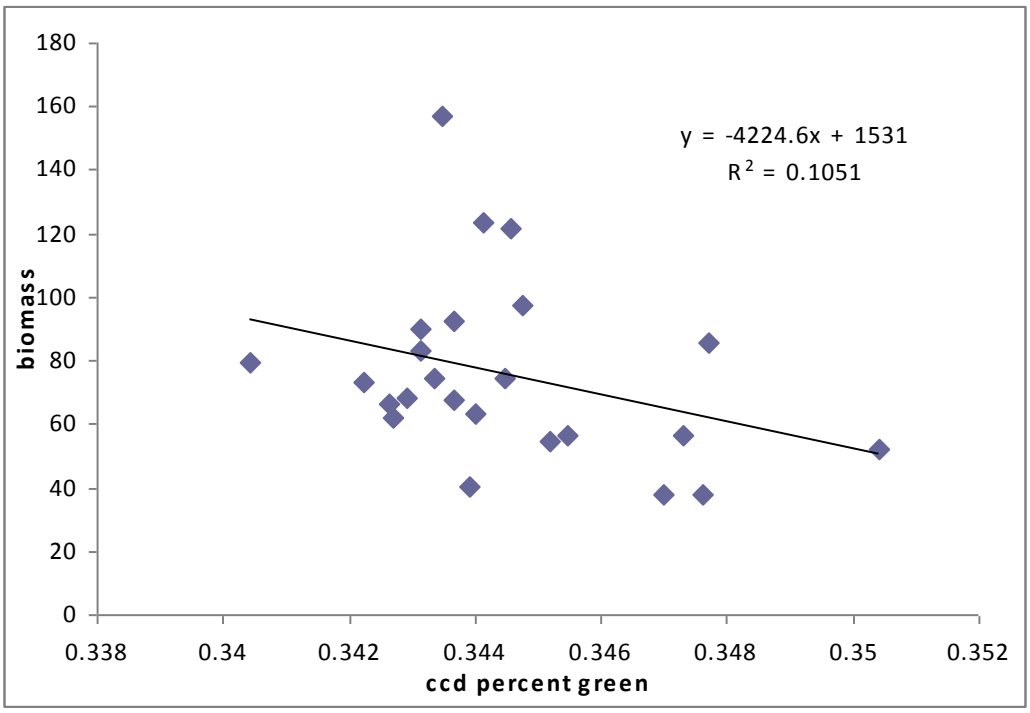
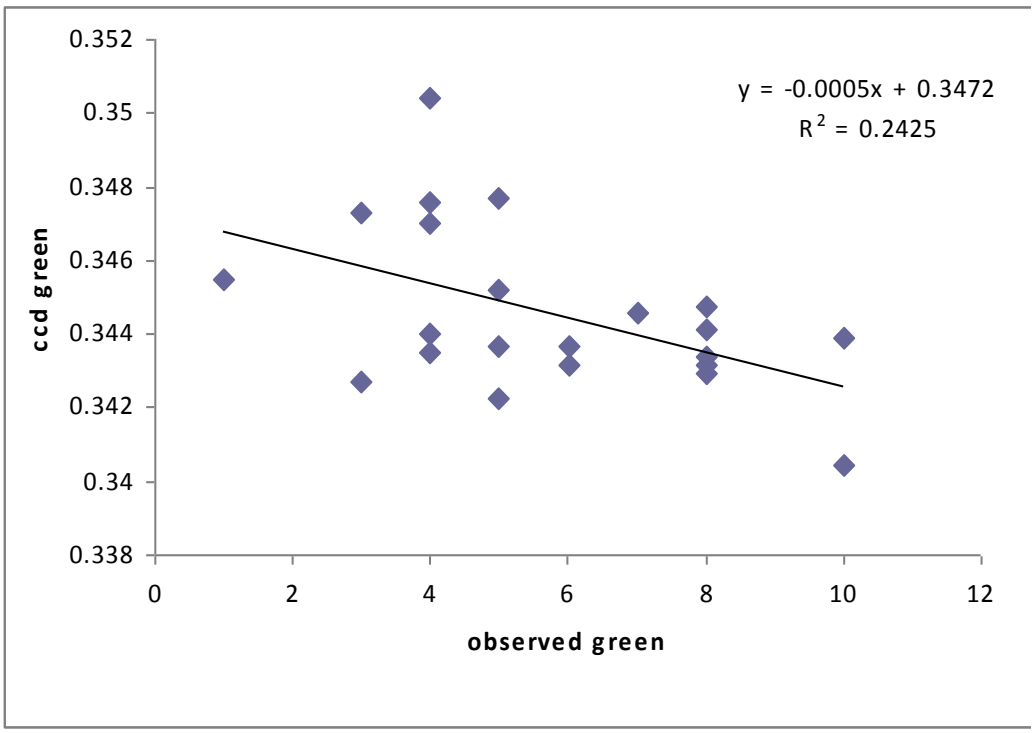


