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Quantifying the Effects of Dredged Sediment Application on Soil Properties and Plant Responses in Combination with Common Agricultural Field Management Practices

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QUANTIFYING THE EFFECTS OF DREDGED SEDIMENT APPLICATION ON SOIL PROPERTIES AND PLANT RESPONSES IN COMBINATION WITH COMMON AGRICULTURAL FIELD MANAGEMENT PRACTICES

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I HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER MY SUPERVISION BY Ashley N. Julian ENTITLED Quantifying the effects of dredged sediment application on soil properties and plant responses in combination with common agricultural field management practices BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Doctor of Philosophy.

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ABSTRACT

Julian, Ashley N. Ph.D. Environmental Sciences Ph.D. Program, Wright State University, 2023. Quantifying the effects of dredged sediment application on soil properties and plant responses in combination with common agricultural field management practices

Successful crop production relies on soils with balanced physical, chemical and biological properties. Demand for greater crop yields has led to the breakdown of soil properties through detrimental agricultural practices. To combat soil degradation, farmers employ field management practices including cover crop application, crop rotation strategies and organic soil amendment addition. These practices, used independently or in combination, can improve soil stability, increase soil nutrient content and functions of beneficial soil microbiota while increasing crop yield. Despite showing promise as an organic soil amendment, dredged sediments are still not well understood, due in part to the fresh or weathered conditions dredged sediments can be applied. Specifically, there is currently no research combining dredged sediments with cover crops, comparing different dredged sediments conditions in a single study or evaluating dredged sediment condition coupled with cropping strategies. To address these knowledge gaps, my dissertation evaluates changes in soil properties and crop responses when dredged sediments are coupled with these practices. I evaluated changes in dredged sediment property responses and corn production following winter rye cover crop application compared to a fallow season in a field experiment where I found cover crop application increased corn yields compared to a fallow season. These differences were driven by microbial-associated nutrient mineralization. Additionally, I quantified soil property and corn responses to different application ratios of fresh and weathered dredged sediments in a greenhouse experiment and determined applications of dredged sediments calculated

based on the nutrient recovery ratio are not sufficient to provide benefits to agricultural soils. However, in 100% applications, weathered dredged sediments were more beneficial to corn growth than agricultural soils, while fresh dredged sediments proved detrimental to corn growth. Finally, when assessing soil property and crop responses to a corn / soybean rotation strategy as a function of dredged sediment condition in a greenhouse experiment, results indicate a second growing season, regardless of crop species, can improve overall benefits of dredged sediments. Outcomes of this dissertation advance the knowledge of potential benefits dredged sediments can provide in agricultural systems when combined with current field management practices to increase crop production while mitigating soil degradation.

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Chapter 1

Introduction

As the global population increases, set to reach 9.8 billion people by 2050 (United Nations 2017), the demand on agricultural soils to produce larger crop yields is greater than it has ever been. Due to this increased need for agricultural production, agricultural systems are continuously utilized and often consist of intensive row-cropping practices typically involving large, monocrop systems that leads to the systemic break down of soil properties known as soil degradation (Scherr and Yadav 2020). An estimated 52% of all agricultural land is affected by soil degradation, causing poor plant growth and production, resulting in global economic losses of $400 billion annually (Hellerstein et al. 2019; Kopittke et al. 2019). In the US, the loss of productive agricultural soils results in a 14% reduction in crop yield which leads to a ~$2.8 billion decline in annual revenue (den Biggelaar et al. 2001; Eswaran et al. 2001; Thaler et al. 2021). Soil is a non-renewable resource, so consequently, research which seeks to restore degraded soil properties to their full potential is vital for both the economy and sustaining of the American people.

Soil properties include the physical structure and stability of soil, chemical aspects of soil and microbiota of the soil and are directly linked to plant growth and production in agricultural systems (Stott 2019). Each property consists of several measurable characteristics which contribute to the ability of plants to grow, reproduce and survive. Soil physical structure and stability, which includes texture, bulk density, moisture content and compaction, are responsible for maintaining structural support while providing space for water and air to infiltrate into the soil necessary for plant growth (Phogat et al. 2015; Stott 2019). Soil chemistry includes the nutrients (*i.e.*, carbon, nitrogen, phosphorus, sulfur, magnesium, calcium, potassium) and hospitable environment (*i.e.*, pH, electrical conductivity, cation exchange capacity) necessary for both plants and the soil microbiota to survive and grow to reproduce (Stott 2019; Guo 2021; Ochoa-Hueso et al. 2023). Finally, the soil microbiota, including bacteria, fungi and archaea, provide a vital role in the mineralization and exchange of nutrients with plants to ensure growth and production (Doran and Zeiss 2000; Busby et al. 2017; Stott 2019). The balance of these categories of soil properties has guided farmers in employing field management practices to improve crop production.

Field management practices that are often used to mitigate soil degradation include cover crop application, crop rotation strategies and soil amendments (Hellerstein et al. 2019; NRCS USDA 2020; 2021). Cover crops, or crops grown during the nongrowing-season, provide a plethora of benefits to agricultural systems, including weed and pest suppression, increased nutrient availability, increased beneficial soil microbiota and their associated functions and reduced soil erosion (Gabriel and Quemada 2011; Steele et al. 2012; Kim et al. 2020). Crop rotation, or growing different plant species in an alternating series of growing seasons, can also provide many benefits to agricultural systems, including reduced nutrient depletion, increased soil microbiota and their associated function and increased crop production (Crookston et al. 1991; Jiang and Thelen 2004; Jayasundara et al. 2007; Karlen et al. 2013; Wallander 2013;

McDaniel et al. 2014; Ashworth et al. 2017). Organic soil amendments, or amendments derived from plant, animal or human byproducts, alter the soil environment by increasing nutrients, improving soil's physical structure and enhancing beneficial microbiota while increasing crop production (Stewart et al. 2005; Antonious 2016; Hellerstein et al. 2019). More established organic soil amendments, including manure, biochar and biosolids, have begun replacing traditionally used synthetic fertilizers as a way to reduce the overall negative environmental impacts synthetic fertilizers cause (Stewart et al. 2005; Antonious 2016; Hellerstein et al. 2019).

Dredged sediments, or mechanically removed aquatic sediments, have shown promise as an organic soil amendment by augmenting all three accepted soil properties (Canet et al. 2003; Sigua et al. 2004; Daniels et al. 2007; Darmody and Diaz 2017; Brigham et al. 2021; Kiani et al. 2023; Rúa et al. 2023). When applied to degraded agricultural soils, dredged sediments can improve the physical stability of the soil by altering soil texture ratios, bulk density and moisture content (Canet et al. 2003; Sigua et al. 2004; Daniels et al. 2007; Darmody and Diaz 2017). The use of dredged sediments can also alter the soil chemistry by increasing soil pH and supplying limiting nutrients (Fonseca et al. 1998; Darmody and Marlin 2002; Sigua et al. 2004). Finally, dredged sediments can increase beneficial soil microbiota needed for nutrient mineralization (Rúa et al. 2023), potentially leading to increased crop production. Dredged sediments can be applied in two different conditions: fresh – directly from the water source -- or weathered – sediments stored on land for an extended amount of time for dewatering. Most research examining the use of dredged sediments as a soil amendment focuses on their application in the weathered form, but they may also be applied directly to soils in the fresh form. However, there is currently no research comparing the two conditions in a single study or their use coupled with current field management practices. The overarching goal of my dissertation is to evaluate changes in soil property and crop responses when dredged sediments are coupled with several field management practices. To do this, my dissertation aims to understand how soil property and crop performances respond when: a cover crop is applied to dredged sediments (Chapter 2; Julian et al. *in review*); application ratios based on USDA and EPA guidelines are used with two dredged sediment conditions (weathered, fresh; Chapter 3); and a crop rotation strategy is coupled with the different dredged sediment conditions (Chapter 4). In Chapter 2, I used a field experiment to evaluate differences in soil property responses as well as corn growth and production when a winter rye cover crop is used compared to when a field is left fallow. In Chapter 3, I quantified soil property and corn responses to different application ratios of fresh and weathered dredged sediment conditions in a greenhouse experiment. In Chapter 4, I assessed soil property and crop responses to a corn / soybean rotation strategy when compared to corn / corn continuous cropping coupled with fresh and weathered dredged sediment conditions in a greenhouse experiment. Outcomes of this dissertation advance our knowledge of dredged sediments' ability to increase crop production by altering soil properties. It further provides support for the ability of dredged sediment to combat soil degradation in agricultural systems using current field management practices.

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CHAPTER 2

COVER CROP APPLICATION ON DREDGED SEDIMENTS INCREASES CORN YIELD THROUGH MICROORGANISM-ASSOCIATED ENZYME-DRIVEN NUTRIENT MINERALIZATION

***AS SUBMITTED TO PLANT AND SOIL ON 2 MAY 2023, IN REVIEW,
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ABSTRACT

Background & Aims

Common strategies to mitigate soil degradation of agricultural soils include cover crop application and soil amendment addition. Applying dredged sediments as a soil amendment is gaining popularity since they often provide benefits other amendments lack; however, their use with cover crops is largely unexplored. To understand how cover crop use changes the restorative properties of dredged sediments, we assessed soil physical and chemical properties, enzymatic activities, and corn yield for plots of dredged sediments with and without a cover crop.

Methods

We assessed soil texture, bulk density, water content, pH, nitrogen, phosphorus, cation exchange capacity, calcium, magnesium, potassium and organic matter content, as well as alkaline phosphatase, β-glucosidase, leucine aminopeptidase, peroxidase, polyphenol oxidase and urease enzyme activities and crop responses and yields in manipulated dredged sediments with and without a cover crop over three collection periods: pre-cover crop, post-cover crop, and post-harvest.

Results

Cover crop application on dredged sediments increased corn yields by ~24% when compared to dredged sediments alone. Increases in corn yield were driven by changes in nutrient mineralization, specifically within the nitrogen cycle. The physical and chemical properties of dredged sediments remained unchanged regardless of cover crop application.

Conclusion

Our results suggest that when cover crops are applied to dredged sediments, crop yield increased through microorganism-driven nutrient mineralization. However, the physical and chemical environment remained optimal for corn growth within dredged sediments, regardless of cover crop application. This research is a vital step into understanding the use of dredged sediments in agricultural soil systems.

INTRODUCTION

Row cropping agricultural practices decimate soil systems, contributing to the annual degradation of seventy-five billion tons of productive agricultural soils worldwide (Wallander 2013; Baumhardt et al. 2015; Richardson and Dooley 2017; Scherr and Yadav 2020). Agricultural soils are deemed degraded when there is a breakdown between their physical structures, chemical environment, and/or the function and diversity of their biota (NCRS USDA 2020b). These soil properties are intrinsically linked; therefore, when one or more groups of soil properties degrade, this creates an imbalance within the soil environment which negatively impacts plant growth and agricultural production (Hellerstein et al. 2019). In the US, the loss of productive agricultural soils results in a 14% reduction in crop productions, leading to a roughly $2.8 billion decline in annual revenue (den Biggelaar et al. 2001; Eswaran et al. 2001; Thaler et al. 2021). Economic and environmental losses due to soil degradation establish the need for more beneficial agricultural practices to prevent soil loss and/or restore already degraded soils.

Agricultural soil degradation is a direct result of the continued use of intensive and inefficient agricultural practices such as monocropping, tilling, and synthetic fertilizer amendments that are traditionally used to mass-produce crops. Monocropping, or growing a single crop year after year, can reduce aggregate stability and deplete the soil of nutrients, leading to an inhospitable environment for microorganisms (Crookston et al. 1991; Nevens and Reheul 2001). Direct soil manipulation through tilling can alter the soil’s bulk density (BD) and compaction as well as reduce beneficial fungal communities (Lal et al. 1989; Al-Kaisi and Kwaw-Mensah 2007; Karlen et al. 2013). Traditional synthetic fertilizers supplement only specific nutrients and can also reduce the function of beneficial microorganisms, which often leads to inhospitable environments for crops over the long-term (Liebig et al. 2002; Russell et al. 2006; Hellerstein et al. 2019; Zhu et al. 2016; Srour et al. 2020).

To mitigate soil degradation, farmers have adopted field management practices including applying organic soil amendments and using cover crops (Wallander 2013; Hellerstein et al. 2019; NRCS USDA 2020a; APHIS USDA 2020). Organic soil amendments are additives derived from either animal waste or plants, such as manure, biosolids, or biochar. These additives are used to improve soil structure, increase available nutrients, and enhance the function of beneficial soil biota (US EPA 2013; ERS USDA 2019). The application of organic soil amendments can increase organic matter (OM) within the soils, leading to increased soil aggregate stability and carbon (C) sequestration (Whalen et al. 2003; Tian et al. 2015; Foster et al. 2016; Liu et al. 2020). This increase in soil stability leads to increases in soil cation exchange capacity (CEC) and nutrient availability, as most organic soil amendments, except for biochar, often contain high levels of nitrogen (N), phosphorus (P), potassium (K), and other micronutrients (Barbarick et al. 2012; Filiberto and Gaunt 2013; Hao et al. 2015). As a result, the influx of nutrients added by organic soil amendments can lead to a 2-5x increase in microbial-associated enzymatic activity (Fernández et al. 2009; Jin 2010; Song et al. 2019; Ozlu et al. 2019). Thus, soil property changes due to the application of organic soil amendments can increase crop production by up to 50% compared to unamended agricultural soils, especially for grasses and grains such as corn (Singer et al. 2004; Sigua 2005; Barbarick et al. 2012; Lehmann et al. 2011; Hao et al. 2015; Hellerstein et al. 2019).

A less established organic soil amendment that can benefit degraded agricultural soils is dredged sediments. Dredged sediments are the mechanically removed build-up of sediments in waterways that can have high levels of macro and micronutrients and increased water holding capabilities (Averett et al. 1990). The application of dredged sediments can improve degraded soil physical structures such as BD, texture, and water retention (Canet et al. 2003; Sigua 2005; Darmody and Diaz 2017). When applied to agricultural soils, dredged sediments can also increase available N, P, K, and micronutrient content (Fonseca et al. 1998; Sigua 2005) and neutralize soil pH, depending on their origin source (Sigua 2005; Daniels et al. 2007; Baniulyte et al. 2009; Darmody and Diaz 2017). Dredged sediments also contain beneficial microorganisms which can increase crop yields through increased nutrient cycling (Rúa et al. 2023). Changes in soil properties due to dredged sediments application can lead to increased yield and production for a variety of crops, including corn, soybeans, tomatoes, and lettuce (Sigua 2005; Koropchak et al. 2016; Canet et al. 2003; Daniels et al. 2007; Darmody and Diaz 2017).

Another way to mitigate soil degradation from row cropping agriculture is by using a cover crop during the non-growing seasons. Planting cover crops during non-growing seasons can enhance soil structure by reducing the loss of both soil aggregates and moisture. These enhancements help maintain soil's air and water ratios while increasing BD (Gabriel and Quemada 2011; Steele et al. 2012). Using cover crops also increases OM within the soil, which often leads to improved function of the beneficial soil microbiota (Kim et al. 2020). Additionally, the use of cover crops can improve environmental quality by reducing run-off and nutrient leaching, which retains needed nutrients through the non-growing season (Gabriel and Quemada 2011; Steele et al. 2012).

The use of a cover crop in combination with established organic soil amendments can further improve soil physical and chemical properties as well as the enzymatic activity in agricultural soils, leading to overall greater crop production (Fernandez et al. 2016; Sánchez de Cima et al. 2016; Ashworth et al. 2017; Adeli et al. 2019; Raut et al. 2020). However, interactions between cover crops and dredged sediments are still largely unknown. Here, we investigated the application of a cover crop on dredged sediments, assessing changes in soil properties and corn responses. Following the USDA guidelines for assessing soil properties (NRCS USDA 2020b), we quantified soil texture, BD, and water content (physical properties), nutrient content, CEC, and pH (chemical properties), the activities of six microorganism-associated extracellular enzymes (biological properties), and the growth and reproduction of corn (yield). We hypothesized that adding a cover crop would improve the physical and chemical properties and increase enzymatic activities of dredged sediments compared to dredged sediments left fallow over the winter. We also hypothesized that corn production would be greater after applying a cover crop than corn grown on dredged sediments left fallow over winter. Finally, we hypothesized that changes to the physical properties, chemical properties, and enzymatic activities of dredged sediments due to the application of the cover crop would drive associated changes in corn production. Our results highlight the importance of assessing dredged sediments as an organic amendment intended for use within agricultural systems.

MATERIALS AND METHODS

Site Description

To examine the potential for a cover crop to improve soil health and plant production on dredged sediments, we manipulated the presence of a cover crop in plots at the Great Lakes Dredged Material Center for Innovation (GLDMCI) located in Toledo, Ohio (41.6700354ºN, -83.5029711ºW; Rúa et al. 2023). The GLDMCI was created between 2016 and 2017 by pumping ~ 8,800 m^3 of dredged sediments from the Toledo Harbor and the Maumee River into four ~1 ha plots (Hull and Associates 2018). Dredged sediments in each plot were allowed to dry and acclimate to the on-land environment for two years prior to the experiment (Rúa et al. 2023).

Plot Preparation and Planting

In November 2018, the two available 1 ha plots were mowed and disked to remove existing vegetation. Then one plot was broadcast seeded with the common Midwestern cover crop winter rye (*Secale cereale*; The CISCO Companies, Indianapolis, IN, USA; ‘cover crop’) while the second plot was left fallow (‘control’). Approximately 43 days following the last freeze (CFAES 2022), both plots were tilled such that the existing vegetation was sowed into the dredged material prior to sowing rows of corn (variety: W2903DP; Wellman Seeds Inc., Delphos, OH, USA) via tractor on 15 May 2019. To eliminate weedy vegetation, halfway through the growing season (8 July 2019), both plots were sprayed with the herbicide Glyphosate with a 53.8% concentration at a rate of 0.0004 L/m^2 (1.5 qt per acre; Buccaneer 5; CommoditAg, Effingham, IL, USA). To mirror local farming practices, plots were also fertilized with 48% ammonium sulfate (AMS; CommoditAg, Effingham, IL, USA) on 8 July 2019 at a rate of 0.0002 kg/m^2 into 75.7 L of water per 0.4057 ha (1.5 lbs into 20 gallons of water per acre).

