2021

Exploring the Impacts of No-Till Agriculture and “Three Sisters” Intercropping on Carbon Sequestration and Soil Health

Chloe Wanaselja

Exploring the Impacts of No-Till Agriculture and "Three Sisters" Intercropping on Carbon Sequestration and Soil Health

Chloe Wanaselja

Submitted in partial fulfillment of the requirements for a Bachelor of Arts degree with a major in Biology

Presented to:

Professor Wallace M. Meyer III,
Associate Professor of Biology

Pomona
College,
Claremont,
California

May 10, 2021

1. Objective Statement

This project seeks to explore whether regenerative agriculture can be a tool to mitigate climate change and increase resilience. It asks if specific practices of no-till (NT) and "Three Sisters" intercropping can contribute to a collective climate mitigation "toolkit" across regions of the USA.

2. Problem Statement

Climate change is the existential threat of the century. We are already experiencing shifts in weather and disease patterns and increased food insecurity. Industrial agriculture is a major contributor to climate change, highlighting the imperative of the development of and transition to well-informed regenerative agricultural practices. Despite the urgency, a comprehensive set of agricultural "climate best practices" does not exist for the USA or any specific region. As such, we are currently ill-equipped to give recommendations to farmers or policy makers, and subsequently ill-prepared to address this existential threat.

3. Introduction

3.1 Climate Change and Industrial Agriculture

According to the Intergovernmental Panel on Climate Change, humans have until 2030, 9 years, to dramatically reduce greenhouse gas emissions to keep global temperatures from rising higher than 1.5°C above pre-industrial levels (IPCC, 2018). Limiting this warming to 1.5°C rather than 2°C will be crucial to maintaining global ecosystem function, and will have a noticeable impact on human health and economies (Hoegh-Guldberg et al., 2019). Climate change is predicted to affect most key elements of human livelihood, and in many cases has already done so. Changes in weather patterns associated with climate change can weaken crops' ability to fight infection (Rosenzweig et al., 2001), and expand the range of crop weeds, pests, and diseases into higher latitudes (Dahlsten & Garcia, 1989; Sutherst, 1990). Initially, higher latitudes are expected to experience an increase in human habitability and crop production, while corresponding regions closer to the equator are expected to experience food shortages and malnutrition (McCarthy, 2001; Rosenzweig & Hillel, 1998). Along with weather patterns and sea level-rise impacting human migration, climate-induced food shortages are expected to produce climate refugees, who will experience substandard living conditions and increased risk of disease (Epstein, 1999).

Industrial agriculture is a large contributor to greenhouse emissions. Processes from fertilizer production to packaging and food storage account for one third of all anthropogenic greenhouse gas (GHG) emissions (Gilbert, 2012). As soils store three quarters of the carbon (C) contained in the terrestrial biosphere, soil degradation by agricultural practices also represents a significant climate threat (Lal, 2004). The United States is estimated to be losing roughly 1% of topsoil annually to erosion, with the majority caused by agriculture. The average natural replenishment rate is 2.5 to 5cm every several hundred years, meaning that the United States is losing topsoil at 10 times the replenishment rate (Montgomery, 2012). China and India are losing topsoil at 30 and 40 times the natural replenishment rate, respectively (Pimentel, 2006). Globally, 70% of all drylands are degraded or desertified, decreasing their capacity to store carbon (Dregne, 2002). For the last 12 millenia, land use changes from undisturbed land to agriculture have tended to result in a loss of soil organic carbon, resulting in the accumulation of a substantial carbon ‘debt’, estimated at 133 Gt C (Goldewijk et al., 2011; Paustian et al., 2016; Sanderman et al., 2017).

3.2 Regenerative Agriculture as an Environmental and Climate Solution

Regenerative agriculture has the potential to sequester historically lost soil carbon by using the principles of soil cover, livestock integration, year-round growing season, minimal soil disturbance (no-till), and plant diversity (intercropping) to regenerate soils and re-capture and store carbon (Sykes et al., 2020). Each of these practices individually can have a positive effect, but these processes in combination can have either additive or synergistic effects to reduce greenhouse gas emissions and increase crop productivity and water efficiency (De Gryze et al., 2009; Hu et al., 2017). Additionally, regenerative agriculture practices such as no-till require less machinery usage and therefore a decreased reliance on fossil fuels (De Gryze et al., 2009). As many require minimal inputs and machinery, regenerative agriculture practices are simple to implement and generally lead to a reduction in costs, potentially making farming more accessible (Howitt et al., 2009).

A key part of regenerative agriculture is its slightly diminished focus on crop productivity. Where industrial agriculture seeks to maximize productivity over all else, regenerative agriculture attempts to mimic ecological processes, recoupling food production with pre-existing and surrounding ecosystems. This strategy then enhances and promotes other ecosystem services as well, addressing many problems created by the initial maximization of products associated with industrial agriculture (Jordan, 2013). When standardization is no longer the goal, the necessity to understand the site-specific elements of one’s agricultural land arises. While fossil-fuel optimized industrial farming has many specific guides for how exactly to plant and farm, regenerative agriculture requires complex knowledge of the dynamics of a site. There are many fewer transferable recommendations currently available. Further study is therefore necessary to provide farmers with guidelines who are interested in making a switch to regenerative techniques, but don’t know where to start.

3.3 Tools for Measuring Efficacy of Regenerative Agriculture

3.3.1 *Soil Organic Carbon*

While many other greenhouse gases (GHGs) have higher global warming potential (GWP), carbon dioxide (CO_2) is present in the atmosphere in much greater concentrations than other GHGs (*GHG Emissions and Sinks*, 2017; Sabljic, 2009). CO_2 is used to standardize and simplify climate goals, such as the imperative to keep the concentration of CO_2 equivalents below 430ppm in order to limit warming to 1.5°C (Edenhofer et al., 2014). To achieve this goal given the intensity of the necessary shift in global human activity, we must actively remove carbon from the atmosphere using soils in part to store it (Hilaire et al., 2019). Given soil organic carbon's (SOC) combined significance in climate science and its integral role in soils, SOC is essential to measure how regenerative agriculture practices impact soil carbon content.