Figure 2: CCD Percent Greenness verses aboveground biomass

3. The correlation between observed greenness (eye) and CCD greenness was negative



4. N had an effect on total biomass

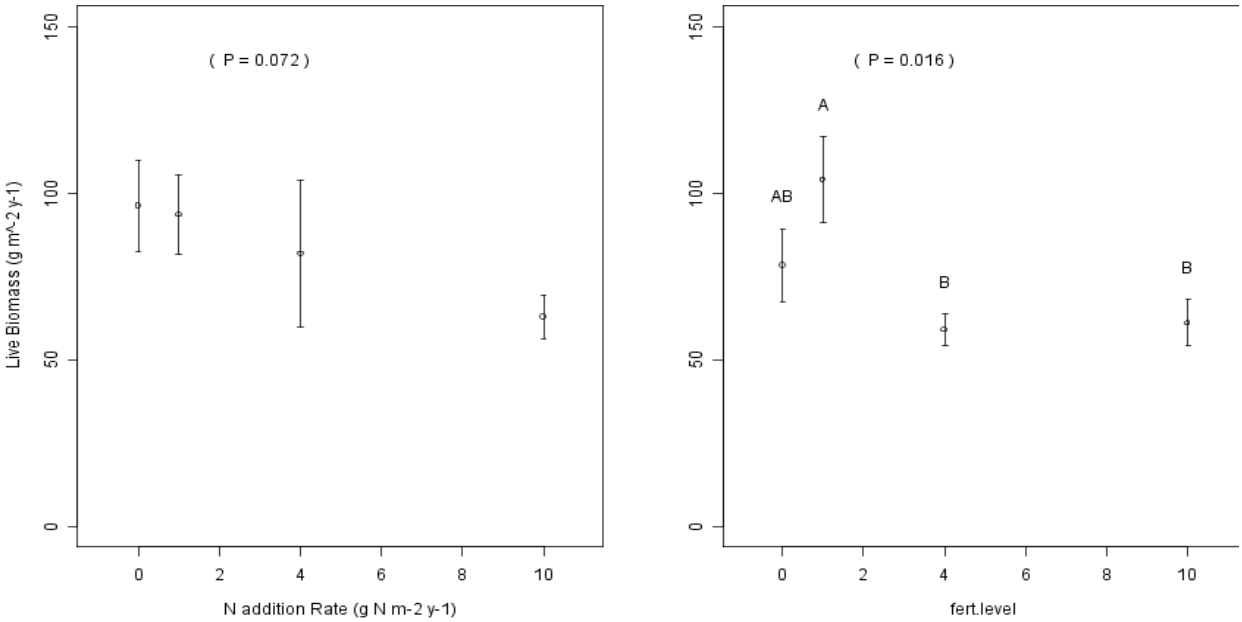


Figure 4: Graph of Live Biomass vs. Nitrogen Addition Rates: Hill Data on the Left and Valley Data on the Right