Soil Sample Collection

To assess differences in soil properties due to the use of a cover crop, we collected soil samples for analyses of physical, biological, and chemical properties from 10 sampling locations per plot at three collection times throughout the experiment: following site prep but prior to cover crop planting (12 November 2018, ‘pre-cover crop’), after completion of the cover crop life cycle / prior to corn planting (12 April 2019; ‘post-cover crop’) and following final corn harvest (12 October 2019; ‘post-harvest’). Soil samples from 10 haphazardly chosen locations were collected per plot (20 samples total), and each location was marked for future sampling. Samples were transported within six hours of collection to the Rúa lab at Wright State University in Dayton, OH, for further analyses.

Upon arriving at the Rúa lab, a bag containing ~750 g of soil from each sampling location (n = 20) was stored at -20 ^{o}C for up to three months prior to analysis for physical and chemical properties. In addition, one 2 mL tube per sampling site (n = 20) was stored on dry ice during transport and then stored at -20 ^oC for up to six months until analyzed for enzymatic activities. Finally, for post-harvest sampling only (12 October 2019), we also collected one soil core (11.5 cm depth x 10.5 cm diameter) from each sampling location and stored the core at room temperature for 18 hours until processing for bulk density measurement.

Soil Physical and Chemical Properties

The soil physical properties, including soil texture, BD, and gravimetric moisture content, were quantified to assess the effects of cover crop application. To assess soil texture, we measured the % sand, % silt, and % clay within each soil sample using the LaMotte soil texture kit (LaMotte, Chestertown, MD, USA). Gravimetric water content ('moisture') was quantified using the mass ratio such that the samples were weighed wet, dried for 48 hours at 105 ^oC, reweighed dry, and then the ratio of dry to weight was taken to determine the percent of water in each sample (Woods et al. 2019).

To measure the BD of soils, we multiplied the ratio of the volume of the soil core ('field moist volume') to the weight of the soil in the core ('field moist mass') by the ratio of the subsample wet mass to the subsample dry mass from the moisture calculation to calculate the bulk density of the sample **(Equation 1;** Onufrak et al. 2019). As soil cores were not used for the first and second collection times, we acquired field moist volume using a 250 mL beaker as the 'core', filled it with the soil sample, took the final weight ('field moist mass') and used these values to calculate the bulk density of the sample (**Equation 1**). To determine any differences between the core and modified methods, we ran the modified method using the same soils from the core method for three samples per treatment of the third collection time and compared the values obtained per method. The side-by-side comparison resulted in a difference of 0.2652 g/mL between the methods that was then used as a correction factor for the first and second collection times.

$$g/ml^{-1} = \left(\frac{field\ moist\ mass\ (g)}{field\ moist\ volume\ (mL)}\right) * \left(\frac{subsample\ dry\ mass\ (g)}{subsample\ wet\ mass\ (g)}\right)$$

Equation 1. Soil bulk density calculation.

We measured several soil chemical properties, including total N, total P, K, magnesium (Mg^{+}), calcium (Ca^{+}), CEC, pH, and % OM content. Soil pH was assessed using a 1:2 soil to water ratio and was measured using a Fisherbrand^{TM} accumet^{TM} AB15 Basic and BioBasic^{TM} pH / mV / ^{o}C meter (ThermoFisher, Waltham, MA, USA; Woods et al. 2019). Three composite samples from each sampling location per plot (Sample A = 1, 2, 3; Sample B = 4, 5, 6; Sample C = 7, 8, 9, 10) for each collection time (n = 9) were sent to A & L Great Lakes Laboratories (Fort Wayne, IN, USA) for CEC, K, Mg^{+}, Ca^{+}, and OM analysis following EPA recommended laboratory protocols. Total N and total P were measured by the Midden Lab at Bowling Green State University (Bowling Green, OH, USA) using alkaline persulfate digestion with automated colorimetric detection of N and P using an AQ2+ discrete chemical analyzer (Seal Analytical, Mequon, WI, USA), as recommended by the EPA and US Geological Survey (Francy et al. 2020).

Bulk Soil Enzyme Activity

We quantified activities of six extracellular enzymes, including the fluorometric enzymes of alkaline phosphatase (AKP), β-glucosidase (BG), and leucine aminopeptidase (LAP), as well as the colorimetric enzymes of peroxidase (PER), polyphenol oxidase (PPO), and urease (UR). All enzyme assays followed protocols modified from Woods et al. (2019) and Sinsabaugh et al. (2000) and used a homogeneous soil slurry with 0.25 g dry weight of soil sample and 31.5 mL of 50 mM sodium acetate buffer (pH 5.6), except for AKP which required 31.5 mL of NaOH Tris buffer (pH 11.0). Soil slurries (‘soil homogenates’) were stored in the dark at 4 ^{o}C for up to one week prior to analysis. All enzymes were assayed in triplicate alongside a buffer blank, substrate blank, soil homogenate blank, and soil homogenate and substrate mix using a 96-well plate. Fluorometric enzyme analyses also included a standard curve for each sample.

For AKP and BG assays, the fluorescence indicator Methylumbelliferone was used in the form of the substrates 4-Methylumbelliferyl phosphate and 4-Methylumbelliferyl beta-D-glycopyranoside. For LAP, assays were conducted with the substrate L-Leucine-7-amid-4-methylcoumarin hydrochloride and 7-Amino-4-methylcoumarin. Once all reagents and samples were added to the 96-well plate, the prepared plates were left in the dark to incubate at room temperature (22 ^{o}C) for the following times: AKP, nine hours; BG, one to eight hours; and LAP, 96 hours. Following incubation, a NaOH stop solution was added to all wells, and the samples were incubated at room temperature for 10 minutes before reading at 360 nm excitation and 450 nm emission on a BioTek

Synergy HT microplate reader (Agilent, Santa Clara, CA, USA). Using the dilution factor (DF = mL volume of buffer / g of soil sample), extinction coefficient (Ɛ; slope of the sample's standard curves), and the calculated net fluorescence units (NFU; **Equation 2**), all fluorometric enzyme activities were expressed as µmol h^{-1} g^{-1} using **Equation 3**.

$$NFU = SH - SHB - B * \left(\frac{slope\ of\ standard\ with\ sample}{slope\ of\ standard\ with\ buffer}\right)$$

Equation 2. Net Fluorescence Unit (NFU) calculation for fluorometric enzymatic activities where 'SH' is the soil homogenate + substrate fluorescence, 'SHB' is the soil homogenate blank fluorescence, and 'B' is the substrate blank fluorescence.

$$\mu\text{mol}\ h^{-1} g^{-1} = \frac{\left(\frac{\text{NFU}}{\frac{\mathcal{E}}{0.25}\,\text{mL}}\right)}{\left(incubation\ time * \left(\frac{1}{DF}\right) * 0.200\ mL\right)}$$

Equation 3. Fluorometric enzymatic activity calculation using the Net Fluorescence Unit (NFU) and Dilution Factor (DF) for alkaline phosphatase, β-glucosidase, and leucine aminopeptidase.

The colorimetric enzymes PER and PPO were tested using 25 µM 3,4-Dihydroxyphenylalanine with the addition of 3% hydrogen peroxide for PER. Plates were incubated in the dark for 24 hours at room temperature (22 ^{o}C). The plates were read at 450 nm emission on a Molecular Devices Corporation SpectraMax 190 microplate reader (Molecular Devices Corporation, Sunnyvale, CA, USA), and activity in µmol h^{-1} g^{-1} was calculated using net absorbance units (NAU = soil homogenate absorbance – soil homogenate blank absorbance – substrate blank absorbance) and the extinction coefficient (Ɛ) 1.8446 for PER and 2.4942 for PPO (**Equation 4**).

$$\mu\text{mol } h^{-1}g^{-1} = \frac{\text{NAU} * Buffer\ volume\ (ml)}{(\mathcal{E} * 0.200\ \text{mL} * Incubation\ Time\ (hr) * sample\ (g)}$$

Equation 4. Colorimetric enzyme activity calculation using the Net Absorbance Unit calculation (NAU) for peroxidase, polyphenol oxidase, and urease.

UR activity was tested using 10 µl of a 400 mM Urea substrate with an incubation time of between two and seven hours at room temperature (22 ^{o}C) in the dark before adding 40 µl of both salicylate and cyanurate acid and a one-hour incubation time after the addition. We read the plates at 610 nm on a Molecular Devices Corporation SpectraMax 190 microplate reader (Molecular Devices Corporation, Sunnyvale, CA, USA). We used net absorbance units (NAU = soil homogenate absorbance – soil homogenate blank absorbance – substrate blank absorbance) and the extinction coefficient (Ɛ) of 0.2403 to calculate UR activity in µmol NH_4 g^{-1} h^{-1} (**Equation 4**).

Plant Responses

We assessed the response of corn to the cover crop treatment by measuring plant height, leaf count, reproduction status, photosynthetic efficiency, total (above and below ground) biomass, and final yield for 10 focal plants located at the previously sampled soil locations in each plot (total of 20 plants). We measured leaf count and plant height weekly throughout the growing season, starting from plant emergence on 24 May 2019 and ending with the final harvest on 12 October 2019. Plant height (cm) was assessed using a tape measure from the ground to the top of the arch of the highest fully formed leaf (APHIS USDA 2020). Leaf count was measured following the guidelines from the USDA (2020) by counting only the fully formed leaves with an arch. Leaf count was also used to indicate vegetative growth stages (*i.e.*, for V6, the corn plant had six fully developed leaves). Reproductive growth stages of tassel (VT) and kernel development (R1-R6) were assigned when applicable (Nafziger 2017).

Photosynthetic efficiency, which evaluates the efficiency of a plant's photosystem II to capture sunlight and transfer energy, was measured twice during the growing season, once during the vegetation stage (21 June 2019, day 63 of growth) and again during kernel production (31 August 2019, day 110 of growth). We measured photosynthetic efficiency (F_{VM}) using an OS-30 chlorophyll fluorometer (Opti-Sciences, Hudson, NH, USA) following the procedure outlined in Friedman et al. (2020). Briefly, we placed a closed clip on two different leaves, facing the top of the leaf, and allowed the clipped area to equilibrate for 30 minutes prior to light exposure. We then exposed the leaf enclosed by the clip to a low light emission followed by a high light emission and recorded the F_{VM} for each clip per plant. Photosynthetic efficiency was then calculated by averaging the F_{VM} of both clipped leaves for each plant.

To assess yield, we quantified number of ears, kernel count per ear and per plant, the weight of kernels per ear (g/ear) and per plant (g/plant), and total plant biomass (g). Above and below ground biomass were measured twice during the experiment, once to mimic silage harvest (31 August 2019) and once at final harvest (12 October 2019). For silage harvest, plants of similar height and leaf count located near the focal plants were used, while the final harvest plants were the focal plants themselves. For both harvest collections, plants were hand harvested, and the roots were disconnected from the stalk and cleaned to remove excess soil. All plant material was then dried for 48 hours at 60 ^{o}C, and the above and below ground plant material was weighed separately (g). Ears were counted on each corn plant at final harvest. Next, kernels were hand removed from the cob, counted for each ear, and totaled for each plant. Finally, kernels were weighed (g) per ear and per plant.

Statistical Analysis

To determine if the application of a cover crop improved dredged sediment soil property and corn responses compared to a plot left fallow, we ran univariate and multivariate statistical analyses. All statistical analyses were performed in the statistical programming environment R, version 4.2.1 (R Core Team 2022). Data were normalized through log transformation as needed to match model assumptions. All data visualizations were created using the *ggplot2* (Wickham et al. 2022) and *ggbiplot* (Vu 2011) packages.

We created separate linear models for each soil property metric to quantify changes in soil physical properties, chemical properties, and enzymatic activities due to adding a cover crop compared to a plot left fallow. We created linear models using the *lm()* function in base R for % OM, CEC, Ca^{+}, K, and Mg^{+} since these properties were assessed using only three composite samples per plot per collection period. We created linear mixed effects models using the *lme()* function in the *nlme* package (Pinheiro et al. 2021) for % sand, % silt, % clay, and pH and the log transformed data for BD, soil moisture, and activities for AKP, BG, LAP, PER, PPO, and UR with a random effect in each model for unique plant ID. All soil property models were created such that each soil property metric was a function of the interaction of treatment (cover crop vs. control) and collection time [pre-cover crop (12 November 2018), post-cover crop (12 April 2019), post-harvest (12 October 2019)]. We then tested each model with an analysis of variance (ANOVA) using the *anova()* function in base R. In cases with significant relationships between response variables and interaction terms, we performed post-hoc analyses using the *emmeans()* function in the *emmeans* package (Lenth et al. 2022), where all pair-wise comparisons a Tukey's p-value adjustment method. To determine if soil property responses differed between groupings of treatments (cover crop vs. control), we used separate principal component analyses (PCAs) for physical, chemical, and enzyme properties were also created with the *prcomp()* function in base R to condense changes in properties into two linear principal components. We performed an analysis of similarity (ANOSIM) for all PCAs using the *adonis2()* function in *vegan* package (Oksanen et al. 2022).

To quantify changes in corn vegetative development between plots with or without a cover crop application, we created separate linear models for plant stage, height, leaf count, relative growth rate, and root to shoot ratio. We evaluated differences in development time by week due to cover crop use by creating a linear model using the *lm()* function in base R, using week of plant data collection as a function of the interaction between treatment (cover crop vs. control) and plant stage (VE - R6).

To assess differences in plant height over time, we created a linear model using the *lme()* function in the *nlme* package (Pinheiro et al. 2021), where height was a function of the interaction of treatment (cover crop vs. control) and week of data collection with unique plant IDs was a random effect. We then tested our model with repeated measures ANOVA using the *anova()* function in base R, with post-hoc analyses using the *emmeans()* function in the *emmeans* package (Lenth et al. 2022) where all pair-wise comparisons used a Tukey's p-value adjustment method. We created a generalized linear mixed effects model with a Poisson distribution using the *glmer()* function in the *lme4* package (Bates et al. 2022) to evaluate leaf count as a function of the interaction between treatment (cover crop vs. control) and week, with individual plant ID as the random effect. We tested our model with an ANOVA using the *Anova()* function in the *car* package (Fox et al. 2022) and post-hoc analyses for significant interactions using the *emmeans()* function in the *emmeans* package (Lenth et al. 2022) where all pair-wise comparisons used a Tukey's p-value adjustment method.

To quantify changes in plant matter allocation, we calculated the ratio of roots (below ground biomass) to shoots (above ground biomass). We then created a linear model using the *lm()* function in base R, where the root to shoot ratio was a function of treatment (cover crop vs. control) and was evaluated for both silage and final harvests. We then tested our models with an ANOVA using the *anova()* function in base R. Significant interactions were subjected to post-hoc analyses using the *emmeans()* function in the *emmeans* package (Lenth et al. 2022) where all pair-wise comparisons used a Tukey's p-value adjustment method.

Finally, we calculated the relative growth rate (RGR) by week for each plant by taking the height of the current week, subtracting it from the height in the previous week, and dividing that value by the height in the previous week

(**Equation 5**). We then evaluated changes in weekly RGR with a linear mixed effects model using the *lme()* function in the *nlme* package (Pinheiro et al. 2021), where RGR was a function of the interaction between treatment (cover crop vs. control) and current week with individual plant ID as the random effect. We then tested our model with an ANOVA using the *anova()* function in base R and significant interactions were subjected to post-hoc analyses using the *emmeans()* function in the *emmeans* package (Lenth et al. 2022), where all pair-wise comparisons used a Tukey's p-value adjustment method.

$$RGR\ for\ Week\ n = \frac{Height\ (Week\ n+1) - Height\ (Week\ n)}{Height\ (Week\ n)}$$

Equation 5. Calculation for weekly relative growth rate (RGR).

We assessed photosynthetic efficiency (F_{VM}) in both vegetative (V5-V7) and reproductive stages (R3) by creating a linear mixed effects model using the *lme()* function in the *nlme* package (Pinheiro et al. 2021), where photosynthetic efficiency (F_{VM}) was a function of treatment (cover crop vs. control) and plant stage (vegetative vs. reproductive) with the individual plant ID as a random effect. We again tested our model with an ANOVA using the *anova()* function in base R and significant interactions were subjected to post-hoc analyses using the *emmeans()* function in the *emmeans* package (Lenth et al. 2022), where all pair-wise comparisons used a Tukey's p-value adjustment method.

We quantified differences between corn reproductive development when planted on a plot with a cover crop application compared to a plot left fallow by assessing final ear count, kernel count per plant, and kernel weight per plant. For final ear count, we created a generalized linear model with a Gaussian distribution that better captured ears where only half the kernels developed, using the *glm()* function in base R, where ear count was a function of treatment (cover crop vs. control). Final kernel count was assessed with a generalized linear model with a Poisson distribution using the *glm()* function in base R, with kernel count as a function of treatment (cover crop vs. control). Differences in final kernel weight were assessed with a linear model using the *lm()* function in base R, where kernel weight was a function of treatment (cover crop vs. control). All corn reproductive models were tested with an ANOVA using the *anova()* function in base R. We then performed post-hoc analyses using the *emmeans()* function in the *emmeans* package (Lenth et al. 2022), where all pair-wise comparisons used a Tukey's p-value adjustment method for all significant interactions.

Furthermore, since corn yield can encompass both kernel production and the whole plant (silage harvest), yield was also captured with above ground, below ground, and total plant biomass measurements. To evaluate differences in biomass, we created three separate linear models using the *lm()* function in base R, with biomass (above ground, below ground, or total) as a function of treatment (cover crop vs. control) and these models were used for both silage and final harvest. All biomass models were tested with an ANOVA using the *anova()* function in base R, and any significant interactions were tested using post-hoc analyses using the *emmeans()* function in the *emmeans* package (Lenth et al. 2022) where all pair-wise comparisons used a Tukey's p-value adjustment method.