Soil organic carbon is the carbon component of biological compounds in soil, and its change over time is used in the literature extensively as a proxy for carbon storage. To be considered sequestration, the carbon must originate from the atmospheric CO_2 pool, and be integrated into the soil by plants. Regenerative agriculture practices are meant to maximize the time that carbon remains in the soil. Until recent advances in chemistry allowed a direct look at SOC location and formation in the soil, it was thought that SOC persisted in soil via the formation of aggregates, or stabilized collections of soil that are less affected by soil management or microbial decomposition (Six et al., 2004). The new paradigm suggests that simple molecules persist in soil because of their physical location and chemical attraction to mineral surfaces (Bradford et al., 2019; Lehmann & Kleber, 2015). As land use practices shift, especially tillage practices, SOC concentration at various depths in the soil profile can change, making it essential to measure SOC across soil profiles, even at depths deeper than those disturbed by a plow (Olson et al., 2014).

In addition to SOC's role in carbon sequestration, higher SOC concentrations confer other benefits to soils. Increased SOC has been shown to change physical soil conditions to improve water retention in soils, increasing their resilience in the face of intensifying weather (Rawls et al., 2003). SOC is also a key determinant for soil productivity and quality, as it provides a nutrient reservoir and is related to a more diversified soil biology (Deb et al., 2015). Additionally, global data sets support the idea that increased SOC increases crop yield (Oldfield et al., 2019).

3.3.2 *Indicators of Soil Health and Quality*

Soil quality is defined as a soil's capacity to sustain productivity and health and to maintain or improve water and air quality (Karlen et al., 1997). Soil health cannot be determined directly. Instead, various measurements must be taken as proxies or indicators, broken into biological, chemical, and physical indicators. Here, biological and chemical indicators are measured, and the physical effects of SOC on soil structure will serve as a physical indicator.

3.3.2a *Microbial Biomass*

To maintain a healthy and sustainable soil system, organisms (bacteria, fungi, and other microbes and invertebrates) must recycle soil detritus into a usable form. Soil microbes are the main ecosystem engineers that supply plants with nutrients through rapid cycling (Wall et al., 2001). In addition to nutrient cycling, microbial biomass potentially plays a role in nutrient transformation and pesticide degradation (Dalal, 1998).

Many plants also have symbiotic relationships with microbes, exuding carbon and other compounds from their roots to develop specialized beneficial microbial communities from the larger soil microbial community (Bakker et al., 2020). In return for the nutrients provided, the selected microbiome provides the plant with many services and nutrients. These include obtaining plant nutrients and water, conducting nitrogen fixation, promoting plant growth, protecting against infection, and providing defense against predators (Timmis et al., 2019). Through this pathway, plant roots deposit between 10 to 44 percent of their photosynthetically fixed carbon (Bais et al., 2006).

Measures of microbial biomass are sensitive enough to record differences in land-use change such as revegetation (An et al., 2013), intercropping (Kumar & Babalad, 2018), and change from tillage to NT (Helgason et al., 2010). However, there is no benchmark value for what biomass indicates a healthy soil, and so an increase in biomass associated with changes in agricultural practices is the best indicator. However, more microbial biomass can also lead to higher rates of microbial respiration, which can offset soil carbon sequestration. In order for the soil to have a net increase of SOC over time, more carbon must be stored than is being lost by increased microbial activity.

3.3.2b *Nitrogen and Phosphorous Concentrations*

Nitrogen (N) and Phosphorus (P) concentrations, and pH (detailed in 3.3.2.c) were identified as three of the most important chemical parameters to assess soil health and quality in a study of 39 physical, chemical, and biological parameters of soil health correlated to plant growth and yield, under different tillage, rotation, and cover-cropping regimes (Idowu et al., 2008). N and P both play critical roles in photosynthesis, cell growth, metabolism, and protein synthesis (Chapin et al., 2000). They are the most common rate-limiting nutrients in plant growth across earth's major biomes (Elser et al., 2007). N is available in the atmosphere and converted to a usable form for plants in part via rhizobia, bacteria from multiple genera which engage in a symbiotic relationship with many genera of legumes (Doin de Moura et al., 2020). In natural systems, P comes from rock phosphate which is renewed with the uplift of continental rock (Guignard et al., 2017). N is often assessed as mineral N in soils, particularly nitrate, organic N, or mineralizable N stored in organic matter. Along with other factors, climatic conditions can affect N soil dynamics in ways that render measurements of N availability for plants less reliable (Cardoso et al., 2013). Available P is present as orthophosphates in soil, but microbial P and organic P can easily become bioavailable.

3.3.2c *pH*

pH is the measure of the acidity or alkalinity of the soil and its optimal range for plant growth is typically between 6 and 7, with a more acidic or alkaline soil being less favorable (*Soil Quality Indicators: PH*, 1998). Soil pH correlates directly with nutrient availability and solubility, and can influence microbial activity (Cardoso et al., 2013). As such, pH assessment allows for a prediction of potential nutrient availability for crops (Souza et al., 2007). Additionally, pH is correlated with the soil's capacity to support high-yield crops (Kelly et al., 2009). Soil pH can be affected by various agricultural practices, with lower pH resulting from the application of nitrogen and sulfur fertilizers (*Soil PH*, 2014). Soils with a higher SOC content buffer pH shifts more effectively (Magdoff & Bartlett, 1985).

3.4 Regenerative agriculture and cultural techniques

3.4.1 *No-till*

Under a no-till (NT) agriculture system, the soil is not overturned to leave bare earth for a new crop, but instead seeds are planted in soil with crop/plant residue remaining on the surface. NT has been shown to reduce erosion, slowing the loss of topsoil (section 3.1; Seitz et al., 2019). In addition to erosion prevention, NT can improve nutrient cycling (Jones et al., 1994). In regions where water is a limiting factor of crop growth, NT can also conserve water in the soil by improving filtration and reducing evaporation (Jones et al., 1994; Triplett & Dick, 2008).