5. N had an effect on observed greenness

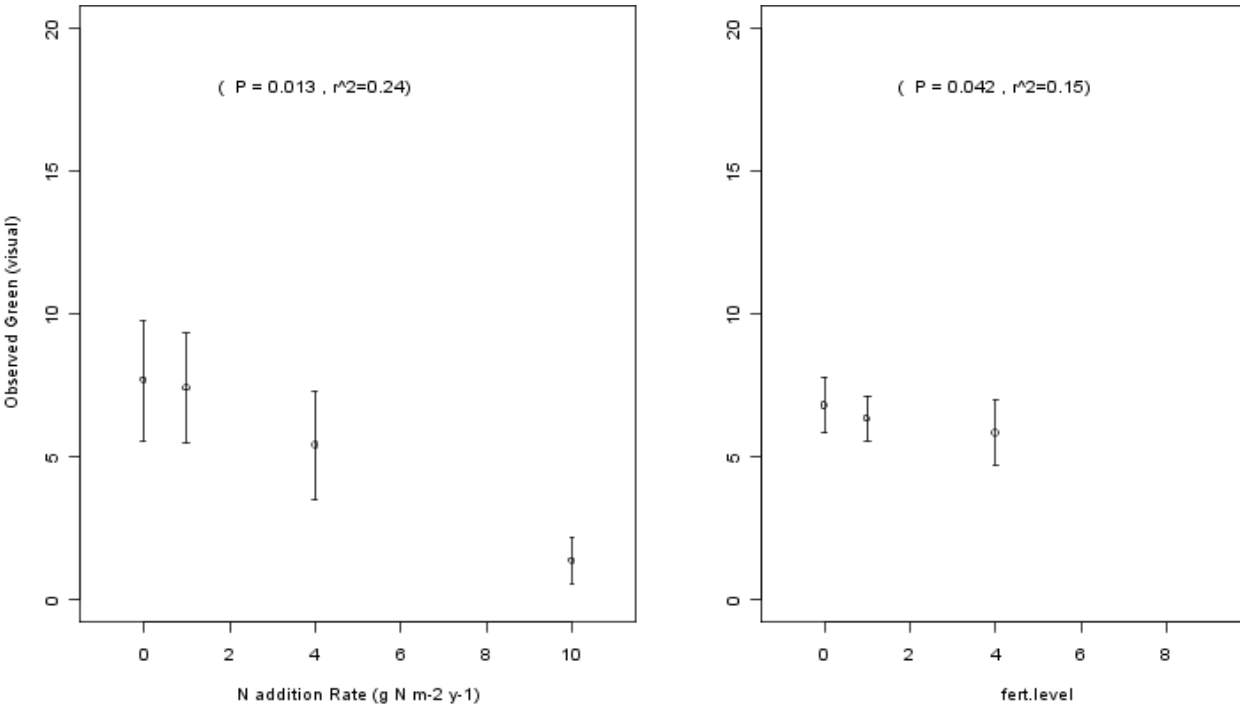


Figure 5: Graph of Observed Green Verses Nitrogen Addition Rates: Hill Data on the Left and Valley Data on the Right