Finally, we assessed the relationship of crop responses associated with production (biomass, kernel count) with individual soil properties. We created linear models using the *lm()* function in base R, where total biomass was a function of a single soil property (Physical: % sand, % silt, % clay, % OM, BD, moisture; Chemical: pH, CEC, N, P, K, Mg^{+}, Ca^{+}; Enzyme Activity: AKP, BG, LAP, PER, PPO, and UR) and treatment (cover crop vs. control). We used generalized linear models using the g*lm()* function in base R for kernel count with a Poisson distribution, where kernel count was a function of a single soil property. We then tested each model with an ANOVA using the *anova()* function in base R, and any significant interactions were assessed using the *emmeans()* function in the *emmeans* package (Lenth et al. 2022), where all pair-wise comparisons used a Tukey's p-value adjustment method.

RESULTS

Soil physiochemical properties

Soil pH significantly changed over time and was dependent on cover crop treatment ($F_{2,36} = 40.00$, $P < 0.0001$, $R^2 = 0.86$, **Fig 2.1A**). While soil pH was initially higher in the plot with the cover crop compared to the control plot, pH increased at post-cover crop collection for both plots so that they were statistically indistinguishable from each other (**Fig 2.1A**). By post-harvest, overall soil pH decreased slightly when compared to the spring collection time but remained statistically indistinguishable between plots (**Fig 2.1A**). Finally, soils from the cover crop treatment had the same pH at the pre-cover crop and post-harvest collection times, while soil pH in the control plot increased over the same period (**Fig 2.1A**).

Differences in soil texture were dependent on treatment but not time. We found a significant decrease in the % sand ($F_{1,18} = 10.35$, $P = 0.0048$, $R^2 = 0.15$, **Fig S2.1A**) in the cover crop plot compared to the control plot, independent of time. In contrast, for % clay, there was a significant difference in the cover crop plot compared to the control plot, independent of time ($F_{1,18} = 2.7380$, $P = 0.0135$, $R^2 = 0.11$, **Fig S2.1B**). We found no significant differences in % silt ($P = 0.1141$), moisture content ($P = 0.7984$) or BD ($P = 0.9597$) between the interaction of treatment and time, or by time ($P > 0.05$), or treatment ($P > 0.05$) independently.

Soil texture (% sand, % silt, % clay), BD, moisture, and pH were all condensed into two principal components, which explained 24.8% and 34.4% of the total variation in the data. The analysis of similarity on the resulting two linear principal components revealed significant differences in soil properties based on both treatment and time, separately (P = 0.003, R^2 = 0.14 **Fig 2.1B**; P = 0.004, R^2 = 0.11, **Fig 2.1C**); however, the interaction of treatment and time was not significant (P = 0.210).

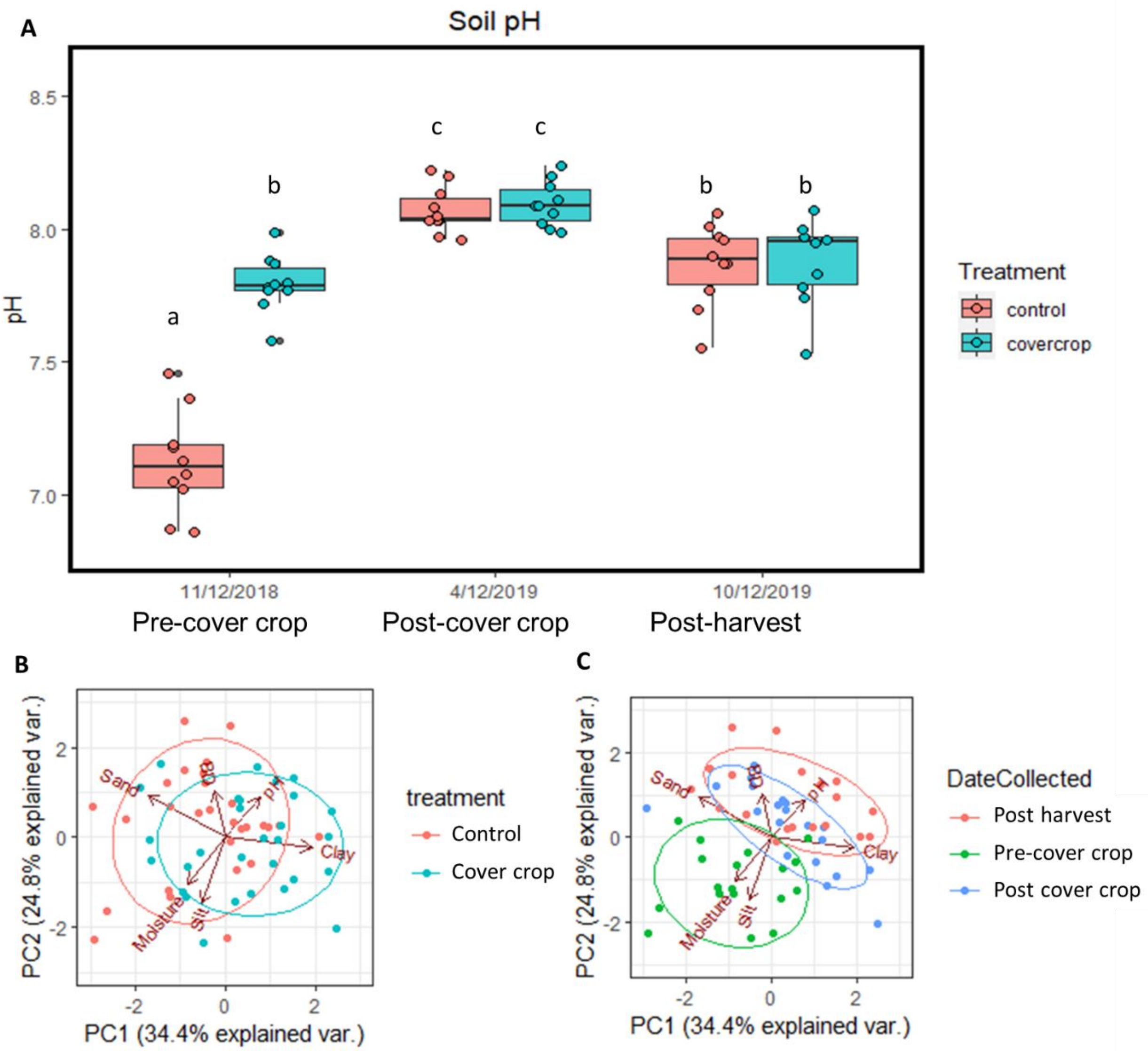

Fig 2.1. (A) Soil pH changed over time and by cover cropping treatment. Soil pH was higher in the plot with a cover crop than the control plot at the initial collection time but increased to the same value in each treatment in the spring, followed by a slightly decreased overall pH in the fall with no difference between treatments ($F_{2,36}$ = 40.00, P < 0.0001, R^2 = 0.86). Treatment is represented by color, where pink is the control plot and blue is the plot with a cover crop applied. Boxes are 50% quartiles, with the thick black line indicating the median pH value. Each point represents one soil sample (n = 10 per treatment), and letters indicate

significant differences based on Tukey's post hoc analyses ($P < 0.05$). Both **(B)** treatment ($P = 0.003$, $R^2 = 0.14$; cover crop = blue, control = pink) and **(C)** time $P = 0.004$, $R^2 = 0.11$ (pre-cover crop = green, post cover crop = blue, post-harvest = pink) were significant when accounting for differences in soil physical properties and pH. Ellipses represent 95% confidence intervals, and points represent individual soil samples (n = 30 per treatment).

When assessing differences in chemical properties of the soil by treatment over time, we found no significant effects of treatments on soil CEC ($P = 0.1054$) or the K content ($P = 0.6504$), Mg^+ ($P = 0.2899$), and Ca^+ ($P = 0.0942$). Similarly, % OM did not change with the use of a cover crop over time ($P = 0.1886$). Total N ($P = 0.8134$) and total P ($P = 0.5323$) also remained stable between treatments over time. The soil chemical properties of CEC, Mg^+, Ca^+, K, N, P, and OM were all condensed into two principal components that explained 33.8% and 39.2% of the variation within this data. An analysis of similarity discerned differences in soil chemical properties based on time ($P = 0.001$, $R^2 = 0.85$, **Fig 2.2**) but not cover crop use ($P = 0.626$) or their interaction ($P = 0.958$).

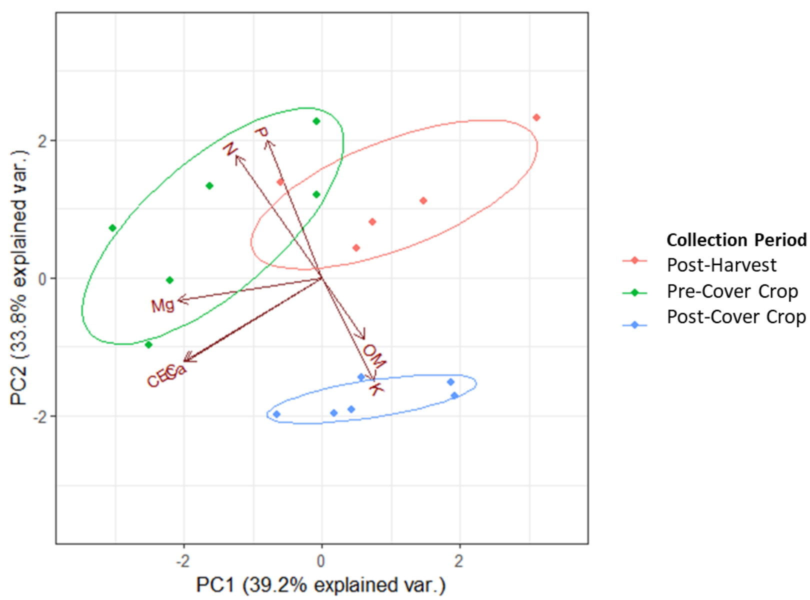

Fig 2.2. Collection time (pre-cover crop = green, post-cover crop = blue, post-harvest = pink) was significant when accounting for differences in soil chemical

properties ($P = 0.001$, $R^2 = 0.85$). Ellipses represent 95% confidence intervals, and points represent individual soil samples ($n = 6$ per collection time).

Enzyme Activities

Differences in enzyme activities due to cover crop treatment and time were enzyme specific. LAP activity significantly changed with collection time by cover crop use ($F_{2,36} = 7.277$, $P = 0.0022$, $R^2 = 0.54$, **Fig 2.3A**). During the pre-cover crop collection time, LAP activity was greater in the plot designated for cover crop planting compared to the control plot left fallow (**Fig 2.3A**). Over the winter, LAP activity increased in the control plot but remained the same in the plot with cover crop application, removing any differences between plots during the post-cover crop collection time (**Fig 2.3A**). However, by post-harvest collection, overall LAP activity decreased in both plots, maintaining no differences between treatments (**Fig 2.3A**). AKP activity did not significantly change by treatment (cover crop vs. control) over collection periods ($P = 0.7930$); however, AKP activity increased over collection period alone ($F_{2,38} = 23.92$, $P < 0.0001$, **Fig 2.3B**). PPO activity did not differ by treatment with collection time ($P = 0.1404$) but was greater in the cover crop plot compared to the control plot, independent of time ($F_{1,18} = 7.250$, P 0.0149, $R^2 = 0.11$, **Fig 2.3C**). We found no significant differences in BG ($P = 0.8021$), PER ($P = 0.1215$), or UR ($P = 0.5272$) activities between treatments over time or independent of time ($P > 0.05$).

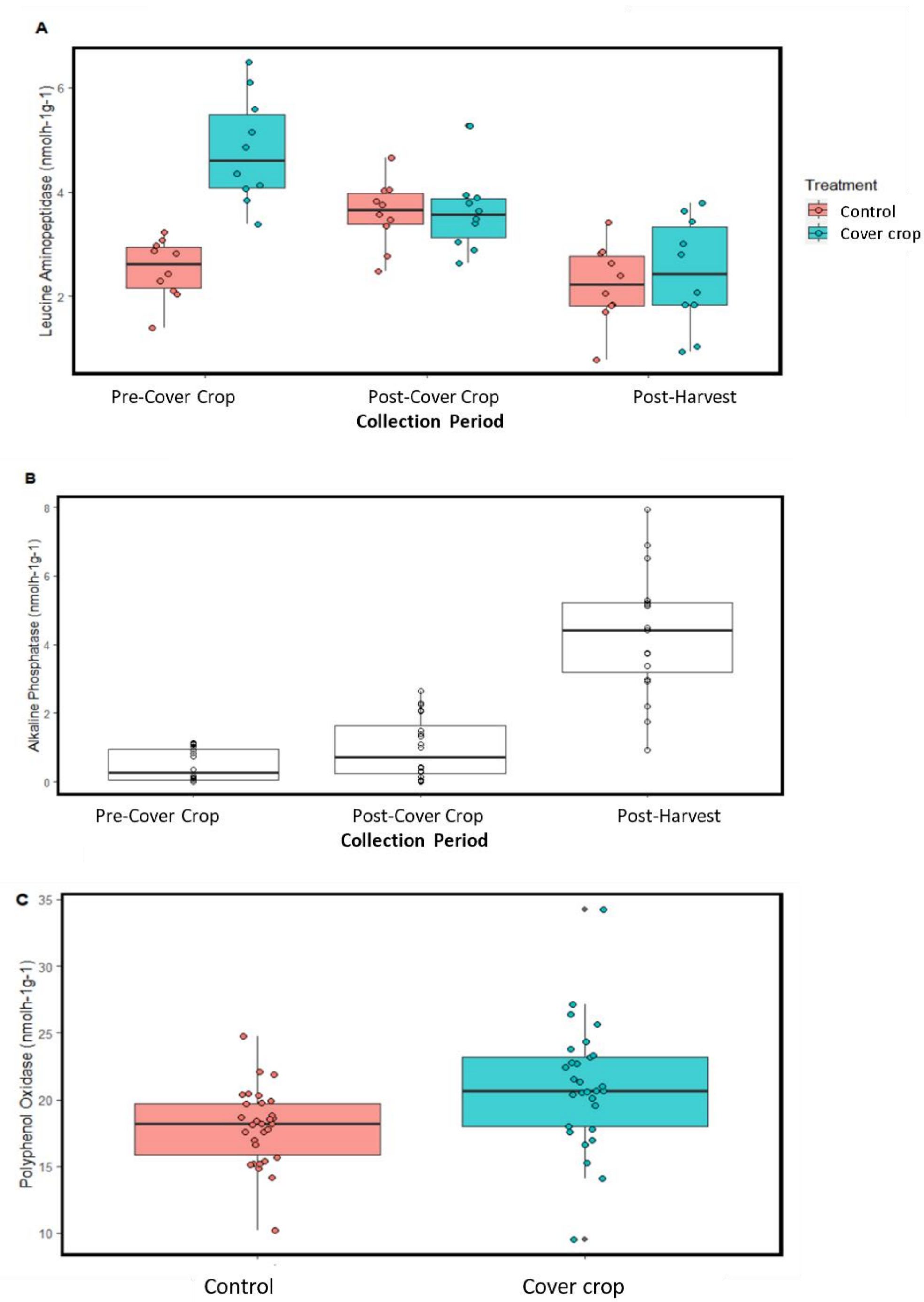

Fig 2.3. (A) Leucine aminopeptidase (LAP) activity decreased in the cover crop applied plot, while LAP activity in the control plot was the same at post-harvest as pre-cover crop. LAP was higher in the plot with a cover crop applied (blue) than in the control plot (pink) at the initial collection time; however, LAP activity decreased in the cover crop treatment to become the equivalent to LAP activity in the control plot in the spring. This change was followed by a slight decrease in LAP activity in both treatments in the fall, maintaining no difference between treatments, returning the control plot to initial activity levels ($F_{2,36}$ = 7.277, P = 0.0022, R^2 = 0.54). Boxes are 50% quartiles, with the thick black line

indicating the median LAP activity. Each point represents one soil sample (n = 10 per treatment). **(B)** Alkaline phosphatase (AKP) activity changed over time but was not different between treatments. AKP activity increased from post-cover crop to post-harvest collections ($F_{2,38}$ = 23.92, P < 0.0001) independent of treatment. Boxes are 50% quartiles, with the thick black line indicating the median AKP activity. Each point represents one soil sample (n = 20 per date collected). **(C)** Polyphenol Oxidase (PPO) activity was significantly different between treatments independent of time. PPO activity was higher in the plot with a cover crop applied (blue) than in the control plot (pink; $F_{1,18}$ = 7.250, P 0.0149, R^2 = 0.11); however, time was not a factor. Boxes are 50% quartiles, with the thick black line indicating the median PPO activity. Each point represents one soil sample (n = 30 per treatment).

AKP, BG, LAP, PER, PPO, and UR activities were condensed into two principal components that explained 20.6% and 30.3% variation within the data. The analysis of similarity on the resulting two linear principal components discerned significant differences in soil enzyme activities based on the use of a cover crop (P = 0.031, R^2 = 0.05, **Fig 2.4A**) and time (P = 0.013, R^2 = 0.10, **Fig 2.4B**); however, the interaction of treatment and time was not significant (P = 0.262).

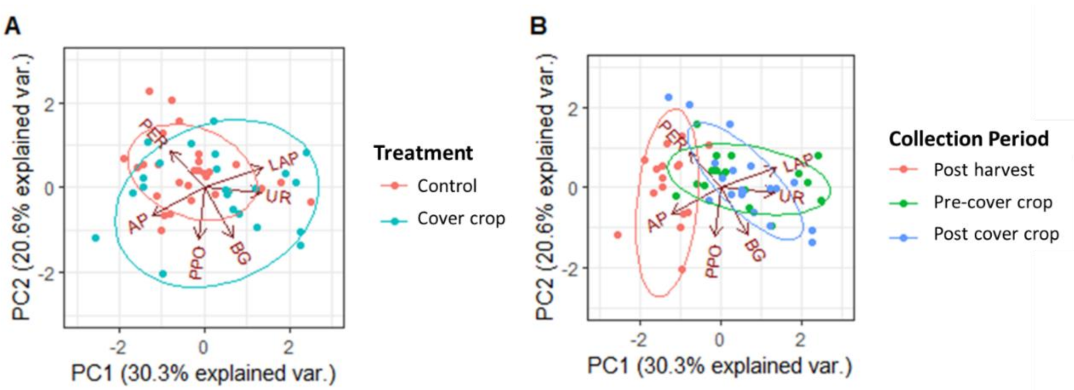

Fig 2.4. Both (**A**) treatment (cover crop = blue, control = pink; P = 0.031, R^2 = 0.05) and (**B**) time (pre-cover crop = green, post-cover crop = blue, post-harvest = pink; P = 0.001, R^2 = 0.10) were significant when accounting for differences in soil enzyme activities. Ellipses represent 95% confidence intervals, and points represent individual soil samples (n = 30 per treatment).