Carbon has been thought to increase in the soil's plow layer under NT because of soil structure changes and the physical protection of C in aggregates, which reduces the C decomposition rate (Jastrow et al., 1996; Six et al., 1998). Tillage has been thought to disrupt this process (Six et al., 2000). With recent debate around the mechanism of C storage in soils, I was not able to find research explaining NT's success at storing carbon under this new paradigm (section 3.3.1; Lehmann & Kleber, 2015). In general, NT has been found to have higher SOC in the top 20cm of the soil, whereas conventional tillage has higher SOC at depths greater than 20cm. This is thought to be because tillage moves organic matter deeper into the soil profile (Ogle et al., 2019)

A meta-analysis conducted on NT efficacy across 178 experimental studies indicated that in wet (mean annual precipitation ≥ 1000mm) temperate climates of all soil types SOC was increased with the adoption of NT. SOC was higher with NT adoption in both warm (mean annual temperatures ≥ 20°C) and cool (<20°C) regions. Predictions for other climates and soil types also indicated an increase in SOC, but the confidence intervals included zero (Ogle et al., 2019). A previous meta-analysis showed that crop productivity can increase with NT adoption, but productivity was occasionally reduced specifically in cooler and/or wetter temperate climates. Though cool and wet temperate regions occasionally have lower soil organic C stocks and C inputs, decomposition rates are also low resulting in net C sequestration (Ogle et al., 2012). Combining these findings, we see that NT is expected to increase SOC across temperature ranges in wetter temperate regions, but warmer areas may be more productive while storing this SOC.

NT has also been shown to increase bacterial, fungal, and total microbial biomass by up to 32% and alter community profiles in soil aggregates compared to conventionally tilled fields (Helgason et al., 2010).

3.4.2 *Intercropping Using the "Three Sisters" Polyculture*

In principle, intercropping is the practice of growing different plant types next to each other, either touching or in adjacent rows. Limiting resources, including water, light, and nutrients, can be used more effectively in intercropping systems compared to respective monocultures, leading to higher yields (Bedoussac et al., 2015; Li et al., 2006; Lithourgidis et al., 2011). Intercropping can improve soil physical properties, controlling soil disintegration, increasing water filtration, and reducing runoff volume (Seran & Karunarathna, 2010). Intercropping has been demonstrated to improve soil chemical properties as well. In N-deficient soil, a maize and cowpea intercropping improved available N and P compared to a maize monocrop (Vesterager et al., 2008). Legumes are thought to lower soil pH, increasing nutrient accessibility for cereals in the rhizosphere-the soil area directly surrounding plant roots (Yan et al., 1996). Legumes can also improve microbial environment in soils, potentially improving biological soil health in intercropping systems which include legumes (Kumar & Babalad, 2018).

Interactions between NT and intercropping in relation to SOC and soil health are rare in the literature. In Subtropical Karnataka India in a pigeonpea and soybean intercropping system, all conservation tillage methods studied resulted in higher soil carbon sequestration and SOC than conventional methods (while soil microbial biomass was higher in tillage systems) (Kumar & Babalad, 2018). In the semi-arid region of Brazil, a combination of no-till and intercropping with cotton, maize, beans, sesame and pigeon pea resulted in equal or greater SOC than adjacent native grassland (Maia et al., 2019). As carbon is typically lost when native habitat is converted to agriculture, this result is particularly salient.

This study will use the “Three Sisters” or a corn, bean, and squash intercropping system. This polyculture has been cultivated by Indigenous people across the Americas since at least 1500 CE (Engelbrecht, 2005), and has been shown to have greater yield and increased biomass production when grown together than when grown individually as monocultures, with proposed reasoning that differences in root foraging strategies between species increase total soil exploration (Zhang et al., 2014). Despite an extensive literature search, I was unable to find any published literature on the effects of Three Sisters intercropping on SOC or soil health. As such, this line of research will provide crucial foundations for further research with Three Sisters polyculture, SOC, and other measures of soil health.

4. Research Questions Addressed

Does NT enhance carbon sequestration and soil health relative to conventional tillage practices?

- No till agriculture has increased SOC, N, and P concentrations across the soil profile.
- No till agriculture has enhanced microbial biomass across the soil profile.
- No till agriculture has more neutral soil pH.

Does Three Sisters intercropping enhance SOC and soil health compared to each crop grown as a monoculture?

- Intercropping increases SOC and soil P concentrations.
- Legume monoculture has the highest soil N concentration.
- Intercropping enhances microbial biomass.
- Intercropping results in soils with neutral pH.

Does the combination of intercropping and NT enhance SOC and soil health?

- SOC, microbial biomass, N and P concentrations are elevated in soils that receive both NT and intercropping practices in combination. These soils also have a more neutral pH.

Do warmer (mean annual temperatures ≥ 15°C) and wetter (mean annual precipitation ≥ 1000mm) regions enhance SOC and soil health?[1]

- For warmer and wetter regions, intercropping further improves NT's enhancement of SOC and soil health.
- For cooler and wetter regions, SOC storage rates are lower than in warmer wetter regions, but intercropping still further improves NT's enhancement of SOC and soil health.
- For all other climatic regions in the US, intercropping enhances SOC and soil health.

5. Methods

5.1 Determination of Testing Sites

Climatic regions will be delineated using similar criteria as Ogle et al. (2005). While this paper studied the differences in temperate versus tropical climates, there is much variation within temperate climates, thus highlighting the need for exploring this line of inquiry. Using data made available by the NOAA National Climatic Data Center and collected throughout each state in the years 1972-2000, states will be classified into 4 categories: wet/warm(mean annual temperatures ≥ 15°C, mean annual precipitation ≥ 1000mm), wet/cool, dry/warm, and dry/cool (Figure 1). Generally wet and warm states fall in the Southeast, wet and cool states in the Northeast, dry and warm states in the Southwest and West, and dry cool states in the Midwest (Figure 1). Working based on these climactic criteria, 6 tilled organic farms in each of the 4 climatic regions will be selected upon which to conduct the various treatments. These farms will be found and coordinated with through the networks of the Agricultural Research Service from the USDA.

[1] Ogle et al. (2005) used the cutoff of mean annual temperature ≥ 20°C, but because on the mainland US Florida is the only state that meets these criteria, mean annual temperatures ≥ 15°C will be used instead.

