# Introduction

Earth’s climate is changing because of increases in atmospheric CO2 caused by human fossil fuel use. In the Midwestern United States, temperatures are forecast to increase 2-4 °C from 20th-century averages and [CO2] to exceed 500 ppm by the mid-21st century (Romero-Lankao et al. 2014, Melillo et al. 2016). These changes will have profound effects on the ecology and biogeochemistry of all ecosystems, and therefore on the policies and land use decisions needed to manage them effectively. Land use decisions in turn change ecosystem C storage and will therefore feed back to global climate; these feedbacks may be stabilizing if a new land use stores more C or destabilizing if it releases C (Anderson-Teixeira and DeLucia 2011). Therefore when considering possible land-use scenarios, quantifying their C storage potential is fundamental to predicting climate responses and is an essential step toward choosing policies that promote positive climate outcomes.

The dominant ecosystems of the Midwest are agricultural, and are currently managed almost exclusively for the production of maize and soybean, generally grown in rotation. In 2016 a total of 71.3 million Ha were planted to maize and soybean (NASS 2016), making it the largest ecosystem type in the continental US by area. Since their aboveground biomass is removed or tilled in each year, C storage in these ecosystems consists exclusively of the belowground C pool. A large proportion of new C inputs come from root and root-exudate C and the overall annual ecosystem C balance depends greatly on tillage decisions (Allmaras et al. 2004, Bernacchi et al. 2005). Therefore, understanding the C storage potential of agroecosystems is primarily a question of understanding root and soil C cycling.

The largest current use for US-produced maize (~40% of total production; NASS 2016) is as a feedstock for the production of ethanol. It has been suggested that changing some of this acreage to produce cellulosic fuel crops could potentially have climate and economic benefits. In general, cellulosic crops that take a “land sparing” approach (Anderson-Teixeira et al. 2012) by maximizing ethanol production on a small number of acres appear most likely to be successful in current economic conditions (Heaton et al. 2008, Davis et al. 2012, Hudiburg et al. 2016), while lower-productivity but lower-input systems such as prairie restorations may still have considerable benefits if other ecosystem services, such as biodiversity and resilience to unpredictable climates, are considered (Fornara and Tilman 2009, Gelfand et al. 2013). However, considerable uncertainty remains about the accumulation rate and turnover time of soil C in both high- and low-input biofuel crops (Anderson-Teixeira et al. 2013, Agostini et al. 2015, Bach and Hofmockel 2016).

The central aim of my dissertation research was to quantify how the mass, placement, and chemical & taxonomic makeup of roots will affect the trajectory of belowground C storage in the warmer, higher CO2 Midwest of the coming decades. Within this overarching framework, I focused on three land-use scenarios that can be characterized as conventional tillage, high-yield perennial grasses, and low-input restored prairie. Because these scenarios differ greatly in their depth of previous study, the outstanding questions about belowground C cycling differ in each system. This means that in each system I collected very different measurements, not all of which can immediately be converted into units of carbon. But in each case I have attempted to assess what my results mean for the long-term C status of the system.

## Scenario 1: Maize-soybean rotation

I began with a business-as-usual scenario, where the Midwestern United States continues to be mostly managed as a conventional maize-soybean rotation and farmers do their best to maintain maximum yields in an increasingly warm and high-CO2 environment. In this scenario the responses of crop roots are relatively well-studied: Elevated CO2 increases soybean plant size, including the root system (Ainsworth and Long 2005, Ruiz-Vera et al. 2013, Gray et al. 2016), and appears to have little effect on maize plant size (Leakey et al. 2006, Ruiz-Vera et al. 2015). It is important to note that from an agronomic perspective, these increases in biomass appear unlikely to translate into reliable yield increases (Ruiz-Vera et al. 2013, 2015, Gray et al. 2016), but from a carbon cycling perspective it is likely that maize-soybean ecosystems will have higher C inputs to the soil in the future. Therefore in this chapter I focused on the question of outputs: How do elevated temperature and CO2 affect the rate of C *loss* from the soil? By measuring soil CO2 flux for three years, partitioning it into root-derived and microbe-derived components, and comparing the results against the predictions of a 100-year in silico experiment using an ecosystem biogeochemistry model (Parton et al. 1998), I found that elevated temperature increases the microbial breakdown of soil organic matter and that increases in biomass input from elevated CO2 appear to prime additional increases in respiration, suggesting that the long-term result of the business as usual scenario will be substantial losses of soil C.