Plant Responses

Plant development varied with the use of a cover crop ($F_{1, 21}$= 1.654, P = 0.0454, R^2 = 0.99, **Fig 2.5A**), with corn in the control plot taking longer to reach plant stages V5 and V6, while corn in the cover crop plot taking longer to reach V11 (**Fig 2.5A)**. There was no difference between corn grown in the cover crop applied plot and the control plot left fallow over time for RGR (P = 1.000, **Fig S2.2**), plant height (P = 0.2242) or leaf count (P = 0.9998), or by treatment or week independently (P > 0.05).

Photosynthetic efficiency was ~56% greater during vegetative development for the control plot compared to the plot with the cover crop application; however, this difference was lost once corn reached reproductive stages in the control plot as photosynthetic efficiency decreased ($F_{3,35}$ = 23.34, P < 0.0001, R^2 = 0.64, **Fig S2.3**).

During both harvests, corn biomass allocation after cover crop application had a ~65% smaller root to shoot ratio than corn grown in the control plot left fallow over the winter (silage harvest: $F_{1,18}$ = 5.458, P = 0.0312, R^2 = 0.19, **Fig 2.5B**; final harvest: $F_{1,18}$ = 6.150, P = 0.0233, R^2 = 0.21, **Fig 2.5C**). However, despite these differences in biomass allocation, we found no significant differences in above ground (silage harvest: P = 0.4364; final harvest: P = 0.0671), below ground (silage harvest: P = 0.0844; final harvest: P = 0.0894) or total plant biomass (silage harvest: P = 0.5638; final harvest: P = 0.0972) between treatments at harvest.

Corn grown after cover crop application had a significant increase in both the number of ears ($F_{1,18}$ = 1.525, P = 0.015, R^2 = 0.29, **Fig 2.5D**) and kernel count per plant ($F_{1,18}$ = 15.00, P < 0.0001, R^2 = 0.32, **Fig 2.5E**) compared to corn grown in the control plot after a fallow season; however, kernel count per ear (P = 0.618), kernel weight per ear (P = 0.157) and kernel weight per plant (P = 0.1749) were not significantly different between treatments.

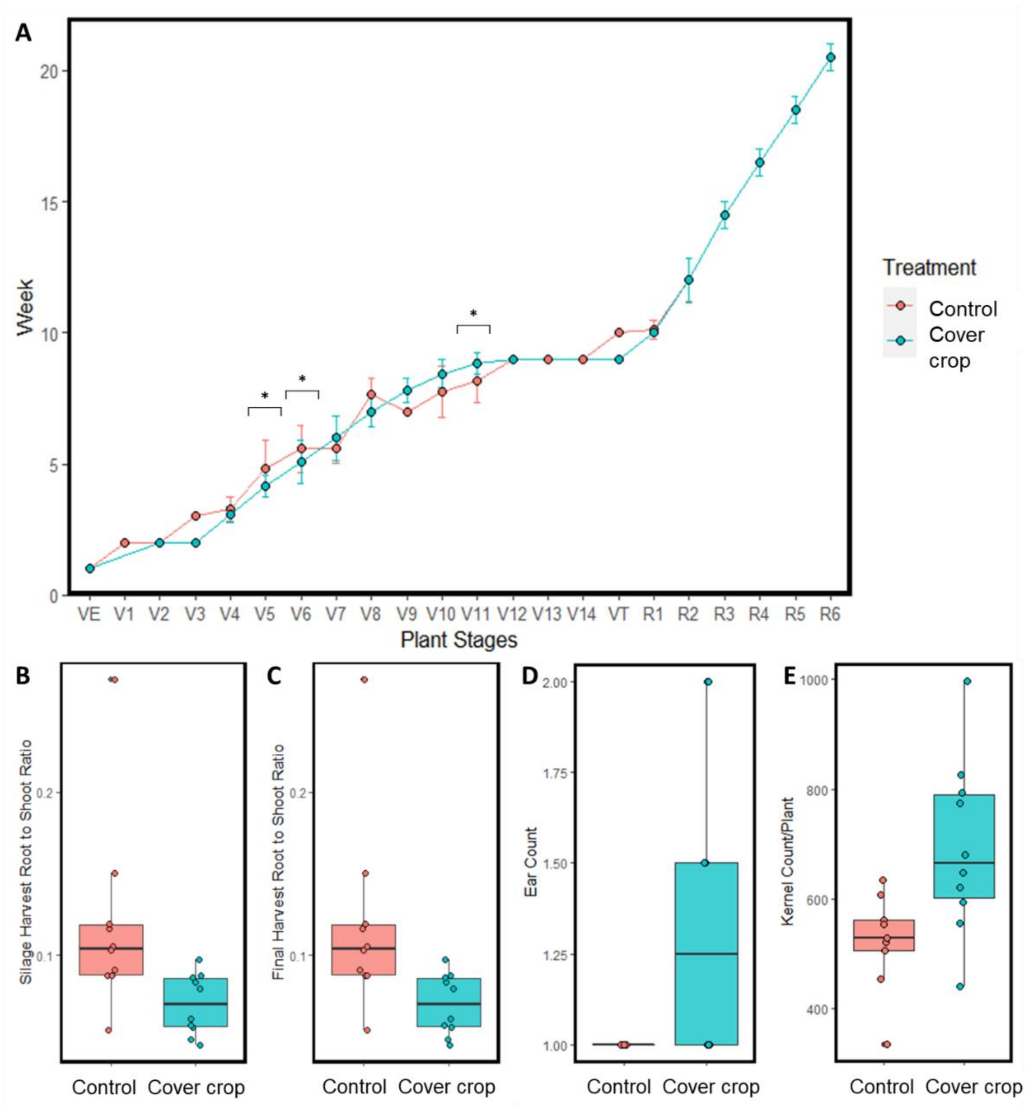

Fig 2.5. (A) Plant stage development over time varied between treatments. Corn grown in the control plot (pink) took longer to reach two vegetative stages and less time for a single vegetative stage than corn grown in the cover crop applied plot (blue; $F_{1, 21}$= 1.654, P = 0.0454, R^2 = 0.99). Each point represents the

average week each plant stage was reached, and asterisks (*) indicate significant differences between treatment by week based on Tukey's HSD ($P < 0.05$). The root to shoot ratio of corn grown in the cover crop plot (blue) was lower than corn grown in the control plot (pink) at **(B)** silage harvest ($F_{1,18} = 5.458$, $P = 0.0312$, $R^2 = 0.19$) and **(C)** final harvest ($F_{1,18} = 6.150$, $P = 0.0233$, $R^2 = 0.21$). Corn yields as **(D)** number of ears per plant ($F_{1,18} = 1.5250$, $P = 0.015$, $R^2 = 0.29$) and **(E)** kernel count per plant ($F_{1,18} = 15.0$, $P < 0.0001$, $R^2 = 0.32$) were higher for corn grown in the cover crop plot (blue) compared to corn grown in the control plot (pink). Boxes are 50% quartiles, with the thick black line indicating the median count, and each point represents one plant (n = 10 per treatment).

Soil properties and plant responses

The impact of soil chemistry on plant growth was dependent on treatment, with several soil chemical properties significantly influencing final kernel count and total plant biomass. We found that CEC ($F_{3,2} = 9.369$, $P = 0.0384$, $R^2 = 0.83$, **Fig S2.4A**), Ca^+ ($F_{3,2} = 20.43$, $P = 0.0470$, $R^2 = 0.92$, **Fig S2.4B**) and pH ($F_{3,16} = 4.134$, $P = 0.0239$, $R^2 = 0.33$, **Fig S2.4C**) significantly impacted plant biomass as a function of cover crop application such that when the soil property increases, the total biomass of the plant from the control plot increases while total biomass in plants from the cover crop plot decreases.

Six of the physical and chemical soil properties we assessed influenced kernel count. For example, as N increased, kernel count increased for the corn grown after the cover crop, while kernel count decreased for corn grown in the control plot ($F_{5,8} = 16.03$, $P < 0.0001$, $R^2 = 0.67$, **Fig 2.6A**). Additionally, as K increased, the kernel count of corn grown after the cover crop application also increased; however, the kernel count of corn grown in the control plot decreased ($F_{2,5} = 11.73$, $P = 0.0115$, $R^2 = 0.87$, **Fig 2.6B**). Total P ($F_{5,8} = 13.52$, $P < 0.0001$, $R^2 = 0.66$, **Fig 2.6C**), CEC ($F_{2,5} = 1.840$, $P = 0.0069$, $R^2 = 0.98$, **Fig 2.6D**), Ca^+ ($F_{2,5} = 1.821$, $P = 0.0024$, $R^2 = 0.98$, **Fig 2.6E)**, BD ($F_{2,18} = 6.601$, $P = 0.0101$, R^2

= 0.37, **Fig 2.6F**) and % silt ($F_{2,18}$ = 38.16, P < 0.0001, R^2 = 0.40, **Fig 2.6G**) all had similar effects such that as the soil property increased, the kernel count for corn grown in the control plot increased, whereas the kernel count for the corn grown in the cover crop plot decreased.

Soil enzyme activities also drove differences in kernel count. As BG activity increased, kernel count in the cover crop and control plots decreased ($F_{2,18}$ = 10.19, P = 0.0014, R^2 = 0.40, **Fig 2.6H**). As LAP activity increased, kernel count increased for corn grown on the cover crop plot, whereas kernel count for corn grown in the control plot decreased ($F_{2,18}$ = 42.53, P < 0.0001, R^2 = 0.39, **Fig 2.6I**). Finally, as PER activity ($F_{2,18}$ = 9.601, P = 0.0019, R^2 = 0.44, **Fig 2.6J**) and UR activity ($F_{2,18}$ = 7.423, P = 0.0065, R^2 = 0.64 **Fig 2.6K**) increased, the kernel count of corn grown in both the cover crop and control plots decreased.

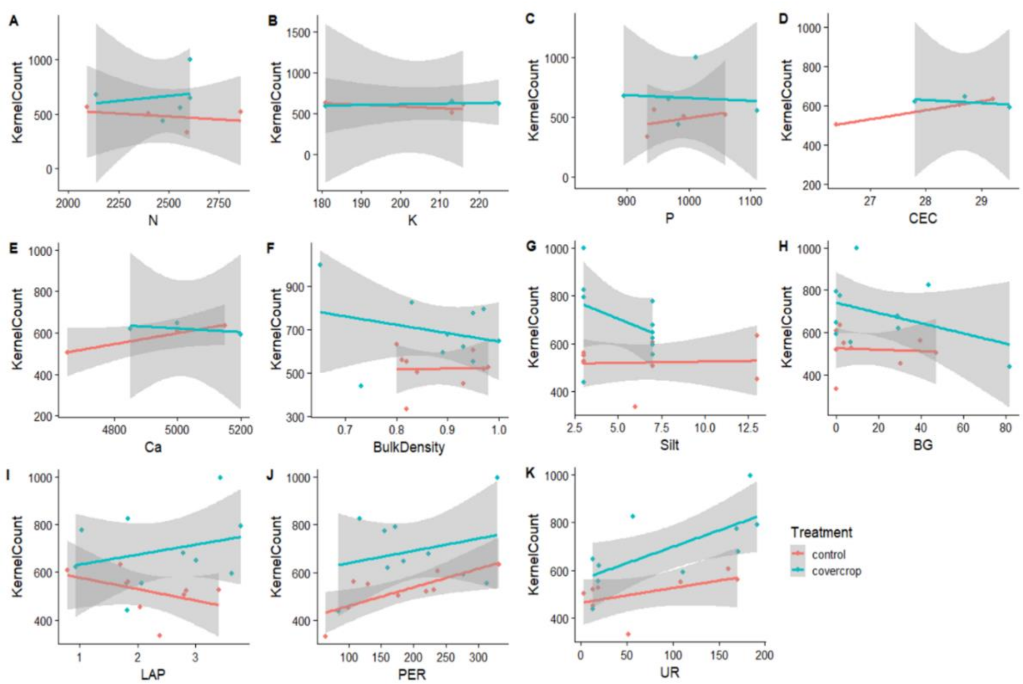

Fig 2.6. (A) Total nitrogen (N; $F_{5,8}$ =16.03, P < 0.0001, R^2 = 0.67), **(B)** potassium (K; $F_{2,5}$ = 11.73, P = 0.0115, R^2 = 0.87), **(C)** total phosphorus (P; $F_{5,8}$ =13.52, P < 0.0001, R^2 = 0.66), **(D)** Cation Exchange Capacity (CEC; $F_{2,5}$ = 1.840, P = 0.0069, R^2 = 0.98), **(E)** Calcium (Ca^+; $F_{2,5}$ = 1.821, P = 0.0024, R^2 =

0.98), **(F)** bulk density (BD; $F_{2,18} = 6.601$, $P = 0.0101$, $R^2 = 0.37$), **(G)** % silt ($F_{2,18} = 38.16$, $P < 0.0001$, $R^2 = 0.40$), **(H)** β-glucosidase **(**BG; $F_{2,18} = 10.19$, $P = 0.0014$, $R^2 = 0.40$), **(I)** leucine aminopeptidase (LAP; $F_{2,18} = 42.53$, $P < 0.0001$, $R^2 = 0.39$), **(J)** peroxidase (PER; $F_{2,18} = 9.601$, $P = 0.0019$, $R^2 = 0.44$), and **(K)** urease (UR; $F_{2,18} = 7.423$, $P = 0.0065$, $R^2 = 0.64$) activity significantly corresponded with kernel count of both the cover crop (blue) and control (pink) treatments. Shaded regions represent 95% confidence intervals, and each point represents one plant (n = 10 per treatment for BD, % silt, BG, LAP, PER, and UR; n = 5 for N and P; n = 3 for K, CEC and Ca^{+}).

DISCUSSION

Cover crop application has gained momentum as a beneficial soil management practice, doubling in use from 3% of all agricultural land in 2012 to 7.3% in 2021 (Hellerstein et al. 2019; Wallander et al. 2021). This increase is directly related to the wide variety of benefits cover crops provide, including: enhancing soil structure, increasing organic matter, reducing soil erosion, and promoting pest management (Gabriel and Quemada 2011; Steele et al. 2012; Kim et al. 2020; Wittwer and van der Heijden 2020). Additionally, previous research has shown that combining cover crop application with soil amendments further increased soil stability, crop production, and yields (Fernandez et al. 2016; Sánchez de Cima et al. 2016; Ashworth et al. 2017; Adeli et al. 2019; Raut et al. 2020). However, despite these benefits, the effect of using a cover crop with dredged sediments on soil properties and its subsequent relationship with crop production is still largely unknown. Here we show that using a cover crop on dredged sediments increased corn yields compared to corn grown on dredged sediments left fallow. We further show that this effect was mediated through increased nutrient mineralization as a direct result of increased microorganism-associated enzyme activities.

Nutrient Dynamics Drive Corn Biomass and Production

Cover crop application positively influenced the relationship between N cycling properties and corn yields. Following cover crop application, we found a positive relationship between total kernel count and total N content as well as the enzymatic activities associated with N mineralization (LAP, PER, and UR). However, we failed to find a significant increase in total plant biomass with N mineralization-associated enzymes or total N content. The increase in kernel count, but not biomass, suggests that cover crop application promotes the conversion of bioavailable N for plant uptake beneficial for increased kernel count by increasing microorganism enzymatic activities associated with nutrient mineralization. Despite our findings aligning with previous findings in agricultural systems (Jayasundara et al. 2007; Nyiraneza et al. 2010) the use of winter rye cover crop provided beneficial relationships different than those previously described following winter rye use. Specifically, we found that corn yields increased with increased N mineralization following cover crop application, which contrasts previous research using winter rye (Chim et al. 2022). The increases in kernel count driven by N-mineralization-associated enzyme activities supports our hypothesis for coupling a cover crop with dredged sediments for agricultural use, which is the goal of using soil amendments in agricultural systems.

Although we found significant relationships between soil properties related to N mineralization and increases in kernel count following cover crop application, we failed to find similar relationships for soil properties related to P cycling. Specifically, total kernel count decreased as total P content increased following cover crop application, and there was no significant relationship found between total P content and plant biomass. This negative relationship between kernel production and total P content and lack of relationship between plant biomass and total P content or AKP activity was surprising as P is vital for corn plant growth and kernel production (Hellerstein et al. 2019; USDA 2019). Additionally, there was no significant relationship between total kernel count or plant biomass and AKP activity, which is associated with P mineralization. The lack of change in AKP activity suggests the microorganism community is failing to mineralize the cover crop-driven increase in total P, making it unavailable for plant uptake. This mirrors other cover crop research in which the P content in soils can either be taken up directly by plants (Mori et al. 2023) or used by microorganisms in other ways such as increased biomass (Hallama et al. 2019). Another potential explanation for high total soil P content coupled with a lack of AKP activity, is that P may be fixed within the soil, potentially bound to heavy metals creating non-bioavailable forms such as iron phosphate (USDA 2019; Durrer et al. 2021). The lack of any positive relationship between kernel production and P cycling suggests that cover crops applied to dredged sediments may not support P mineralization needed for increasing kernel production. However, to evaluate the relationship of the P cycle dynamics in dredged sediments more effectively, future research should focus on P uptake in plants and how those patterns differ from uptake in traditional agricultural systems.