6. Effect of N on the CCD measurement of greenness did not follow these patterns

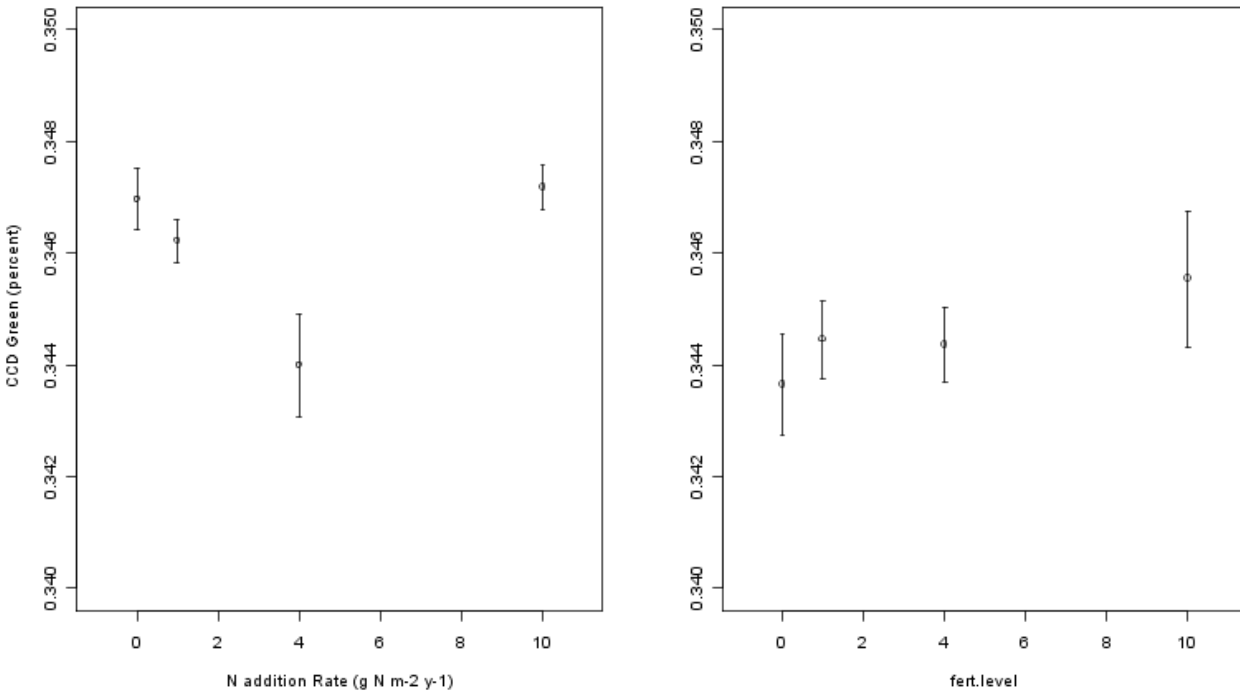


Figure 6: Graph of CCD Green Percent vs. Nitrogen Addition Rates: Hill Data on the Left and Valley Data on the Right

Discussion and Conclusion:

This work provides additional data for the measurement of grassland responses to N during a long term N addition experiment. Grass biomass production response to N in 2008 was consistent with previously reported patterns [2]. Nitrogen induced shifts in plant community composition has led to a negative relationship between N and biomass in this ecosystem. Now that there is four years of biomass data, it is clear that there is has been a shift in the biogeochemical cycling of this ecosystem that result from changes in the composition of the plant community.

There is evidence that greenness could be used as a non-destructive proxy for measuring aboveground biomass. The next step would be to do a multiple regression that includes a greenness index as well as percent cover by each of the dominant species. For instance, one of the most clear changes in plant community composition is the near complete removal of *Nassella pulchra* (a perennial) cover at high N addition rates. Other plants, including less productive forbs and annual grasses increase in cover at these sites. Because these measurements were taken in June, the greenness that we observed in 2008 was primarily the perennial *Nassella pulchra* while the other plant species were already senesced. If we used images taken earlier in the season, for example March, the results of percent greenness may be more closely related to biomass It is important that the results might differ depending on where in the image the RGB split analysis was done.

CCD greenness could be different from observed greenness because the human eye is not as sensitive as the CCD sensor. The CCD scanner can see many more grains than the human eye. Alternatively, the CCD

sensors are sensitive to a range of light. Furthermore, although the green sensor is most sensitive at green wavelengths (~550nm), it has substantial sensitivity to adjacent wavelengths, e.g. the green sensor is half as sensitive at 500nm (blue-green) and 600 nm (orange) as it is at 550nm. On the other hand, this source of error should be minimized by normalizing the value of the green sensor with the reflectance recorded by the red and blue sensors.