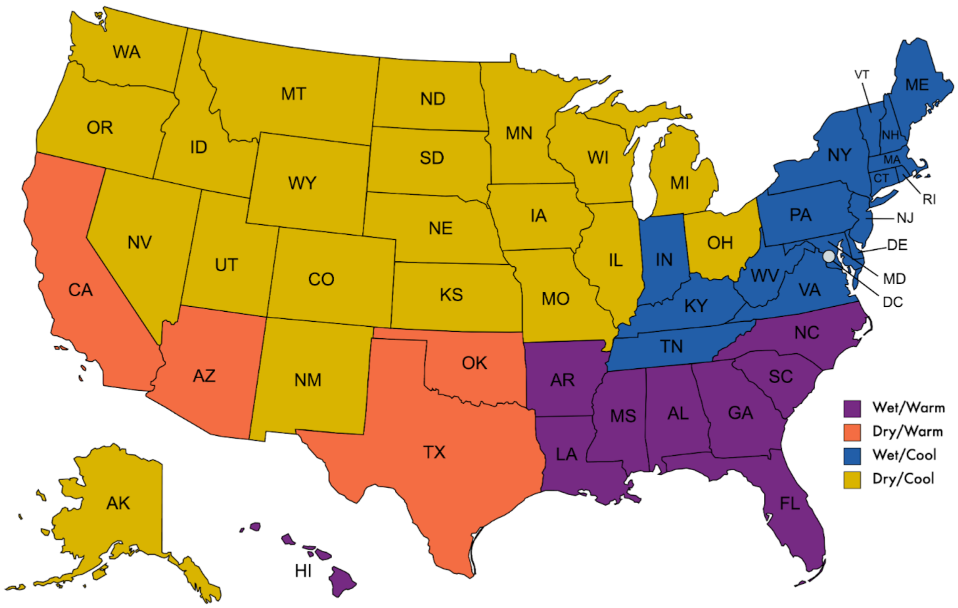

Figure 1. The United States broken into 4 ecoregions.
wet/warm is mean annual precipitation ≥ 1000mm, mean annual temperatures ≥ 15°C;
dry/warm is precipitation ≤1000mm, temperature ≥ 15°C; wet/cool is precipitation ≥ 1000mm, temperature ≤ 15°C;dry/cool is precipitation ≤1000mm, temperature ≤15°C.

5.2 Experimental Design

At each site, we will use a split-plot design where 1 acre will be allocated to a NT system and 1 acre to a system tilled once in the fall with a moldboard plow for deep (20-30cm) plowing, and once before planting with a tandem disk (Hanna et al., 2018). Within each tillage system (NT and Till), plots will be divided into four ¼ acre plots and randomly assigned to different planting regimes, as outlined in Table 1. Combined, the two tillage systems and 4 planting regimes result in 8 agricultural treatments (Table 1). Tillage and planting regimes will continue on the same land for the duration of the study (8 years). One varietal of corn, legume, and squash will be planted for each region (Figure 1) based on what varietals are known to grow well in the region. Methods for choosing varietals and for the Three Sisters planting process will be taken from The Old Farmer's Almanac (Boeckmann, 2020). With this extended time period, the opportunity to consider effects of climate change and/or extreme weather patterns such as droughts and flooding may arise. If large inter-annual shifts or aspects associated with drought affect the experiment, more funding may be requested to continue the experiment.

Table 1. The 8 treatment types associated with planting and tillage regimes

	Treatment Groups: Tillage and crops	
Crops Planted	NT	Till
Corn	NT/ corn monoculture	Till/ corn monoculture
Legumes	NT/ legume monoculture	Till/ legume monoculture
Squash	NT/ squash monoculture	Till/ squash monoculture
Three Sisters	NT/ Three Sisters	Till/ Three Sisters

5.3 Data Collection

Measurements will be taken for each site once a year in spring for the duration of the study. Additional measurements will be taken once in each of the four seasons during the first and last year of the experiment. Mean annual temperature and precipitation will be measured at each site, along with soil type (eg. sandy, loamy). During each sampling event, four subsamples will be taken for each of the 8 treatment groups.

Combining soil for each of the 4 subsamples per treatment, 500g of soil will be extracted from the top 5-10cm of soil (within the plow layer), and another 500g will be collected at a depth of greater than 30cm (below the plow layer). These samples will be sent immediately to the UC Davis Analytical Laboratory to determine SOC, N, P, pH, and soil texture. SOC concentration is measured from soil organic matter using the loss-on-ignition technique (Ben−Dor & Banin, 1989; Nelson & Sommers, 1996). Total N concentration is determined using a combustion system with an induction furnace combined with a thermal conductivity detector (TCD) system and an infrared (IR) detector system ("AOAC Official Method 972.43," 1997). To determine P concentration, the soil sample is first digested using nitric acid/hydrogen peroxide closed vessel microwave digestion. Analysis is then conducted using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Sah & Miller, 1992). PH is measured by creating a saturated paste from the soil and measuring with a pH meter ("PH Reading of Saturated Soil Paste," 1954).

An additional 50g of soil will be used to make a composite from the 4 subsamples, with 25g taken from the top 5-10cm, and 25g taken from a depth greater than 30cm. These samples will be sent immediately to Earthfort Laboratories (Corvallis, Oregon) to determine total/active bacteria and total/ active fungi, here used as a proxy for microbial biomass. Total bacteria and fungi (µg/g) are determined using direct enumeration microscopy, and bacteria are identified using the fluorescein isothiocyanate method (Babiuk & Paul, 1970; Van Veen & Paul, 1979). Total fungal biomass is determined by measuring its width and length in the soil sample, and then converting these data to mass. Active bacteria and fungi are assessed by staining samples with fluorescein diacetate, which binds and fluoresces to bacteria and fungi which are metabolically active (Schnürer & Rosswall, 1982; Yang et al., 1995).