The relationship between the breakdown of OM into mineralized C and total kernel count was directly linked to the application of a cover crop; however, we failed to establish this connection with plant biomass. Overall, we found an inverse relationship between BG activity and total kernel count, indicating that an increase in the breakdown of OM does not translate into increased corn production. The trade-off suggests that there is a threshold for mineralized C within agricultural soil systems, and higher levels of OM breakdown due to cover crop application may not be necessary to increase corn yields (Aon and Colaneri 2001). This tradeoff mimics other research which found a negative effect on BG activity following cover crop application in non-amended agricultural soils (Bandick and Dick 1999) and non-dredged sediment amended agricultural soils (Ashworth et al. 2017). However, these findings contradict evidence that applying a cover crop alters the C cycle within soils by providing mineralized C for use within agricultural systems (Schipanski et al. 2014; Aldridge et al. 2019). This disparity may have been caused by the fact the cover crop used in this study did not grow to senescence but was tilled under while still growing. Generally, microorganisms can form microaggregates from OM provided by a cover crop, sequestering C within the soil (Gougoulias et al. 2014); however, this relationship was not seen in our study, likely due to the life-cycle of the cover crop. While our results suggest that cover crop applications are not necessary for increasing kernel production as dredged sediments alone change C cycling in ways sufficient to increase corn yield, future research should evaluate the relationship between multiple cover crop growing seasons and corn grown on dredged sediments to understand how the C cycle dynamics are impacted.

In addition to nutrient cycling, cover crop application on dredged sediments drives changes in the relationships between other chemical properties and plant responses. Our results indicate an inverse relationship between both total kernel count and total plant biomass to Ca^{+} content, CEC, and soil pH with cover crop application. This suggests high levels of Ca^{+} are not needed for kernel production and higher cations levels and soil pH are not conducive to increase plant biomass; however, these patterns oppose those found in non-dredged sediment amended agricultural systems (Tarkalson et al. 2006). The positive relationship between K content and total kernel count with cover crop application contrasts both the relationships with other cations within this study as well as previous research where higher K content reduced overall corn production in both traditionally farmed agricultural systems (Rhem 1994) and cover crop applied systems (Jian et al. 2020). Total plant biomass did not demonstrate any significant relationship with K content. Taken together, these findings suggest increased soil pH, Ca^{+} content, and CEC in dredged sediments due to cover crop application are not necessary for overall kernel production or plant biomass. Consequently, based on changes in soil chemistry, cover crop application on dredged sediments may not be necessary to improve overall plant development. These outcomes were surprising as soil chemistry is typically an important driver of plant development in agricultural soils (Russell et al. 2006; Gaspar 2019).

Similar patterns emerged when quantifying the relationships between physical or chemical soil properties and plant responses, which is not surprising as these properties themselves are interconnected. Total kernel count decreased with increasing physical properties of BD and silt content due to cover crop application; however, this relationship did not extend to plant biomass. These results suggest higher BD is bad for kernel production when grown on dredged sediments following cover crop application, which is unusual in agricultural systems; previous studies have shown that generally corn production on agricultural soils decreases when BD declines following cover crop application (Koudahe et al. 2022). This differential response in the relationship between BD and kernel production with cover crop application suggests there are overall differences in soil stability between agricultural soil and dredged sediments (Marlin 2002; Vermeulen et al. 2005; Oliveira et al. 2017). The negative relationship following cover crop application between BD and kernel production shows cover crop application is unnecessary to change BD to increase crop production.

Cover Crop Application Alone Drives Corn Yield Increases

Several corn responses increased directly due to the application of a cover crop but were not mediated by physical, chemical, or biological soil properties. Corn grown in the cover crop applied plot produced 1.5-2x more ears compared to the plot left fallow. In addition to the increased corn yields following cover crop application, the combined corn yield from both experimental plots from our dredged sediment experiment was 20% greater than surrounding agricultural fields (Lucas County, Ohio; NASS USDA 2020). The increased yields from corn grown in dredged sediments lead to differences in biomass allocation between treatment plots.

Plant biomass allocation, but not total biomass, was influenced by the application of a cover crop. Differences in plant biomass distribution is typical in low nutrient environments, where plants require more roots compared to high-nutrient environments, in which plants distribute biomass towards reproduction (Poorter et al. 2012). Similarly, in our study, corn in the fallow control plot developed ~65% more roots to obtain the necessary nutrients that cover crops aid in providing to promote corn production, which aligns with plant allocation patterns typically found in agricultural systems (Postma et al. 2014; Gabriel and Quemada 2011). These findings demonstrate that combining a cover crop and dredged sediments can increase overall corn production.

The time it took to reach a vegetative or reproductive stage (plant stage development) varied in response to cover crop application but was not driven by changing soil properties. Specifically, the time to reach plant stage V5 and V6 was significantly faster in the cover crop applied plot while V11 was reached faster in the control plot left fallow. However, the time to reach any of the reproductive stages did not significantly vary. We found no significant differences in either RGR, the number of leaves or height for the corn, as vegetative stages are representative of developed leaves and RGR is representative of plant height. Altogether, our results suggest that when cover crops are applied to dredged sediments they can enhance early corn growth and development, which align with corn responses to cover crop application in non-dredged sediment-supplemented agricultural soil systems (Abendroth et al. 2011; Gabriel and Quemada 2011; Steele et al. 2012). However, the short-lived differences in plant stage development between the cover crop and fallow treatments suggest cover crop application may not be needed to stimulate corn growth when coupled with dredged sediments.

In this study, corn production varied following cover crop application on dredged sediments. There was a significant increase in corn yield following cover crop application compared to the control plot left fallow. However, despite the yield differences between the cover crop and non-cover crop plots, we failed to find meaningful differences in plant growth and development for plant height, RGR, leaf count, or plant stage development. Additionally, cover crop application was not beneficial for photosynthetic efficiency as efficiency was significantly greater in the control plot left fallow during vegetative development and decreased over time regardless of treatment. Responses for corn grown in dredged sediments following cover crop application varied from those documented within non-dredged sediment amended agricultural systems, with many benefits missing (Mahama et al. 2016; Acharya et al. 2020; Chim et al. 2022). Although corn yield was the only plant response to benefit from cover crop application due to changes in soil properties, the combination of cover crop application with dredged sediments may be beneficial but not necessary to improve corn production.

Soil Properties Differ in Response to Cover Crop Application

Several dredged sediment soil properties failed to significantly vary between the cover crop and non-cover crop plots. Total N, UR activity, and LAP activity remained consistent throughout the growing season, regardless of cover crop application, suggesting constituents of the N cycle are unaltered by the application of a cover crop in dredged sediments. This pattern is opposite to other patterns for non-dredged sediment agricultural soil systems where N mineralization rates increase following cover crop application (Christopher et al. 2021). Additionally, LAP activity, which is linked to both N and C mineralization through the breakdown of protein in soils (Greenfield et al. 2021), remained unchanged in the plot left fallow but decreased over time in the cover crop plot. These results suggest cover crop application aids dredged sediments in maintaining sufficient nutrient availability, leading to a reduced need for microorganism associated LAP activity (Gabriel and Quemada 2011; McDaniel et al. 2014; Wittwer and van der Heijden 2020).

Overall, there were varied responses in the soil properties within the C cycle between plots. Specifically, there were no differences between the treatment plots for OM content, or BG and PER activity, which are two enzymes associated with OM breakdown to mineralized C. However, PPO activity, which is responsible for more complex C breakdown, significantly increased within the cover crop applied plot compared to the plot left fallow, regardless of time. Increases in PPO activity may suggest that as C becomes limited in the sediments, it may be necessary for the breakdown of more complex OM provided by a cover crop (Aon and Colaneri 2001; Hungria et al. 2009; Jamir et al. 2019). The lack of difference in total OM content between the treatments coupled with the increases in PPO activity differed from results in most agricultural soils systems where cover crop application is known to increase OM content (Hellerstein et al. 2019; Acharya et al. 2020). These patterns together suggest cover crop application on dredged sediments is not necessary to alter OM content due to the already high amount of OM found in dredged sediments (Sigua 2005; Oliveira et al. 2017; Kiani et al. 2021).

Constituents of the P cycle also did not vary between the cover crop and non-cover crop plots. Total P content remained consistent throughout the growing season, regardless of cover crop application. However, AKP activity, which is associated with P mineralization, increased over time regardless of cover crop application. These findings suggest that regardless of cover crop application, mineralized P is limited in dredged sediments throughout a growing season (Hallama et al. 2019), which mirrors typical outcomes in agricultural soil systems with (Zhu et al. 2018; Hellerstein et al. 2019) or without cover crop application (Hou et al. 2012; Zhu et al. 2018; Hellerstein et al. 2019). The results from this study suggest that using dredged sediments in agricultural systems coupled with cover crop applications may not address the limited P issues currently in agriculture. Since this study was only over one growing season, additional research investigating multiple growing seasons may provide further insight.

While moisture content remained consistent between the cover crop applied plot and the plot left fallow, this outcome has unique context. Dredged sediments are known to have high water holding capacity, as they are sediments originally removed from an aquatic environment (Averett et al. 1990), so there is significant concern about their ability to retain water during high precipitation events (Vermeulen et al. 2005). Our study took place in Toledo, Ohio, USA (Lucas County) following several severe weather events between November 2018 through April 2019 that caused the United State Department of Agriculture to declare a natural disaster area (USDA 2019). The study site and surrounding county experienced 72% more rainfall in November 2018 compared to the 30-year average, followed by extreme cold, dry, and windy weather from the polar vortex from January until April, and concluding with another period of intense precipitation during which the region experienced a 37% increase in rainfall compared to the 30-year average in both March and April 2019 (USDA 2019; Toledo Weather Records 2019). Despite these circumstances, soil moisture content remained consistent at ~30% from pre-cover crop to post-cover crop collection periods and decreased over the corn growing season, regardless of cover crop application. These findings support previous research showing that dredged sediments can withstand significant precipitation events and not become oversaturated (Vermeulen et al. 2005; Oliveira et al. 2017).

The ability of dredged sediments to maintain consistent moisture content is essential for their use in agricultural systems, as changes in moisture content are linked to changes in soil chemistry (Rengasamy et al. 2022). The CEC and associated cations K, Ca^{+}, and Mg^{+} all remained consistent within the plots, regardless of cover crop application. Additionally, soil pH also remained consistent at the high end of the neutral range for corn, between 7.5-8, regardless of cover crop application. This consistency in soil chemistry supports the hypothesis that dredged sediments, regardless of cover crop application, can create and maintain a hospitable chemical environment for corn production (Oliveira et al. 2017; Darmody and Diaz 2017). This is particularly valuable since the slightly basic dredged sediments could be used to neutralize agricultural soils which have become increasingly acidic due to repeated synthetic fertilizer application (Darmody and Marlin 2002; Ozlu and Kumar 2018; Iqbal et al. 2021).

Aligning with the patterns seen within the soil chemistry, dredged sediment structure and stability remained unchanged over time regardless of cover crop application. BD remained consistent at 0.8803 ± 0.0106 g/ml^{-1} regardless of cover crop application, which is just below the USDA recommended range of 1.0 -1.4 g/ml^{-1} (NRCS USDA 2019). The consistency in BD indicates dredged sediments can maintain soil porosity regardless of cover crop application, which is necessary for nutrient and water retention and vital for root development (Rhem 1994; Arshad et al. 1997; Jiang and Thelen 2004; Daniels et al. 2007; Darmody and Diaz 2017; Gaspar 2019). The ratio of soil textures did not change with the addition of a cover crop, and initial texture ratios were maintained throughout the corn growing season. No change in soil texture is consistent with prior research showing the texture of dredged sediments remains stable in field applications, with or without cover crop applications (Villamil et al. 2006; Steele et al. 2012; Darmody and Diaz 2017). The ability of dredged sediments to maintain their physical soil structure and stability regardless of cover crop application supports prior research demonstrating dredged sediments' physical properties often align with agricultural standards (Sigua 2005; Daniels et al. 2007; Darmody and Diaz 2017).

Cover crop application has the potential to increase crop yields when coupled with dredged sediments and has many additional benefits in agricultural soil systems, including the improvement of BD and water content, microbial-driven nutrient mineralization, and crop yield increases (Gabriel and Quemada 2011; Steele et al. 2012; Hellerstein et al. 2019; Kim et al. 2020; Wittwer and van der Heijden 2020; Acharya et al. 2020). Within this study, cover crop had little influence on soil properties, with only the most notable relationship found in microbial-driven enzymatic activities leading to increased corn yields. The differences in outcomes from cover crop application may be due to the specific cover crop we used in this study to reflect local growing practices, winter rye, which has been known to only moderately improve soil properties and actually decrease overall corn production (Acharya et al. 2020). Consequently, the results from our study may not fully reflect the potential for cover crops to improve dredged sediments for agricultural use but instead reflect the unique effect of using winter rye prior to planting corn.

Conclusion

In investigating the impact of cover crop application on dredged sediments, corn yields increased following cover crop application compared to dredged sediments left fallow. Yield increases were mediated through increased microorganism-associated enzyme-driven nutrient mineralization, specifically N mineralization, which was not evident in the plot left fallow. However, we failed to find positive relationships between either kernel count or plant biomass and most other soil physical, biological, and chemical properties following cover crop application. Together, soil property and plant responses indicate that dredged sediments, especially in combination with a cover crop, have the potential to increase crop yields while maintaining soil stability and microorganism activity compared to traditionally farmed agricultural soils (Sigua 2005; Darmody and Diaz 2017; Villamil et al. 2006). Furthermore, dredged sediments, regardless of cover crop application, demonstrated a consistent water holding capacity even when inundated with excessive precipitation events. The work presented here represents an important step in advancing dredged sediment research and expanding current agricultural practices to further increase crop production and potentially repair degraded agricultural soils.

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CHAPTER 3

THE AMENDMENT OF WEATHERED DREDGED SEDIMENTS OUTPERFORMED FRESH DREDGED SEDIMENTS BY IMPROVING SOIL PROPERTIES IN AGRICULTURAL SOILS

ABSTRACT

To mitigate soil degradation, farmers are constantly improving field management practices including crop rotations, cover crop applications and soil amendment additions. Dredged sediments have gained attention as a potential soil amendment due to their high nutrient content and water holding capacity. They can be applied in two conditions: fresh from the aquatic environment or following a weathering period on land. However, much is unknown about the optimal condition of dredged sediments when used as a soil amendment. Furthermore, there is limited research investigating realistic application rates of dredged sediments following the USDA and EPA guidelines or how these rates may vary should dredged sediments be applied as fresh or weathered. Our research aims to evaluate what application ratio is optimal for corn growth and determine whether dredged sediment condition alters that ratio. To do so, we factorially manipulated dredged sediment condition and dredged sediments: agricultural soil application ratios (1:99, 3:97, 5:95, 10:90) in a greenhouse experiment using corn as a focal species. We also included treatments with 100:0 agricultural soil and 100:0 fresh and weathered dredged sediment treatments. Soil samples were collected prior to corn planting and again at final harvest to assess 12 physical and chemical properties and nine enzyme activities related to soil function. Corn responses were assessed throughout the growing season and at harvest. We found that dredged sediment condition drove changes in agricultural soil properties

and corn performance more than application ratio. Additionally, the 100:0 weathered dredged sediments provided the most beneficial environment for corn growth compared to the agricultural soil while the 100:0 fresh dredged sediments were the least beneficial for plant survival. The research presented here is the first to quantify differences in dredged sediment condition when applied to agricultural soils and provide a better understanding of dredged sediment use in US agricultural systems.

INTRODUCTION

Dredged sediments have been shown to be an effective organic soil amendment to help alleviate soil degradation in row-cropping agriculture and increase crop production (Canet et al. 2003; Sigua 2005; Daniels et al. 2007; Darmody and Diaz 2017; Brigham et al. 2021; Julian et al. *in review*). Despite the growing area of research surrounding the use of dredged sediments in agriculture, there is still so much that remains unknown. For example, applying a soil amendment should be based on the USDA recommended nutrient recovery ratio (Hellerstein et al. 2019) and the US EPA recommended amendment application guideline (2013). However, there is little research regarding the most beneficial rates of applications of dredged sediment based on these recommendations, with most using large application rates (20-100%; Canet et al. 2003; Ebbs et al. 2006; Darmody and Diaz 2017; Brigham et al. 2021; Kiani et al. 2021) or mixing with other soil amendments (Sigua 2009; Oliveira et al. 2017). Dredged sediments can be used in two different conditions: fresh which are from their aquatic source, and weathered which are stored on land to de-water. Currently, there is no research comparing the use of these dredged sediment conditions for use in agricultural systems using application guidelines. Here we used a greenhouse experiment to investigate changes in soil properties and crop responses with the use of dredged sediments in different conditions which were applied at different application rates to agricultural soils.

Dredged sediments can improve soil physical, chemical and biological soil properties when applied in an agricultural system (Fonseca et al. 1998; Darmody and Marlin 2002; Canet et al. 2003; Sigua et al. 2004; Sigua 2005; Daniels et al. 2007;

Darmody and Diaz 2017; Rúa et al. 2023). Dredged sediments can alter soil texture ratios to better match ideal crop growing conditions (Canet et al. 2003; Daniels et al. 2007; Darmody and Diaz 2017) while also increasing bulk density (BD) and water holding capacity and reducing soil compaction, giving plant roots adequate air, water and space to grow (Canet et al. 2003; Sigua et al. 2004; Gabriel and Quemada 2011; Steele et al. 2012). These nutrients Carbon (C) and nitrogen (N) can be severely depleted in agricultural systems (Nair 2019; Chowdhury et al. 2021) and exacerbated by erosion (Hellerstein et al. 2019; Kopittke et al. 2019). Applying dredged sediments to agricultural soils can alleviate this nutrient limitation, as these sediments often contain high levels of organic matter (OM), N, phosphorus (P), and micronutrients (Fonseca et al. 1998; Darmody and Marlin 2002; Sigua et al. 2004). Additionally, dredged sediments contain microorganisms beneficial for crop growth (Rúa et al. 2023) and these microorganisms can increase nutrient mineralization in dredged sediments (Julian et al. *in review*), leading to increases in crop yields.