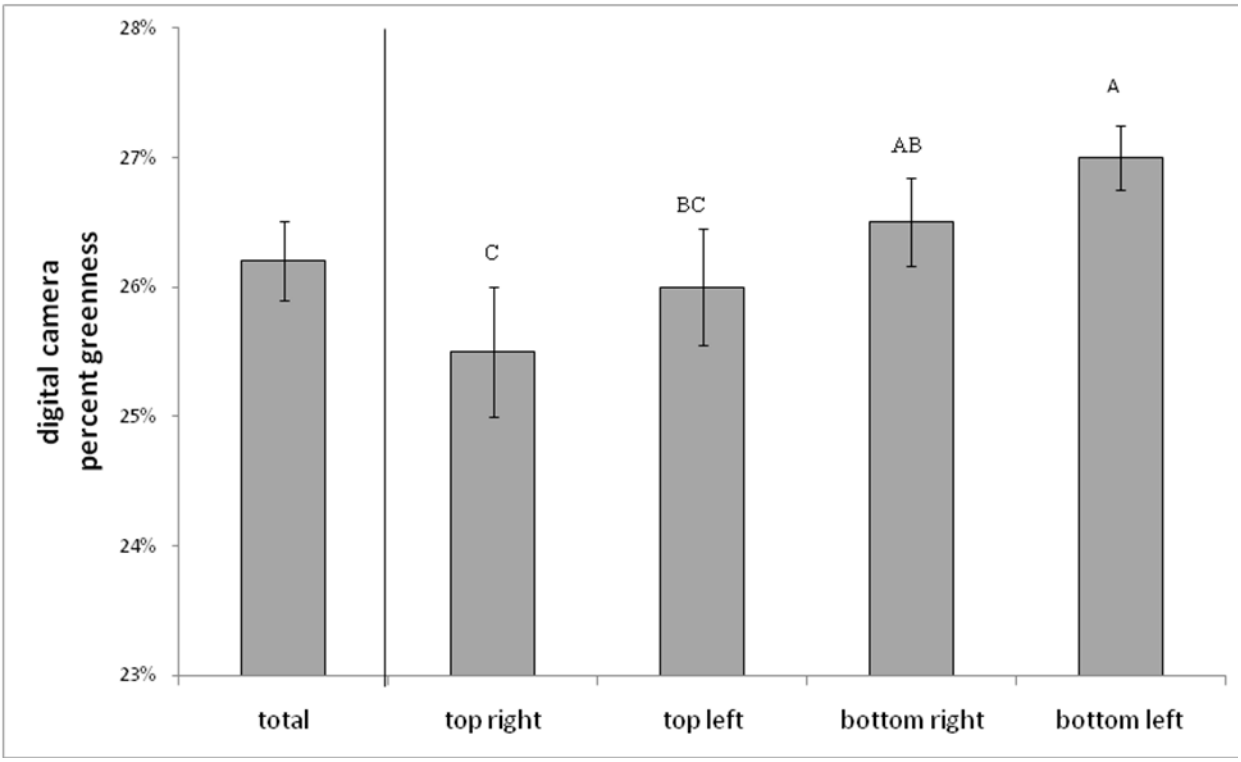


Figure 7: Effect of image area on percent greenness (P = 0.002). Mean values of image sections were compared using Tukey's HSD mean comparison, sections with significantly different percent greenness values are indicated by different letters above bars. Error bars represent ± 1 SE around the mean.

Additional Work

Although the results are not presented here, I also compared fully destructive root length samplings with the root-screen intercept method (described in methods). To measure root length, I converted scanned images of previously harvested roots into image binaries in gimp (black and white). The next step would be to use a root measurement software such as WinRhizo to calculate root length per meter squared. If the root screens provide an accurate estimate of root length, it will be possible to monitor below ground productivity with substantially less effort and minimally destructive harvesting, and this will permit more accurate and long term study of net primary productivity in these plots.

[1] LeBauer, DS and Treseder, KK. Nitrogen Limitation of Net Primary Production in Terrestrial Ecosystems is globally distributed. 2008. Ecology

[2] Ibid.