5.4 Analysis

To examine if tillage and planting regimes influence SOC and indicators of soil health and quality (microbial biomass, N concentrations, P concentrations and pH) at shallow (5-10cm) and deep (20-30cm) depths, I will run 10 four-factor univariate PERMANOVA tests using regions, tillage regimes, planting regimes, and years as fixed factors. Five PERMANOVAs will use data from shallow soils and five will use data from deeper soils. All analyses will use the program PRIMER-E (Clarke & Gorley, 2006). Similarity matrices will be created using the Euclidean distance algorithm. Year will be included as a fixed factor as we expect that drought and wet years will impact many of the response variables. To explore seasonal effects, we will run another ten five-factor PERMANOVAs using season data collected during the first and last year of the project including season as a new fixed factor.

6. Broader Impacts

6.1 Climate Change Mitigation and Resilience

In the face of climate change, huge shifts in practice and culture are and will be necessary for human survival and livelihood. Paths for mitigation and resilience are currently lacking, and this research adds needed information to a collection of essential tools for survival. To make a shift to sustainable and regenerative practices, we must understand what methods are effective and radically innovate, centering holistic approaches.

6.2 Re-centering Indigenous Epistemology

Indigenous peoples of what's now known as the United States have a wealth of information grounded on a deep knowledge of hyper-specific environments. This knowledge is created as many generations of people live in the same place, passing down their discoveries to younger generations. As regenerative agriculture practices are rising in popularity, a need for a more granular and nuanced understanding of agricultural landscapes is increasing as well. Indigenous peoples have successfully cultivated these lands for millennia without many of the damaging results of Western Industrial Agriculture (3.1), in fact often increasing fertility at an ecosystem level (Anderson, 2013). Using the Indigenous-engineered Three Sisters polyculture moves towards a knowledge system based more on the long-term effects of human intervention and the support of fertility.

6.3 Making Science More Accessible

Farmers and early-career scientists will be offered the opportunity to collect and analyze the data for this experiment. For junior scientists, this will provide valuable experience in an essential and growing line of scientific research, as well as opening lines of communication between researchers and farmers, establishing relationships and grounding scientific research in the communities that it is meant to serve. For farmers, the opportunity to contribute directly in the scientific process can increase engagement and transparency. This is especially important in the field of regenerative agriculture, as it is anecdotally known that many alternative or regenerative farmers distrust the scientific community and the research it produces, in large part because of disproportionate funding by and towards industrial agriculture. Engaging farmers and scientists in the co-production of this work will increase trust and buy-in. Additionally, with farmers being made central in the research process, the results are more likely to be directly applicable, making these findings more accessible to farmers and policy-makers alike.

References

An, S.-S., Cheng, Y., Huang, Y.-M., & Liu, D. (2013). Effects of Revegetation on Soil Microbial Biomass, Enzyme Activities, and Nutrient Cycling on the Loess Plateau in China. *Restoration Ecology*, *21*(5), 600–607. https://doi.org/10.1111/j.1526-100X.2012.00941.x

Anderson, M. K. (2013). *Tending the Wild*. https://www.ucpress.edu/book/9780520280434/tending-the-wild

AOAC Official Method 972.43 Microchemical Determination of Carbon, Hydrogen, and Nitrogen. (1997). In *Official Methods of Analysis of AOAC International* (16th ed., pp. 5–6). AOAC International. http://m.wdfxw.net/Fulltext69714969.htm

Babiuk, L. A., & Paul, E. A. (1970). The use of fluorescein isothiocyanate in the determination of the bacterial biomass of grassland soil. *Canadian Journal of Microbiology*, *16*(2), 57–62. https://doi.org/10.1139/m70-011

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, *57*, 233–266. https://doi.org/10.1146/annurev.arplant.57.032905.105159

Bakker, P. A. H. M., Berendsen, R. L., Van Pelt, J. A., Vismans, G., Yu, K., Li, E., Van Bentum, S., Poppeliers, S. W. M., Sanchez Gil, J. J., Zhang, H., Goossens, P., Stringlis, I. A., Song, Y., de Jonge, R., & Pieterse, C. M. J. (2020). The Soil-Borne Identity and Microbiome-Assisted Agriculture: Looking Back to the Future. *Molecular Plant*, *13*(10), 1394–1401. https://doi.org/10.1016/j.molp.2020.09.017

Bedoussac, L., Journet, E.-P., Hauggaard-Nielsen, H., Naudin, C., Corre-Hellou, G., Jensen, E. S., Prieur, L., & Justes, E. (2015). Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review.

Agronomy for Sustainable Development, *35*(3), 911–935. https://doi.org/10.1007/s13593-014-0277-7

Ben−Dor, E., & Banin, A. (1989). Determination of organic matter content in arid−zone soils using a simple “loss−on−ignition” method. *Communications in Soil Science and Plant Analysis*, *20*(15–16), 1675–1695. https://doi.org/10.1080/00103628909368175

Boeckmann, C. (2020, May 14). *The Three Sisters: Corn, Beans, and Squash*. Old Farmer’s Almanac. https://www.almanac.com/content/three-sisters-corn-bean-and-squash

Bradford, M. A., Carey, C. J., Atwood, L., Bossio, D., Fenichel, E. P., Gennet, S., Fargione, J., Fisher, J. R. B., Fuller, E., Kane, D. A., Lehmann, J., Oldfield, E. E., Ordway, E. M., Rudek, J., Sanderman, J., & Wood, S. A. (2019). Soil carbon science for policy and practice. *Nature Sustainability*, *2*(12), 1070–1072. https://doi.org/10.1038/s41893-019-0431-y

Cardoso, E. J. B. N., Vasconcellos, R. L. F., Bini, D., Miyauchi, M. Y. H., Santos, C. A. dos, Alves, P. R. L., Paula, A. M. de, Nakatani, A. S., Pereira, J. de M., & Nogueira, M. A. (2013). Soil health: Looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Scientia Agricola*, *70*(4), 274–289. https://doi.org/10.1590/S0103-90162013000400009

Chapin, F. S., Zavaleta, E. S., Eviner, V. T., Naylor, R. L., Vitousek, P. M., Reynolds, H. L., Hooper, D. U., Lavorel, S., Sala, O. E., Hobbie, S. E., Mack, M. C., & Díaz, S. (2000). Consequences of changing biodiversity. *Nature*, *405*(6783), 234–242. https://doi.org/10.1038/35012241

Clarke, K., & Gorley, R. (2006). Primer v6: User Manual/Tutorial. In *PRIMER-E*. PRIMER-E.