Fresh dredged sediments can provide a variety of benefits to agricultural systems. In addition to the high nutrient content found in most fresh dredged sediments (Darmody and Marlin 2002; Sigua et al. 2004), they can also improve soil moisture properties with increased moisture content and increased water holding capabilities, potentially providing benefits to drier agricultural systems (Vermeulen et al. 2005; Darmody and Diaz 2017). However, fresh dredged sediments may also contain high levels of harmful contaminants of concern such as heavy metals, pharmaceuticals and others (Lee et al. 1982; Tang 1999; Vácha et al. 2011). Fresh dredged sediments can also be extremely difficult to transport to the field, as they are water-saturated and heavy. The drawbacks of containments and transport issues when using fresh dredged sediments have led to the development of methods for weathering these sediments before use in agricultural systems (Cappuyns et al. 2004; Vermeulen et al. 2003, 2005, 2007; Oliveira et al. 2017).

The weathering processes of dredged sediments co-occur over time and fall into three main categories of ripening based on the mechanism of change to the sediments: physical, chemical, and biological. Physical ripening occurs when the particles and aggregates of dredged sediments shift towards more terrestrial soil-like consistency (Cappuyns et al. 2004; Vermeulen et al. 2003, 2005, 2007; Daniels et al. 2007; Oliveira et al. 2017). Chemical ripening of dredged sediments changes the pH and electrical conductivity (EC) of sediments, creating a more hospitable chemical environment which is necessary for plant growth (Cappuyns et al. 2004; Vermeulen et al. 2003, 2005, 2007; Oliveira et al. 2017). Biological ripening occurs when soil biota shift from mostly aquatic communities to mostly terrestrial communities, and this happens concurrently with the physical and chemical ripening processes (Cappuyns et al. 2004; Vermeulen et al. 2003, 2005, 2007; Oliveira et al. 2017). As the ripening process is occurring, aerobic bioremediation of contaminants, or the breakdown of chemical compounds through biological processes, is also taking place (Cappuyns et al. 2004; Vermeulen et al. 2003, 2005, 2007; Oliveira et al. 2017). Altogether, weathered dredged sediments share the benefits of high nutrient content and high water holding capacity with fresh dredged sediments, but due to the weathering process, lose most of their aquatic properties, making it easier to blend with existing agricultural soils (Vermeulen et al. 2005). However, the weathering process requires a large area of land for long-term storage, which can be costly and have potentially negative impacts, to the surrounding environment, such as contaminant leeching (Almeida et al. 2001; Averett et al. 1990). While there are pros and cons to using each dredged sediment condition, there is a lack of research that compares the use of weathered and fresh dredged sediments for growing crops.

When using soil amendments in agriculture, the US EPA (2013) has established soil amendment application guidelines to reduce excess nutrients from leaching into nearby waterways. However, these guidelines do not relate to measures of plant performance like growth or reproduction, but instead are calculated based on input of nutrients with the potential for the environmental impacts such as leeching (US EPA 2013). Research examining application rates of dredged sediments to agricultural soils is currently lacking, with most dredged sediment research either using combinations with other soil amendments (Sigua 2009; Oliveira et al. 2017) or using unrealistic rates for current agricultural systems (Canet et al. 2003; Ebbs et al. 2006; Darmody and Diaz 2017; Brigham et al. 2021; Kiani et al. 2021). Additionally, this gap in knowledge does not consider application rates in regard to plant performance limits our understanding of the benefits of using dredged sediments in agricultural systems.

While application rates for dredged sediments should be established based on rates for other soil amendments, obtaining a few key pieces of information is necessary. First, the nutrient content within both the agricultural soils as well as the soil amendment must be ascertained (US EPA 2013; Sullivan et al. 2015; Hellerstein et al. 2019; ERS USDA 2019). For soil amendments such as biosolids, application rates can vary based on their nutrient content, and as dredged sediments are similar in properties and nutrient content to biosolids (Averett et al. 1990; Sigua 2005), we anticipate that these application rates may also be used for dredged sediment applications. The missing element of estimation for the optimal application rates for dredged sediment application is the nutrient recovery ratio, which is the amount of nutrients needed to maximize crop production (Hellerstein et al. 2019).

To establish rates of application for dredged sediments and determine if the weathering process affects plant productivity, we assessed corn performance in a series of application rates (ratios) calculated using the nutrient recovery ratio for corn using both weathered and fresh dredged sediments in a greenhouse experiment. We hypothesize that weathered dredged sediments, having the benefits of terrestrial acclimation while maintaining high nutrient content and water holding capacity, will improve agricultural soil properties compared to unamended agricultural soils. If these soil property improvements are seen, this will lead to increased crop yields. Additionally, we hypothesize that fresh dredged sediments will have adverse effects on soil properties and crop yields, especially in higher application rates, as the fresh sediments have not gone through dewatering or any ripening processes and therefore may still contain contaminants. Finally, we hypothesize that application rates (as applied in ratios), which align with the nutrient recovery ratio and US EPA soil amendment application guidelines, of 3:97 and 5:95 (dredged sediment to agricultural soil) will be the most effective applications overall, with maximum improvements to soil properties and crop yield increases compared to unamended agricultural soils.

MATERIALS AND METHODS

Experimental Design

To investigate how applied dredged sediments of different conditions (weathered vs. fresh) and different application ratios may influence agricultural soil properties and crop responses, we factorially manipulated dredged sediment condition and application ratio (0:100, 1:99, 3:97, 5:95, 10:90, and 100:0) in a greenhouse study at Wright State University in Dayton, Ohio, USA using corn as a focal plant. We determined dredged sediment application ratios first by quantifying the 'nutrient recovery ratio' needed for corn growth on our conventionally farmed agricultural soils (Don Nelson, personal communication). We then calculated the amount of dredged sediments needed to fulfill the nutrient recovery ratio based on the known nutrient content (nitrogen and phosphorus) of the sediments and the agricultural soils. Based on these calculations, we determined that a dredged sediments to agricultural soil application rate of 3:97 was necessary to fulfill the nutrient recovery requirements for corn within our soil system. We then chose a lower application rate that reflected the absolute minimum application rate (1:99), and a maximum application rate based on feasibility of 10:90. We also included three experimental controls: 100% unamended agricultural soil (0:100) to reflect unamended agricultural soil conditions, 100% fresh dredged sediments (100:0) and 100% weathered dredged sediments (100:0).

Weathered dredged sediments (originally collected in 2016 from the Toledo Harbor, Ohio) were collected from the Great Lakes Dredged Material Center for Innovation in Toledo, Ohio, USA (41.6700354^{o}N, -83.5029711^{o}W; Rúa et al. 2023; Julian et al. *in review*) on 8 January 2019. Fresh dredged sediments were collected by

Luedtke Engineering during annual dredging of the Toledo Harbor (41.729301 ^{o}N, -83.395612 ^{o}W) in winter 2019 (November and December). Finally, on 8 January 2019 and 20 January 2020, agricultural soil was collected from a conventionally farmed field of continuous corn and in the same soil series as the dredged sediments in Oregon, OH (41.6751180 ^{o}N, -83.4272100 ^{o}W). For all soil and sediment sources, all plants, rocks and mollusk shells were removed prior to potting and homogenized by hand.

All materials were transported to Wright State University campus via car following collection and stored in the greenhouse (weathered dredged sediments and agricultural soils) or the cold room (fresh dredged sediments) for up to 1.5 years until use. The greenhouse was maintained throughout the experiment at an average temperature of 23.9 ^{o}C with an average humidity of 29.8%. Each treatment combination (application rate x dredge condition) was replicated 20 times with the exception of the 10:90 rate which was replicated only 10 times due to an insufficient amount of agricultural soil, totaling 200 pots [(6 application rates x 20 replicates x 2 soil types) + (1 application rate x 20 replicates x 1 soil types) = 200 pots]. Every soil type-application rate mixture was made in a single batch, homogenized by hand, and cut with 20% sand (Garick, LLC, Cleveland, OH, USA) to improve drainage. The soil type-application rate blends were then potted into 1-gallon pots, weighing ~2.5 kg each, and pots were randomly distributed with an equal number of each treatment combination across five blocks (A-E).

All pots were initially planted on 20 August 2020 (experimental day 0) with two corn seeds obtained from Pioneer® (P33T60; Corteva Agriscience™, Wilmington, DE, USA). We thinned the pots to a single seedling ~2 weeks after germination by removing the seedling at a lower vegetative stage (Nafziger 2017). If there were two plants of the same plant stage and height, then we chose the center-most plant. If no seeds germinated, then a seedling from the same soil treatment combination was transplanted, so that each pot contained a single plant by experiment day 18. All pots were fertilized on experiment day 88 (16 November 2020) to mimic applications in the field which occur prior to corn reaching reproductive stages. All pots received 100 ml of a 24:8:16 N:P:K solution (Scotts Miracle-Gro Products, LLC, Marysville, OH, USA). All plants were repeatedly treated for spider mites with Safer® Brand Insect Killing Soap (Woodstream Corporation, Lititz, PA, USA) following application instructions after they were initially found on experiment day 116 (14 December 2020). The corn was allowed to grow until senescence on experiment day 152 (25 January 2021).

<u>Soil Sample Collection</u>

We collected soil samples from each pot prior to corn planting (20 August 2020, ‘before planting’) and again at harvest (25 January 2021, ‘harvest’) for soil properties analyses. For physical and chemical properties, we collected ~750 g of soil from the center of each pot with a scoopula and stored these samples at -20 ^{o}C for six months until analysis. We also collected two 2 ml tubes from the center of each pot to quantify bulk soil enzyme activities, held them on dry ice in the greenhouse, and stored samples for 16-18 months at -20 ^{o}C until processing.

<u>Soil Physical and Chemical Properties</u>

We assessed soil physical properties of soil texture, bulk density, soil compaction, and water content to determine the effects of dredged sediment application on agricultural soil. Soil texture analysis (% sand, % silt and % clay) was conducted following the protocol provided for the LaMotte soil texture kit (LaMotte, Chestertown, MD, USA). Water (moisture) content was measured using the mass ratio protocol in which we took ~20 g of soil, recorded the initial 'wet' mass (g), dried the soil sample for 48 hours at 105 ^{o}C, then recorded the final 'dry' mass (Woods et al. 2019). Moisture content (%) was calculated by subtracting the 'dry' mass from the 'wet' mass, then dividing by the 'wet' mass, and finally multiplying by 100 (**Equation 1**).

$$\% = \left(\frac{wet\ mass\ (g) - \ dry\ mass\ (g)}{wet\ mass\ (g)}\right) * 100$$

Equation 1. Moisture content (%) calculation.

BD was also measured following established protocols with the exception that soil from 1-gallon pots were used as a soil 'core' (Onufrak et al. 2019). We first calculated the volume of each pot as the 'field moist volume', then measured the weight of the pot containing soil as 'field moist mass,' and finally used the moisture content values as the 'subsample' values to calculate the bulk density of each sample (**Equation 2**).

$$g/ml^{-1} = \left(\frac{field\ moist\ mass\ (g)}{field\ moist\ volume\ (mL)}\right) * \left(\frac{subsample\ 'dry'\ mass\ (g)}{subsample\ 'wet'\ mass\ (g)}\right)$$

Equation 2. Soil bulk density calculation.

Soil compaction followed the protocol provided for the Dickey-john penetrometer (Churchill Industries, Minneapolis, MN, USA). We added 100 ml of water to each pot to ensure the soil was moist prior to using the ½ inch tip recommended for firm soil types and recorded the pressure range (green < 200 psi, yellow = 200 – 300 psi, red >300 psi) at a depth of 3 inches (7.2 cm) in each pot.

Several soil chemical properties were measured to assess differences driven by dredged sediment application to agricultural soils. These chemical properties included pH, conductivity (EC), cation exchange capacity (CEC), organic matter (OM) and nutrient content [phosphorus (P), potassium (K), calcium (Ca^+), magnesium (Mg^+)]. Soil pH was measured following the 2:1 water to soil preparation method where 20 ml of water was added to 10 g of soil and shook by hand for 30 seconds (Woods et al. 2019). After allowing samples to settle for 30 minutes, we recorded pH using a Fisherbrand™ accumet™ AB15 Basic and BioBasic™ pH / mV / °C meter (ThermoFisher, Waltham, MA, USA). EC analysis used the same samples prepared for soil pH and followed the protocol provided for the Traceable Conductivity/Total Dissolved Solids Meter (Cole-Palmer, Vernon Hills, IL, USA). Soil samples for each collection date were also analyzed for CEC, P (Mehlich), K, Mg^+, Ca^+, and OM following EPA guided laboratory protocols at A & L Great Lakes Laboratory (Fort Wayne, IN, USA).

<u>Extracellular Enzyme Activities</u>

Enzyme activities were assayed for four specific fluorometric enzymes [alkaline phosphatase (AKP), arylsulfatase (ARS), β-glucosidase (BG) and leucine aminopeptidase (LAP)] and four colorimetric enzymes [invertase (IV), peroxidase (PER), polyphenol oxidase (PPO) and urease (UR)]. All enzyme assays used a soil homogenate consisting of a mixture of 0.25 g dry weight of soil sample and 31.5 mL of 50 mM sodium acetate buffer (pH 5.6) except AKP which instead used 31.5 mL of NaOH Tris buffer (pH 11.0). Soil homogenates were stored in the dark at 4 °C for up to one week prior to analysis. All enzyme assays were run in triplicate using 96-well plates with a buffer blank, substrate blank, soil homogenate blank, soil homogenate and substrate mix. Fluorometric enzymes also included a standard curve for each sample (Sinsabaugh et al. 2000; Brockett et al. 2012; Hoehn 2016; Woods et al. 2019; Wan et al. 2020; Julian et al. *in review*).

The fluorometric enzymes AKP, ARS and BG used the fluorescence indicator Methylumbelliferone in the form of 50 µL of substrates 4-Methylumbelliferyl phosphate, 4- Methylumbelliferyl sulfate, and Metheylumbelliferyl beta-D-glycopyranoside while LAP used the fluorescence indicator 7-Amino-4-methylcoumarin in the form of 50 µL of substrate L-Leucine-7-amid-4-methylcoumarin hydrochloride. Once all samples were plated and prepared, they were incubated in the dark at room temperature (22 ^{o}C) for the following times: AKP, four to six hours; ARS, eight hours; BG, 24 to 72 hours; and LAP, 96 hours. After incubation, the prepared plates were read at 360 nm excitation and 450 nm emission using a Synergy H1 BioTek microplate reader (BioTek, Winookski, VT, USA). All fluorometric enzyme activities were calculated in µmol h^{-1} g^{-1} as shown in **Equation 3** using the dilution factor (DF = mL volume of buffer / g of soil sample), extinction coefficient (Ɛ; the slope of the sample's standard curves) and the calculated net fluorescence units (NFU; **Equation 4**).

$$\mu\text{mol } h^{-1}g^{-1} = \frac{\left(\frac{\text{NFU}}{\frac{\varepsilon}{0.25}\text{mL}}\right)}{\left(incubation\ time * \left(\frac{1}{DF}\right) * 0.200\ \ mL\right)}$$

Equation 3. Fluorometric enzymatic activity calculation for alkaline phosphatase, arylsulfatase, β-glucosidase and leucine aminopeptidase using the Net Fluorescence Unit (NFU) and Dilution Factor (DF).

$$NFU = SH - SHB - B * \left(\frac{slope\ of\ standard\ with\ sample}{slope\ of\ standard\ with\ buffer}\right)$$

Equation 4. Net Fluorescence Unit (NFU) calculation for fluorometric enzymatic activities where 'SH' is the soil homogenate + substrate fluorescence, 'SHB' is the soil homogenate blank fluorescence and 'B' is the substrate blank fluorescence.

PER and PPO activities were assayed using 50 µl of 25 µM 3,4-Dihydroxyphenylalanine with an additional 10 µl of 3% hydrogen peroxide for PER. Plates were incubated in the dark at room temperature (22 ^{o}C) between 16 and 24 hours. Following incubation, plates were read at 450 nm emission on a Synergy H1 BioTek microplate reader (BioTek, Winookski, VT, USA). Activities for PER and PPO were calculated in µmol h^{-1} g^{-1} following **Equation 5** using net absorbance units (NAU = soil homogenate absorbance – soil homogenate blank absorbance – substrate blank absorbance) and the extinction coefficient (Ɛ) 1.8446 for PER and 2.4942 for PPO.

$$\mu\text{mol}\ h^{-1}g^{-1} = \frac{\text{NAU} * Buffer\ volume\ (ml)}{(\varepsilon * 0.200\ \text{mL} * Incubation\ Time\ (hr) * sample\ (g)}$$

Equation 5. Colorimetric enzyme activity calculation for invertase, peroxidase, polyphenol oxidase and urease using the Net Absorbance Unit calculation (NAU).

IV activity was quantified using 50 µl of the substrate 50 mM sucrose. Plates containing the samples for IV analyses were incubated in the dark at room temperature (22 ^{o}C) for four hours until read at 340 nm emission on a Synergy H1 BioTek microplate reader (BioTek, Winookski, VT, USA). Using the net absorbance units (NAU) and the extinction coefficient (Ɛ) of 6.3, we calculated IV activity in µmol g^{-1} h^{-1} (**Equation 5**).

To assay UR activity, we used 10 µl of a 400 mM Urea substrate with an incubation time of 24 hours in the dark at room temperature (22 ^{o}C), prior to the addition of 40 µl of both salicylate and cyanurate acid, followed by a one-hour incubation time after the addition. UR enzyme plates were read at 610 nm using a Synergy H1 BioTek microplate reader (BioTek, Winookski, VT, USA). To calculate UR activity in µmol NH4 g^{-1} h^{-1}, we used net absorbance units (NAU) and an extinction coefficient (Ɛ) of 0.2403 (**Equation 5**).