## Scenario 2: High-yield biofuels

Next, I asked whether some of the predicted soil C losses can be avoided by changing management strategies. High-yielding perennial grasses have large root systems and reduced disturbance cycles and have been proposed as a promising biofuel feedstock that may also increase soil C storage (Agostini et al. 2015). However, the stability of this C may depend greatly on the timing of its arrival and its physical placement in the soil profile (De Deyn et al. 2008). To explore root C inputs in more depth, improved nondestructive methods of root monitoring are needed (Rewald et al. 2012, Topp et al. 2016). I developed and calibrated a Bayesian statistical model written in Stan (Stan Development Team 2016) to estimate the root volume of a full soil profile from minirhizotron images, and combined it with results from direct sampling to show that perennial monocultures of switchgrass and *Miscanthus* can have root systems comparable in size to native prairies, and importantly that they store much of this mass in the deepest soil layers where it may be highly resistant to decomposition. This suggests that these root carbon inputs can be maintained as soil carbon for longer than if they were sent to shallower soil.

## Scenario 3: Low-input high-diversity biofuels

Finally, I turned to a multispecies grassland more like the native vegetation of our region of the Midwest. As an ethanol crop, restored prairies produce far less biomass per acre than high-yielding monocultures, and therefore they would require substantially more land diverted from food production (Heaton et al. 2008), making their widespread adoption seem unlikely at present. However, as the biome that literally created the fertility of the Corn Belt, the ability of prairies to store soil C over the long term is indisputable (David et al. 2009), and their adoption could accelerate if climate policy shifts to increase attention to other management goals such as yield stability in extreme conditions, supporting trophic diversity, and preserving locally adapted species (Fargione et al. 2009). Compared to the monocultures of the other two scenarios, my challenge in quantifying root inputs to this complex community mixture was very basic: “Here are some roots, but which species grew them?” I used DNA metabarcoding to disentangle the species in the root mixture and asked whether there is any spatial structure to the root stand: How do the species partition the niche space, and how does that affect the potential for C storage? I found that grasses increased in prevalence deeper in the soil profile, implying that deep-soil C inputs are likely also enriched in grass roots. Since grasses tend to have lower turnover than forb roots, and since deep soil layers have the highest chance of long-term C storage, this combination suggests that deep-soil C storage under prairies could be greater and more stable than suggested by shallow sampling.

## Scaling up: Exploiting computation to match small samples to big questions

In addition to their link of arising from an interest in soil C, these projects also share a common methodological theme: In each case the scale of the inference I wanted to make was poorly matched to the scale of the available measurement methods. Quantifying soil C contents or root mass is easy for any given sample, but obtaining each sample is laborious and usually destructive, and belowground systems are characterized by extreme heterogeneity on all scales. This means that questions about changes in belowground C across time or space are inherently difficult, often to the point of impracticality, to answer by direct measurement alone. Therefore each of the following chapters includes a substantial computational component. My approach was to collect what direct measurements were available, then use models to synthesize the available information to match the scale of the question.

The model is different for each question: In chapter 2, I scaled root and microbial respiration from instantaneous flux rates to century-scale changes in pool size by using a well-tested and mechanistically validated simulation of ecosystem biogeochemical processes. In chapter 3, I used a novel Bayesian statistical model to correct biases in image-based estimates of root distribution, therefore bringing an indirect measurement method into closer agreement with more laborious direct observations. Finally, in chapter 4, I applied bioinformatic tools from the rapidly emerging field of environmental metagenomics to filter noisy, mixed-sample DNA sequences into estimates of root identity and to attribute spatially partitioned niches to their taxonomic groups.

All of these computational analyses used different sets of programming tools, but a critical common theme is that the assumptions I made while programming them are *at least* as important for the quality of my inferences as are the methods I used for my direct measurements. However, unlike collecting soil cores, it is easy to redo computations! To that end, all of the code and data for these chapters are freely available online at my public code repository at https://github.com/infotroph/ and also at locations listed in each chapter. I encourage anyone to reproduce my analyses, test my assumptions for themselves, or alter the code for their own purposes. If you find errors, please tell me.

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