Dahlsten, D. L., & Garcia, R. (Eds.). (1989). *Eradication of exotic pests: Analysis with case* *histories*. Yale University Press.

Dalal, R. C. (1998). Soil microbial biomass—What do the numbers really mean? *Australian Journal of Experimental Agriculture*, *38*(7), 649. https://doi.org/10.1071/EA97142

De Gryze, S., Albarracin, M. V., Catalá-Luque, R., Howitt, R. E., & Six, J. (2009). Modeling shows that alternative soil management can decrease greenhouse gases. *California Agriculture*, *63*(2), 84–90. https://doi.org/10.3733/ca.v063n02p84

Deb, S., Bhadoria, P., Mandal, B., Rakshit, A., & Singh, H. (2015). Soil organic carbon Towards better soil health, productivity and climate change mitigation. *Climate Change and Environmental Sustainability*, *3*, 26–34. https://doi.org/10.5958/2320-642X.2015.00003.4

Doin de Moura, G. G., Remigi, P., Masson-Boivin, C., & Capela, D. (2020). Experimental Evolution of Legume Symbionts: What Have We Learnt? *Genes*, *11*(3), 339. https://doi.org/10.3390/genes11030339

Dregne, H. E. (2002). Land Degradation in the Drylands. *Arid Land Research and Management*, *16*(2), 99–132. https://doi.org/10.1080/153249802317304422

Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Farahani, E., Kadner, S., Seyboth, A., Adler, A., Baum, I., Brunner, S., Eickemeier, P., Kriemann, B., Savolainen, J., Schlömer, S., von Stechow, C., Zwickel, T., & Minx, J. C. (2014). *IPCC, 2014: Summary for Policymakers* (Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change). https://www.ipcc.ch/site/assets/uploads/2018/02/ipcc_wg3_ar5_summary-for-policymakers.pdf

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., & Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, *10*(12), 1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x

Engelbrecht, W. (2005). *Iroquoia: The Development of a Native World*. Syracuse University Press.

Epstein, P. R. (1999). Climate and health. *Science (New York, N.Y.)*, *285*(5426), 347–348. https://doi.org/10.1126/science.285.5426.347

Gilbert, N. (2012). One-third of our greenhouse gas emissions come from agriculture. *Nature News*. https://doi.org/10.1038/nature.2012.11708

Goldewijk, K. K., Beusen, A., Drecht, G. van, & Vos, M. de. (2011). The HYDE 3.1 spatially explicit database of human-induced global land-use change over the past 12,000 years. *Global Ecology and Biogeography*, *20*(1), 73–86. https://doi.org/10.1111/j.1466-8238.2010.00587.x

Guignard, M. S., Leitch, A. R., Acquisti, C., Eizaguirre, C., Elser, J. J., Hessen, D. O., Jeyasingh, P. D., Neiman, M., Richardson, A. E., Soltis, P. S., Soltis, D. E., Stevens, C. J., Trimmer, M., Weider, L. J., Woodward, G., & Leitch, I. J. (2017). Impacts of Nitrogen and Phosphorus: From Genomes to Natural Ecosystems and Agriculture. *Frontiers in Ecology and Evolution*, *5*. https://doi.org/10.3389/fevo.2017.00070

Hanna, H. M., Melvin, S. W., & R.O. Pope. (2018). *Tillage implements*. Tillage Implements, Purpose and Ideal Use. https://extension.umn.edu/soil-management-and-health/tillage-implements-purpose-and-ideal-use

Helgason, B. L., Walley, F. L., & Germida, J. J. (2010). No-till soil management increases microbial biomass and alters community profiles in soil aggregates. *Applied Soil Ecology*, *46*(3), 390–397. https://doi.org/10.1016/j.apsoil.2010.10.002

Hilaire, J., Minx, J. C., Callaghan, M. W., Edmonds, J., Luderer, G., Nemet, G. F., Rogelj, J., & del Mar Zamora, M. (2019). Negative emissions and international climate goals—Learning from and about mitigation scenarios. *Climatic Change*, *157*(2), 189–219. https://doi.org/10.1007/s10584-019-02516-4

Hoegh-Guldberg, O., Jacob, D., Taylor, M., Bolaños, T. G., Bindi, M., Brown, S., Camilloni, I. A., Diedhiou, A., Djalante, R., Ebi, K., Engelbrecht, F., Guiot, J., Hijioka, Y., Mehrotra, S., Hope, C. W., Payne, A. J., Pörtner, H.-O., Seneviratne, S. I., Thomas, A., … Zhou, G. (2019). The human imperative of stabilizing global climate change at 1.5°C. *Science*, *365*(6459). https://doi.org/10.1126/science.aaw6974

Howitt, R., Català-Luque, R., Gryze, S., Wicks, S., & Six, J. (2009). Realistic payments could encourage farmers to adopt practices that sequester carbon. *California Agriculture*, *63*(2), 91–95.

Hu, F., Feng, F., Zhao, C., Chai, Q., Yu, A., Yin, W., & Gan, Y. (2017). Integration of wheat-maize intercropping with conservation practices reduces CO 2 emissions and enhances water use in dry areas. *Soil and Tillage Research*, *169*, 44–53. https://doi.org/10.1016/j.still.2017.01.005

Idowu, O. J., van Es, H. M., Abawi, G. S., Wolfe, D. W., Ball, J. I., Gugino, B. K., Moebius, B. N., Schindelbeck, R. R., & Bilgili, A. V. (2008). Farmer-oriented assessment of soil quality using field, laboratory, and VNIR spectroscopy methods. *Plant and Soil*, *307*(1), 243–253. https://doi.org/10.1007/s11104-007-9521-0

Inventory of U.S. Greenhouse Gas Emissions and Sinks. (2017, February 8). [Reports and

Assessments]. US EPA. www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks

IPCC. (2018). *Framing and Context. In: Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty*. In Press.