Plant Responses

Plant responses were assessed throughout the corn growing season and at harvest to determine effects of dredged sediments condition when applied to agricultural soils. We recorded emergence daily until day 18 to determine rates of germination. Weekly measurements of leaf count and plant height were taken, starting from plant thinning, and ending with harvest on 25 January 2021. We counted leaves following the guidelines from the USDA (2020) using only the fully formed leaves with an arch. Plant height (cm) was assessed with a tape measure and measured from the top of the soil in the pot to the top of the arch of the tallest fully formed leaf (APHIS USDA 2020). Leaf count was also indicative of vegetative growth stages (*i.e.*, for V9, the corn plant had nine fully developed leaves). Reproductive growth stages of tassel (VT) and kernel development (R1-R6) were also assigned when applicable (Nafziger 2017). Throughout the growing season, plant survivorship was assessed such that if a plant died, final measurements and plant stage were documented, the plant was categorized as dead and then harvested. At harvest, each plant was removed from the pot, the above ground plant material was removed from the below ground material and placed into a labeled paper bag, and the below ground root material rinsed with water until cleaned of any soil and placed into a separate labeled paper bag. The bags containing plant material were then dried for 48 hours at 60 ^{o}C and then weighed (g). The above ground and below ground biomass was then used to calculate total biomass as well as the ratio of roots (below ground biomass) to shoots (above ground biomass). Ear count was also documented for each plant, if applicable, however, there were no viable kernels produced.

Statistical Analysis

To determine how applied dredged sediments of different conditions (weathered vs. fresh) and different application ratios (0:100, 1:99, 3:97, 5:95, 10:90, 100:0) may influence agricultural soil properties and crop responses, we performed both univariate and multivariate statistical analyses using the statistical programming environment R, version 4.3.0 (R Core Team 2023). Data were normalized as needed with log transformation to match model assumptions. We used a variety of linear models, generalized linear mixed effects models, and linear mixed effects models for univariate analyses. Each linear model was tested with a Type III ANOVA using the *anova()* function in base R, and any significant interactions were evaluated using the *emmeans()* function from the *emmeans* package (Lenth et al. 2023), where all pair-wise comparisons used a Tukey's HSD test. Data visualizations for all linear models were created using the *ggplot2* package (Wickham et al. 2023).

We first separated the application ratios into two subsets [(0:100, 1:99, 3:97, 5:95, 10:90) and (0:100, 100:0)], as initial analysis indicated the 100:0 dredged sediments (weathered, fresh) were masking any variation in the calculated application ratios. We then created separate linear mixed effects models for soil properties using either the *lme()* function in the *nlme* package (Pinheiro et al. 2023) or the *lmer()* function in the *lme4* package (Bates et al. 2023), such that each soil property metric [physical (sand, silt, clay, BD, moisture), chemical (pH, EC, OM, P, CEC, Ca^+, K, and Mg^+), biological (AKP, ARS, BG, IV, LAP, PER, PPO, UR)] was a function of the interaction between soil type (agricultural soil, fresh dredged sediments, weathered dredged sediments), application ratio [(0:100, 1:99, 3:97, 5:95, 10:90) or (0:100, 100:0)], and date collected [before planting (20 August 2020), harvest (25 January 2021)] with random effects in each model for unique plant ID.

To determine if soil property responses differed between groupings of soil type and application ratios, we used a principal component analysis (PCA) created with the *prcomp()* function in base R to condense changes in the soil properties into two linear principal components, except OM, Ca^+ and PER which were removed due to their high correlation with other properties. We then performed an analysis of similarity (ANOSIM) for all PCAs using the *adonis2()* function in the *vegan* package (Oksanen et al. 2022). PCA data were visualized using the *ggbiplot* package (Vu et al. 2011).

To evaluate the compaction of the soil type and application ratio combinations, we first established the relative frequency of each level of compaction (*i.e.*, 'low', 'moderate', 'high') and then performed the chi-squared test using the *chisq.test()* function in base R to determine significance with regards to the interaction of soil type, application ratio and time.

To assess germination rates for corn grown in the different soil type and application ratio combinations, we first calculated the rate of germination by dividing the number of seeds germinated by the total number of seeds planted for each of day germination was recorded. We then created a linear mixed effects models using the *lme()* function in the *nlme* package (Pinheiro et al. 2023), where germination rate was a function of the interaction of soil type, application ratio and day of data collection, with unique plant ID as the random effect.

To quantify changes in corn development we created a linear model using the *lm()* function in base R, using week of plant data collection as a function of the interaction between soil type, application ratio and plant stage (VE – R1). We created a generalized linear model with a Poisson distribution using the *glm()* function in base R to evaluate leaf count as a function of the interaction between soil type, application ratio and week of data collection. To assess differences in plant height over time, we created a linear mixed effects model using the *lme()* function in the *nlme* package (Pinheiro et al. 2023), where height was a function of the interaction of soil type, application ratio, and the week of data collection with the random effect as unique plant ID.

We calculated the relative growth rate (RGR) by week for each plant by taking the height of the current week, subtracting it from the height in the previous week, and dividing that value by the height in the previous week. We then evaluated changes in the weekly RGR with a linear mixed effects model using the *lme()* function in the *nlme* package (Pinheiro et al. 2023), where RGR was a function of the interaction between soil type, application ratio, and week, with a random effect of individual plant ID.

Corn production was captured with above ground, below ground, and total plant biomass measurements. To evaluate differences in biomass, we created three separate linear mixed effects models using the *lme()* function in the *nlme* package (Pinheiro et al. 2023), where biomass (above ground, below ground, or total) was a function of soil type and application ratio, with a random effect of unique plant ID. To quantify changes in plant matter allocation, we calculated the ratio of roots (below ground biomass) to shoots (above ground biomass) and then created a linear model using the *lm()* function in base R, where the root to shoot ratio was a function of soil type and application ratio.

We used the *survfit()* function in the *survival* package (Therneau et al. 2023) to assess plant survival over the growing season, with the categories alive (status = 1) and dead (status = 2), in response to the different soil type and application ratio combinations. We then used the *ggsurvplot()* function in the *survminer* package (Kassambara et al. 2021) for visualization.

Finally, we assessed the relationship of the crop response associated with production and individual soil properties by creating linear models using the *lm()* function in base R, where total biomass was a function of a single soil property. To determine associations among plant responses and soil properties, we performed a canonical correlation analysis (CCA) using the *CCA()* function in the *CCA* package (González and Déjean 2021) to condense changes in soil properties and plant responses (biomass, leaf count, height) into two matrices. We then determined significance between the canonical covariates based on the combinations of soil type and application ratios by using the 'Wilks' test within the *p.asym()* function in the *CCP* package (Menzel 2022).

RESULTS

Agricultural Soil compared to Application Ratios of Dredged Sediments

Plant Responses to Application Ratios of Dredged Sediments

Germination rates varied for corn grown in the different combinations of soil type and application ratio ($F_{8,\ 2551} = 4.00$, $R^2 = 0.68$, $P = 0.0001$; **Fig 3.1A**). Corn reaching 100% germination with the fastest germination rates was found in four soil type and application ratio combinations: 10:90 fresh dredged sediment application by Day 5 after planting, 10:90 weathered dredged sediment application by Day 9, 5:95 weathered dredged sediments by Day 13, and 1:99 fresh dredged sediments by Day 15 (**Fig 3.1A**). In contrast, no other soil type and application ratio combination had 100% germination, with corn grown in the 3:97 and 5:95 fresh dredged sediments having the overall slowest germination rates (**Fig 3.1A**).

Corn grown in the different soil type and application ratio combinations had varying responses in plant stage development, leaf count, plant height, RGR and production. Plant stage development significantly varied for corn grown in different soil types and application ratio combinations, but only for plant stages V8, V9 and V10, along with corn in the 10:90 weathered dredged sediments reaching V11 before any other treatment combination **(**$F_{121,\ 3100} = 2.37$, $R^2 = 0.82$, $P < 0.0001$; **Fig 3.1B**). However, while there was a difference in development based on vegetative plant stages, there was no difference in the time to reproductive stages (VT and R1; $P > 0.05$; **Fig 3.1B**). Leaf count, which indicates vegetative plant stage, was also not significantly changed by any soil type and application ratio combination ($P = 0.9803$). The RGR was not significantly changed by the interaction of soil type, application ratio and time ($P = 0.6420$) or the combinations of soil type and application ratio independent of time ($P = 0.8404$). Plant height, which is used to calculate RGR, had significant differences only between the 5:95 fresh and 5:95 weathered dredged sediment applications during Weeks 13-15 and 17-18, but was not different for any other soil type and application ratio combination ($P = 0.0278$, $R^2 = 0.25$, $F_{8, 302} = 2.16$; **Fig S3.1**). For corn production, neither above ground ($P = 0.4102$), below ground ($P = 0.7247$) nor total biomass ($P = 0.3920$) were significantly different across any of the soil type and application ratio combinations, which resulted in no difference in the root-to-shoot ratio ($P = 0.8027$). There were no viable kernels produced.

Plant survival was significantly influenced by soil type and application ratio combination ($X^2_{8, 160} = 15.9$, $P = 0.04$; **Fig S3.2**). Throughout the growing season, each combination of soil type and application ratio experienced plant losses before reaching harvest. Specifically, one plant failed to survive in the 10:90 fresh dredged sediment application, while three plants did not survive in the 1:99 fresh, 1:99 weathered, 5:95 weathered, and 10:90 weathered dredged sediment applications, whereas four plants did not make it to harvest in the 100% agricultural soils and 3:97 weathered dredged sediment application, and five plants failed to survive in both the 3:97 fresh and 5:95 fresh dredged sediment applications (**Fig S3.2**).

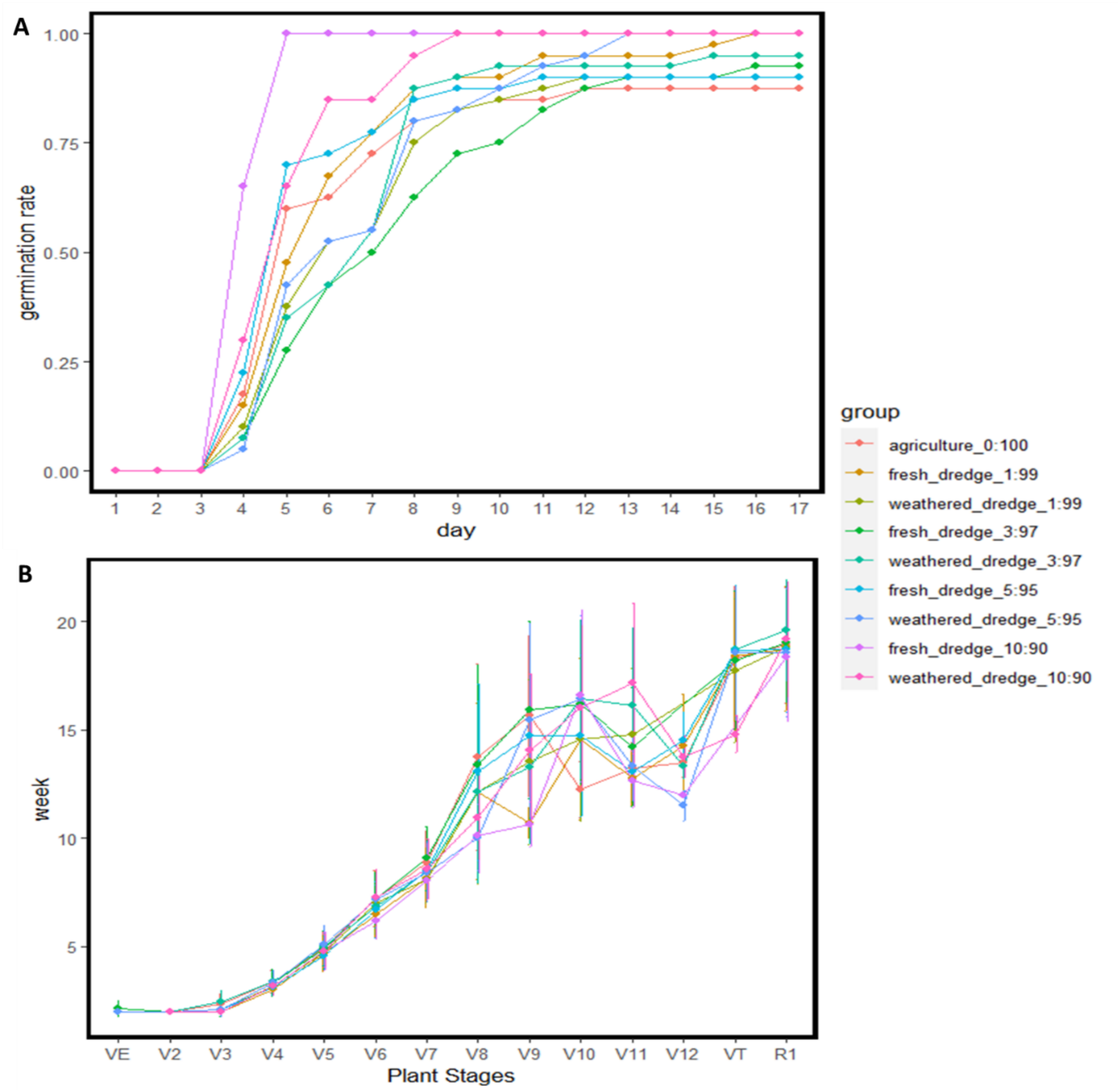

Fig 3.1. (A) Germination rates reached 100% faster in 10:90 fresh dredged sediment application, 10:90 weathered dredged sediment application, 5:95 weathered dredged sediments, and 1:99 fresh dredged sediments, with no other soil type and application ratio combination reaching 100% germination ($F_{8, 2551} = 4.00$, $R^2 = 0.68$, $P = 0.0001$). **(B)** The time it took corn to reach plant stages V8 – V11 varied by soil type and application ratio combinations. However, these differences did not persist into reproduction stages ($F_{121, 3100} = 2.37$, $R^2 = 0.82$, $P < 0.0001$). For both **(A)** and **(B),** each point with its associated error bar represents the average week each germination rate or plant stage was reached, and asterisks (*) indicate significant differences between treatment by week based on Tukey's HSD ($P < 0.05$). Additionally, the colors represent the different soil type and application ratio combinations as follows: 100:0 agricultural soils = light pink, 1:99 fresh dredged sediments = tan, 1:99 weathered dredged sediments = olive, 3:97 fresh dredged sediments = green, 3:97 weathered dredged sediments = teal, 5:95 fresh dredged sediments = light blue, 5:95 weathered dredged sediments = indigo, 10:90 fresh dredged sediments = purple, and 10:90 weathered dredged sediments = hot pink.

Plant Response and Soil Property Relationships

Total biomass for corn grown in any soil type and application ratio combination was not influenced by any soil physical (moisture: P = 0.9911; BD: P = 0.9367; % sand: P = 0.9461; % silt: P = 0.8687; % clay: P = 0.8317), chemical (pH: P = 0.6345; EC: P = 0.4381; CEC: P = 0.5518; Ca^+: P = 0.7553; K: P = 0.0699; Mg^+: P = 0.4054; P: P = 0.0619; OM: P = 0.0732) or biological properties (AKP: P = 0.7448; ARS: P = 0.9316; BG: P = 0.9868; IV: P = 0.9349; LAP: P = 0.9064; PER: P = 0.9697; PPO: P = 0.9374; UR: P = 0.6741).

For application ratio and soil type combinations, all soil properties and the plant responses of total biomass, height and leaf count for the soil type and application ratios were placed into two matrices to assess their associations through the dimensionality of a canonical correlation analysis (**Fig 3.2**). All three canonical dimensions were statistically significant from each other (**Table S3.1A**; P < 0.0001). The first dimension was influenced by the soil properties Mg^+ content (0.40), P content (0.38) and OM content (0.25) and the plant response leaf count (-0.06; **Table S3.1B**). The second dimension was influenced by the soil properties BD (0.05), AKP (-0.05) and % clay (-0.04) and plant response height (-0.08; **Table S3.1B**). The final dimension was influenced by the soil properties Mg^+ content (-0.32), P content (-0.35) and OM content (-0.25) and the plant response biomass (-0.08; **Table S3.1B**).

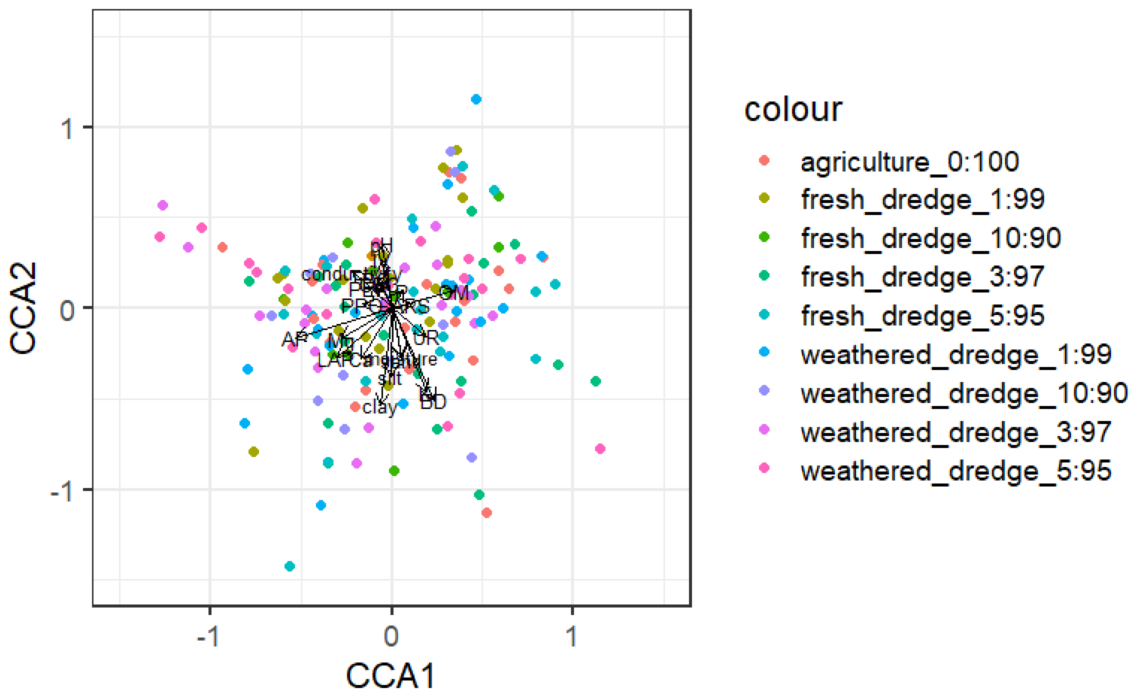

Fig 3.2. Soil properties and plant responses were separated into two matrices to ascertain their associations influenced by the soil type and application ratio combinations through a canonical correlation analysis (CCA). Colors represent the different soil type and application ratio combinations as follows: 100:0 agricultural soils = light pink, 1:99 fresh dredged sediments = tan, 3:97 fresh dredged sediments = olive, 5:95 fresh dredged sediments = green, 10:90 fresh dredged sediments = teal, 1:99 weathered dredged sediments = light blue, 3:97 weathered dredged sediments, = indigo, 5:95 weathered dredged sediments = purple, and 10:90 weathered dredged sediments = hot pink.