Jastrow, J., Miller, R., & Boutton, T. (1996). Carbon Dynamics of Aggregate-Associated Organic Matter Estimated by Carbon-13 Natural Abundance. *Soil Science Society of America Journal - SSSAJ*, *60*, 801–807. https://doi.org/10.2136/sssaj1996.03615995006000030017x

Jones, O. R., Hauser, V. L., & Popham, T. W. (1994). No-tillage Effects on Infiltration, Runoff, and Water Conservation on Dryland. *Transactions of ASAE*, *37*(2), 473–479.

Jordan, C. F. (2013). A History of Unsustainability in Agriculture. In C. F. Jordan (Ed.), *An Ecosystem Approach to Sustainable Agriculture: Energy Use Efficiency in the American South* (pp. 39–62). Springer Netherlands. https://doi.org/10.1007/978-94-007-6790-4_2

Karlen, D. L., Mausbach, M. J., Doran, J. W., Cline, R. G., Harris, R. F., & Schuman, G. E. (1997). Soil Quality: A Concept, Definition, and Framework for Evaluation (A Guest Editorial). *Soil Science Society of America Journal*, *61*(1), 4–10. https://doi.org/10.2136/sssaj1997.03615995006100010001x

Kelly, B., Allan, C., & Wilson, B. P. (2009). Corrigendum to: Soil indicators and their use by farmers in the Billabong Catchment, southern New South Wales. *Soil Research*, *47*(3), 340–340. https://doi.org/10.1071/sr08033_co

Kumar, B. T., & Babalad, H. (2018). Soil Organic Carbon, Carbon Sequestration, Soil Microbial Biomass Carbon and Nitrogen and Soil Enzymatic Activity as Influenced by Conservation Agriculture in Pigeonpea and Soybean Intercropping System. *International Journal of Current Microbiology and Applied Sciences*, *7*, 323–333. https://doi.org/10.20546/ijcmas.2018.703.038

Lal, R. (2004). Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. *Science*, *304*(5677), 1623–1627. https://doi.org/10.1126/science.1097396

Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. *Nature*, *528*(7580), 60–68. https://doi.org/10.1038/nature16069

Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, F. A., & Smith, S. E. (2006). Root distribution and interactions between intercropped species. *Oecologia*, *147*(2), 280–290. https://doi.org/10.1007/s00442-005-0256-4

Lithourgidis, A. S., Vlachostergios, D. N., Dordas, C. A., & Damalas, C. A. (2011). Dry matter yield, nitrogen content, and competition in pea–cereal intercropping systems. *European Journal of Agronomy*, *34*(4), 287–294. https://doi.org/10.1016/j.eja.2011.02.007

Magdoff, F. R., & Bartlett, R. J. (1985). Soil pH Buffering Revisited. *Soil Science Society of America Journal*, *49*(1), 145–148. https://doi.org/10.2136/sssaj1985.03615995004900010029x

Maia, S. M. F., Otutumi, A. T., Mendonça, E. de S., Neves, J. C. L., & Oliveira, T. S. de. (2019). Combined effect of intercropping and minimum tillage on soil carbon sequestration and organic matter pools in the semiarid region of Brazil. *Soil Research*, *57*(3), 266. https://doi.org/10.1071/SR17336

McCarthy, J. J. (2001). *Climate change 2001: Impacts, adaptation, and vulnerability:*

contribution of Working Group II to the third assessment report of the Intergovernmental Panel on Climate Change (Intergovernmental Panel on Climate Change, Ed.). Cambridge University Press.

Montgomery, D. (2012). *Dirt: The erosion of civilizations* (2nd ed.). https://www.ucpress.edu/book/9780520272903/dirt

Nelson, D. W., & Sommers, L. E. (1996). Total Carbon, Organic Carbon, and Organic Matter. In J. M. Bigham (Ed.), *Methods of Soil Analysis. Part 3. Chemical Methods-SSSA* (pp. 1001–1006).

Ogle, S. M., Alsaker, C., Baldock, J., Bernoux, M., Breidt, F. J., McConkey, B., Regina, K., & Vazquez-Amabile, G. G. (2019). Climate and Soil Characteristics Determine Where No-Till Management Can Store Carbon in Soils and Mitigate Greenhouse Gas Emissions. *Scientific Reports*, *9*(1), 11665. https://doi.org/10.1038/s41598-019-47861-7

Ogle, S. M., Breidt, F. J., & Paustian, K. (2005). Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. *Biogeochemistry*, *72*(1), 87–121. https://doi.org/10.1007/s10533-004-0360-2

Ogle, S. M., Swan, A., & Paustian, K. (2012). No-till management impacts on crop productivity, carbon input and soil carbon sequestration. *Agriculture, Ecosystems & Environment*, *149*, 37–49. https://doi.org/10.1016/j.agee.2011.12.010

Oldfield, E. E., Bradford, M. A., & Wood, S. A. (2019). Global meta-analysis of the relationship between soil organic matter and crop yields. *SOIL*, *5*(1), 15–32. https://doi.org/10.5194/soil-5-15-2019

Olson, K. R., Al-Kaisi, M. M., Lal, R., & Lowery, B. (2014). Experimental Consideration, Treatments, and Methods in Determining Soil Organic Carbon Sequestration Rates. *Soil*

Science Society of America Journal, *78*(2), 348–360. https://doi.org/10.2136/sssaj2013.09.0412

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., & Smith, P. (2016). Climate-smart soils. *Nature*, *532*(7597), 49–57. https://doi.org/10.1038/nature17174

PH reading of saturated soil paste. (1954). In *Diagnosis and improvement of saline and alkali soils*. U.S. Government Printing Office.

Pimentel, D. (2006). Soil Erosion: A Food and Environmental Threat. *Environment, Development and Sustainability*, *8*(1), 119–137. https://doi.org/10.1007/s10668-005-1262-8

Rawls, W. J., Pachepsky, Y. A., Ritchie, J. C., Sobecki, T. M., & Bloodworth, H. (2003). Effect of soil organic carbon on soil water retention. *Geoderma*, *116*(1), 61–76. https://doi.org/10.1016/S0016-7061(03)00094-6

Rosenzweig, C., Iglesius, A., Yang, X. B., Epstein, P. R., & Chivian, E. (2001). Climate change and extreme weather events—Implications for food production, plant diseases, and pests. *GLOBAL CHANGE*, *2*(2), 16.

Rosenzweig, & Hillel, D. (1998). *Climate Change and the Global Harvest: Potential Impacts of the Greenhouse Effect on Agriculture*. Oxford University Press.

Sabljic, A. (2009). *ENVIRONMENTAL AND ECOLOGICAL CHEMISTRY - Volume I*. EOLSS Publications.

Sah, R. N., & Miller, R. O. (1992). Spontaneous reaction for acid dissolution of biological tissues in closed vessels. *Analytical Chemistry*, *64*(2), 230–233. https://doi.org/10.1021/ac00026a026

Sanderman, J., Hengl, T., & Fiske, G. J. (2017). Soil carbon debt of 12,000 years of human land use. *Proceedings of the National Academy of Sciences*, *114*(36), 9575–9580. https://doi.org/10.1073/pnas.1706103114

Schnürer, J., & Rosswall, T. (1982). Fluorescein Diacetate Hydrolysis as a Measure of Total Microbial Activity in Soil and Litter. *Applied and Environmental Microbiology*, *43*, 1256–1261. https://doi.org/10.1128/AEM.43.6.1256-1261.1982

Seitz, S., Goebes, P., Puerta, V. L., Pereira, E. I. P., Wittwer, R., Six, J., van der Heijden, M. G. A., & Scholten, T. (2019). Conservation tillage and organic farming reduce soil erosion. *Agronomy for Sustainable Development*, *39*(1), 4. https://doi.org/10.1007/s13593-018-0545-z

Seran, T., & Karunarathna, B. (2010). Review on Maize Based Intercropping. *Journal of Agronomy*, *9*. https://doi.org/10.3923/ja.2010.135.145

Six, J., Bossuyt, H., Degryze, S., & Denef, K. (2004). A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research*, *79*(1), 7–31. https://doi.org/10.1016/j.still.2004.03.008

Six, J., Elliott, E. T., & Paustian, K. (2000). Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry*, *32*(14), 2099–2103. https://doi.org/10.1016/S0038-0717(00)00179-6

Six, J., Elliott, E. T., Paustian, K., & Doran, J. W. (1998). Aggregation and Soil Organic Matter Accumulation in Cultivated and Native Grassland Soils. *Soil Science Society of America Journal*, *62*. https://doi.org/10.2136/sssaj1998.03615995006200050032x

Soil pH (Soil Health Guides for Educators, pp. 1–6). (2014). United States Department of Agriculture. www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_052208.pdf

Soil quality indicators: PH (Soil Quality Information Sheet). (1998). USDA.

Souza, D. M. G., Miranda, L. N., & Oliveira, S. (2007). Acidez do solo e sua correção. *Sociedade Brasileira de Ciência do Solo*, 205–275.

Sutherst, R. W. (1990). *Impact of climate change on pests and diseases in Australasia*. https://publications.csiro.au/rpr/pub?list=BRO&pid=procite:d4e100ae-a750-4ae6-ab1f-c4915e14f32c

Sykes, A. J., Macleod, M., Eory, V., Rees, R. M., Payen, F., Myrgiotis, V., Williams, M., Sohi, S., Hillier, J., Moran, D., Manning, D. A. C., Goglio, P., Seghetta, M., Williams, A., Harris, J., Dondini, M., Walton, J., House, J., & Smith, P. (2020). Characterising the biophysical, economic and social impacts of soil carbon sequestration as a greenhouse gas removal technology. *Global Change Biology*, *26*(3), 1085–1108. https://doi.org/10.1111/gcb.14844

Timmis, K., Cavicchioli, R., Garcia, J. L., Nogales, B., Chavarría, M., Stein, L., McGenity, T. J., Webster, N., Singh, B. K., Handelsman, J., de Lorenzo, V., Pruzzo, C., Timmis, J., Martín, J. L. R., Verstraete, W., Jetten, M., Danchin, A., Huang, W., Gilbert, J., … Harper, L. (2019). The urgent need for microbiology literacy in society. *Environmental Microbiology*, *21*(5), 1513–1528. https://doi.org/10.1111/1462-2920.14611

Triplett, G. B., & Dick, W. A. (2008). No-Tillage Crop Production: A Revolution in Agriculture! *Agronomy Journal*, *100*(S3), S-153-S-165. https://doi.org/10.2134/agronj2007.0005c

Van Veen, J. A., & Paul, E. A. (1979). Conversion of Biovolume Measurements of Soil Organisms, Grown Under Various Moisture Tensions, to Biomass and Their Nutrient Content. *Applied and Environmental Microbiology*, *37*(4), 686–692.

Vesterager, J. M., Nielsen, N. E., & Høgh-Jensen, H. (2008). Effects of cropping history and phosphorus source on yield and nitrogen fixation in sole and intercropped cowpea–maize systems. *Nutrient Cycling in Agroecosystems*, *80*(1), 61–73. https://doi.org/10.1007/s10705-007-9121-7

Wall, D., Adams, D., & Parsons, A. (2001). Soil Biodiversity. In *Global Biodiversity in a Changing Environment: Scenarios for the 21st Century* (pp. 47–82). Springer-Verlag.

Yan, F., Schubert, S., & Mengel, K. (1996). Soil pH changes during legume growth and application of plant material. *Biology and Fertility of Soils*, *23*(3), 236–242. https://doi.org/10.1007/BF00335950

Yang, H.-C., Nemoto, Y., Homma, T., Matsuoka, H., Yamada, S., Sumita, O., Takatori, K., & Kurata, H. (1995). Rapid viability assessment of spores of several fungi by an ionic intensified fluorescein diacetate method. *Current Microbiology*, *30*(3), 173–176. https://doi.org/10.1007/BF00296204

Zhang, C., Postma, J. A., York, L. M., & Lynch, J. P. (2014). Root foraging elicits niche complementarity-dependent yield advantage in the ancient ‘three sisters’ (maize/bean/squash) polyculture. *Annals of Botany*, *114*(8), 1719–1733. https://doi.org/10.1093/aob/mcu191