<u>Soil Physical Property Responses to Application ratios of Dredged Sediments</u>

Moisture content was altered by soil type and application ratio over time ($F_{8, 289}$ = 5.554, R^2 = 0.60, P < 0.0001; **Fig 3.3A**). When compared to the 100% agricultural soils, moisture content was ~ 37% and 27% lower in the 3:97 fresh and 3:97 weathered dredged sediment applications and 62% higher in the 10:90 weathered dredged sediment application prior to planting (**Fig 3.3A**). However, all differences were lost at harvest, as a decrease in moisture content led to no significant differences across all soil type and application ratio combinations (**Fig 3.3A**).

BD changed with the combinations of soil type and application ratios over time ($F_{8, 151} = 11.45$, $R^2 = 0.87$, $P < 0.0001$; **Fig 3.3B**) Prior to planting, BD of the treatment combinations varied with regard to the 100% agricultural soil, such that BD was the same in the 1:99 and 3:97 applications of fresh and weathered dredged sediments as the agricultural soil, whereas BD in the 5:95 and 10:90 fresh and weathered dredged sediment applications was 6-10% lower (**Fig 3.3B**). BD for all soil types and application ratios decreased at harvest, maintaining the patterns established prior to planting (**Fig 3.3B**). Although BD decreased over the corn growing season, most treatments remained within the USDA recommended range (1.0 -1.4 g/ml^{-1}). Only the 5:95 fresh dredged sediments and 10:90 fresh and weathered dredged sediments applications fell below the lowest recommended threshold at harvest (**Fig 3.3B**). As another measure of soi porosity, soil compaction differed between soil type and application ratio combination over time ($R^2_{4, 200} = 20.91$, $P = 0.0003$; **Fig S3.3**). Before planting, all soil type and application ratio combinations had soil compaction classified as 'low' within the pots (**Fig S3.3**). However, all soil type and application ratio combinations had increases to 'moderate' soil compaction at harvest, except the 10:90 weathered dredged sediment applications, which remained at 'low' compaction (**Fig S3.3**).

Soil texture shifted from a higher percentage of clay to a higher percentage of sand over the growing season within the soil type and application ratio combinations. The percentage of sand was significantly different between the soil type and application ratio combinations ($F_{8, 289} = 3.206$, $R^2 = 0.13$, $P = 0.0016$; **Fig S3.4A)** such that prior to planting, the percentage of sand in the 3:97 fresh dredged sediment application was ~16% less than the 100% agricultural soils, however this difference was lost at harvest.

Furthermore, the percentage of silt was higher in the 3:97 fresh dredged sediments compared to the 100% agricultural soils ($F_{8, 298} = 3.662$, $R^2 = 0.36$, $P = 0.0004$; **Fig S3.4B**). Additionally, over time, there was a shift in the soil texture constituents, regardless of soil type or application ratio, such that there was an increase in the percentages of sand ($F_{1, 289} = 10.65$, $P = 0.0012$; **Fig S3.4C**) and silt ($F_{1, 298} = 43.36$, $R^2 = 0.36$, $P < 0.0001$; **Fig S3.4D**) but a decrease in the percentage of clay ($F_{8, 298} = 28.86$, $P < 0.0001$; **Fig S3.4E**). However, the percentages of sand and silt were not changes by the interaction of soil type, application ratio and time ($P > 0.05$).

Soil Chemical Property Responses to Application Ratios of Dredged Sediments

Soil pH was altered by the combination of soil type and application ratio over time ($F_{8, 289} = 5.554$, $R^2 = 0.61$, $P < 0.0001$; **Fig 3.3C**). Prior to planting, soil pH was higher in the 3:97 fresh dredged sediments application, and the 10:90 weathered dredged sediments application had a lower soil pH when compared to the agricultural soils (**Fig 3.3C**). These differences in soil pH disappeared at harvest, with an overall decrease in soil pH, removing any differences between any soil type and application ratio combinations; however, soil pH was maintained within the USDA recommended range (6.5-7.2) throughout the growing season (**Fig 3.3C**).

EC differed between soil type and applications rate combinations ($F_{8, 298} = 2.27$, $P = 0.0226$; **Fig S3.5A**) and time ($F_{1,289} = 305.6$, $P < 0.0001$; **Fig S3.5B**) independent of each other ($P = 0.2451$). While there were differences between soil type and application ratio combinations such that the 5:95 application of fresh dredged sediments had less EC than the 1:99 application of fresh dredged sediments, no application ratio and soil type combination had different EC from the agricultural soil control (**Fig S3.5A**). There was also an overall decrease of 58% in EC at final harvest regardless of soil type and application ratio combinations (**Fig S3.5B**).

Cation contents varied in response to the combination of soil type and application ratio and time. Mg^+ content was 12-14% lower in the 3:97 and 5:95 weathered dredged sediment applications compared to all other soil type and application ratio combinations and the agricultural soil control ($F_{8, 293} = 1.982$, $R^2 = 0.37$, $P = 0.0485$; **Fig 3.3D**). However, at harvest, there was an overall decrease in Mg^+ content such that all application ratios had similar Mg^+ content as the 100% agricultural soils, with the 5:95 and 10:90 fresh dredged sediment applications having 10% and 14% lower Mg^+ content, respectively (**Fig 3.3D**). The 10:90 weathered dredged sediment application had ~20% higher CEC and Ca^+ content than any other soil type and application ratio combinations and 100% agricultural soils (CEC: $F_{8, 141} = 7.7543$, $P < 0.0001$; **Fig S3.6A**, Ca^+: $F_{8, 297} = 7.187$, $P < 0.0001$; **Fig S3.6C**). K content was ~9% and 13% lower in the 5:95 and 10:90 fresh dredged sediment applications than all soil type and application ratios and the 100% agricultural soils ($F_{8, 293} = 6.879$, $P < 0.0001$; **Fig S3.7A**). Additionally, regardless of soil and application ratio combination, initial CEC levels, Ca^+ content and K content decreased at harvest (CEC: $F_{1, 146} = 94.19$, $P < 0.0001$; **Fig S3.6B**, Ca^+: $F_{8, 297} = 7.187$, $P < 0.0001$; **Fig S3.6D**, K: $F_{1, 293} = 521.02$, $P < 0.0001$; **Fig S3.8B**). However, the interaction between the soil type, application ratio and time did not significantly influence CEC levels ($P = 0.5518$), Ca^+ content ($P = 0.3333$) or K content ($P = 0.1279$).

P content was significantly influenced by the combination of soil type and application ratio over time. Prior to planting, P content was ~10-37% lower in the 100% agricultural soils than any soil type and application ratio combination ($F_{8,142} = 2.399$, R^2

= 0.60, P = 0.0186; **Fig 3.3E**). At harvest, P content decreased across all soil type and application ratio combinations, however, all treatment combinations except the 10:90 fresh dredged sediment application maintained higher P content than the 100% agricultural soils (**Fig 3.3E**).

The interaction of soil type, application ratio and time ($F_{8, 148} = 2.985$, $R^2 = 0.28$, P = 0.0436; **Fig 3.3F**) significantly altered OM content. Before planting, the OM content in the 10:90 fresh dredged sediment application was ~30% lower than all other soil type and application ratio combinations and the 100% agricultural soils (**Fig 3.3F**). However, at harvest, OM content significantly decreased in all soil type and application ratio combinations except for the 3:97 both fresh and weathered which remained unchanged (**Fig 3.3F**).

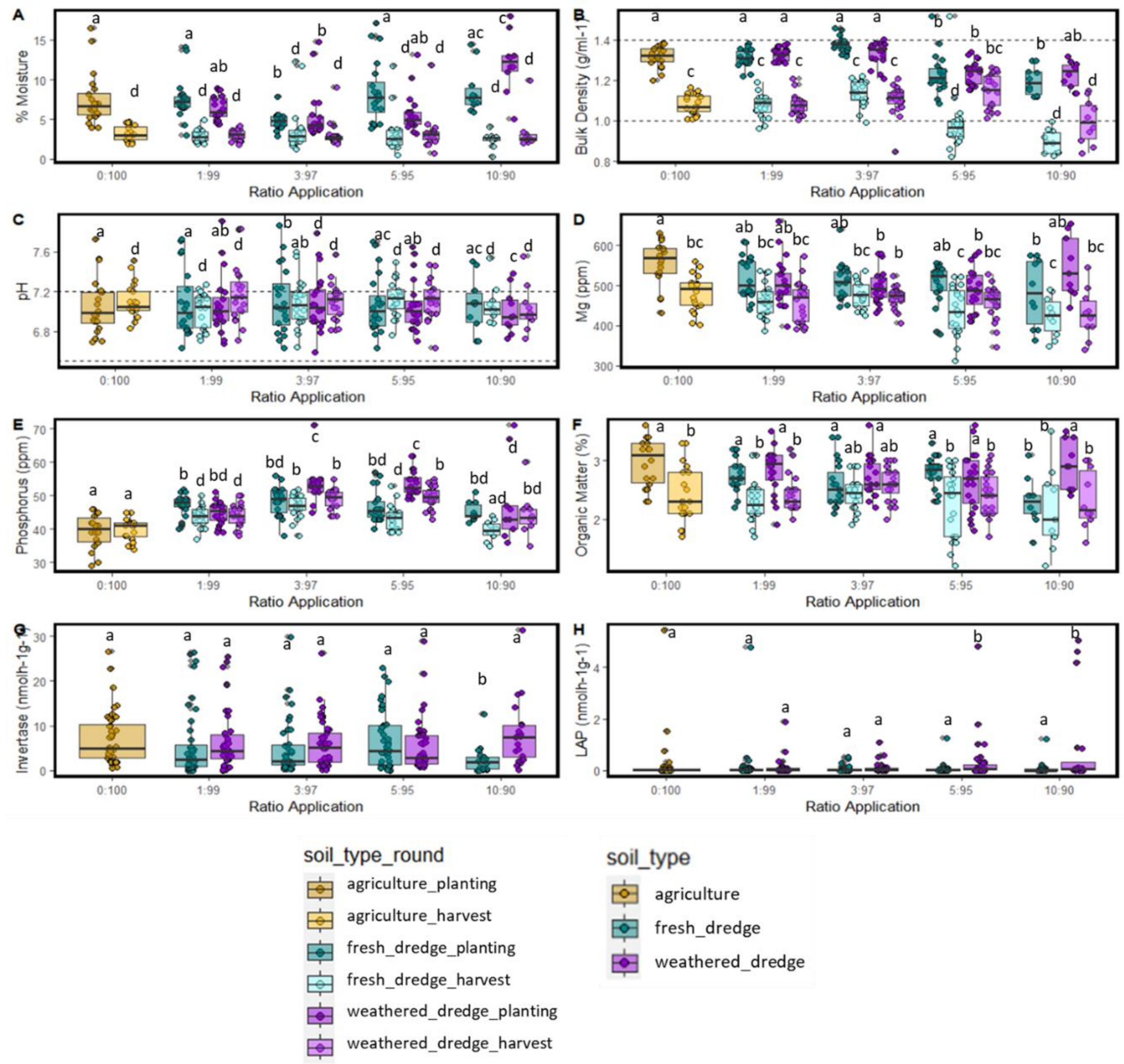

Fig 3.3. (A) Moisture content decreased over time, removing the differences among the soil type and application ratio combinations found before planting ($F_{8, 289}$ = 5.554, R^2 = 0.60, P < 0.0001). **(B)** Bulk Density (BD) decreased at harvest compared to before planting maintaining values within the USDA recommended range of (black dash lines; 1.0 -1.4 g/ml^{-1}), except 5:95 fresh dredged sediments and 10:90 fresh and weathered dredged sediments applications, which fell below the low threshold at harvest ($F_{8, 151}$ = 11.45, R^2 = 0.87, P < 0.0001). **(C)** Soil pH decreased over time, removing the differences among the soil type and application ratio combinations found before planting, but still maintained values within the USDA recommended range (6.5 -7.2; black dashed line; $F_{8, 289}$ = 5.554, R^2 = 0.61, P < 0.0001). **(D)** The differences between 3:97 and 5:95 weathered dredged sediment applications ratios in magnesium (Mg^+) content were lost with a decrease at harvest, where variation between the soil type and application ratio combinations shifted so that Mg^+ content was lower in the 5:95 and 10:90 fresh dredged sediment applications ratios. However, no treatment combination had different Mg^+ content compared to the 0:100 agricultural soils ($F_{8, 293}$ = 1.982, R^2 = 0.37, P = 0.0485).

(E) Phosphorus (P) content was higher in all soil type and application ratio combinations compared to the 0:100 agricultural soils, with an overall decrease in P content at harvest. Also at harvest, the 10:90 fresh dredged sediment application equaled the low P content of the 0:100 agricultural soils ($F_{8,142}$ = 2.399, R^2 = 0.60, P = 0.0186). **(F)** Organic matter (OM) content before planting was higher in the 10:90 weathered dredged sediment application, but at harvest, this difference was lost as there was an overall decrease in OM content in all soil type and application ratios except the 3:97 of both fresh and weathered, which both maintained OM content ($F_{8,\,148}$ = 2.985, R^2 = 0.28, P = 0.0436). **(G)** Invertase (IV) activity was lower in the 10:90 fresh dredged sediment application than in all other soil type and application ratio combinations ($F_{8,\,302}$ = 3.752, P = 0.0003). **(H)** Leucine aminopeptidase (LAP) activity was higher in both the 5:95 and 10:90 weathered dredged sediment applications than any other soil type and application ratio combination ($F_{8,\,298}$ = 1.993, P = 0.0471). For all graphs **(A-H)** boxes are 50% quartiles, with the thick black line indicating the median value. Each point represents one soil sample (soil type and application ratio combinations: n = 40 for 1:99, 3:97 and 5:95 and n = 20 for 10:90 and 0:100; soil type, application ratio and time combinations: n = 20 for 1:99, 3:97 and 5:95 and n = 10 for 10:90 and 0:100). Colors represent soil type and shading represents time (darker colors are prior to 'planting' while lighter colors are following 'harvest'): agricultural soils are brown, fresh dredged sediments are turquoise, and weathered dredged sediments are purple. Letters indicate significant differences based on Tukey's HSD post hoc analyses (P < 0.05).

Soil Biological Property Responses to Application Ratios of Dredged Sediments

N-mineralizing enzymes varied in their response to soil type and application ratio combinations over time. Soil type and application ratio combinations significantly influenced IV activity ($F_{8,\,302}$ = 3.752, P = 0.0003; **Fig 3.3G**), where the 10:90 fresh dredged sediment application had less IV activity than any other soil type and application ratio combination. However, neither time (P = 0.3147) nor the interaction of time, soil type and application ratio (P = 0.1142) were significant. LAP activity was significantly influenced by the combination of soil type and application ratio ($F_{8,\,298}$ = 1.993, P = 0.0471; **Fig 3.3H**) as well as time ($F_{1,\,298}$ = 5.935, P = 0.0153; **Fig S3.8A**), but not their interaction (P = 0.9598). The 5:95 and 10:90 weathered dredged sediments applications had 31% and 242% higher LAP activity compared to the 100% agricultural soils (**Fig 3.3H**). LAP activity before planting was increased at harvest, regardless of soil type and application ratio (**Fig S3.8A**). Neither soil type and application ratio combination (P = 0.9894), time (P = 0.1722) or their interaction (P = 0.9894) influenced UR activity.

Time alone altered the enzymes associated with the P, S and C cycles. There was a significant decrease in activity at final harvest compared to before planting for AKP ($F_{1,298}$ = 11.65, P = 0.0007; **Fig S3.8B**), ARS ($F_{1,298}$ = 35.05, P < 0.0001; **Fig S3.8C**), BG ($F_{1,298}$ = 36.67, R^2 = 0.32, P < 0.0001; **Fig S3.8D**) and PPO ($F_{1,298}$ = 10.95, P = 0.0011; **Fig S3.8E**). However, neither the combination of soil type and application ratio (AKP: P = 0.7704, ARS: P = 0.8372, BG: P = 0.1260, PPO: P = 0.4588) or their interaction with time (AKP: P = 0.6041, ARS: P = 0.7136, BG: P = 0.8910, PPO: P = 0.2084) significantly influenced AKP, ARS, BG or PPO activity. Additionally, PER activity was not influenced by the combination of soil type and application ratio (P = 0.5843), time (P = 0.1907) or their interaction (P = 0.6191).

<u>Changes in Soil Properties Influenced by Application Ratios of Dredged Sediments</u>

The soil physical properties [soil texture (% sand, % silt, % clay), BD, moisture], chemical properties [pH, EC, CEC, P, K, Mg^+], and biological properties [AKP, ARS, BG, IV, LAP, PER, PPO, UR] were all condensed into two principal components, which explained 23.7% and 10.5% of the total variation within the soil type and application ratio combination data. The analysis of similarity on the two linear principal components discerned significant differences in soil properties based on both the soil type and application ratio combinations (P = 0.001, R^2 = 0.26; **Fig 3.4A**) and time (P = 0.001, R^2 = 0.67; **Fig 3.4B**); however, the interaction of soil type, application ratio and time was not significant (P = 0.226).

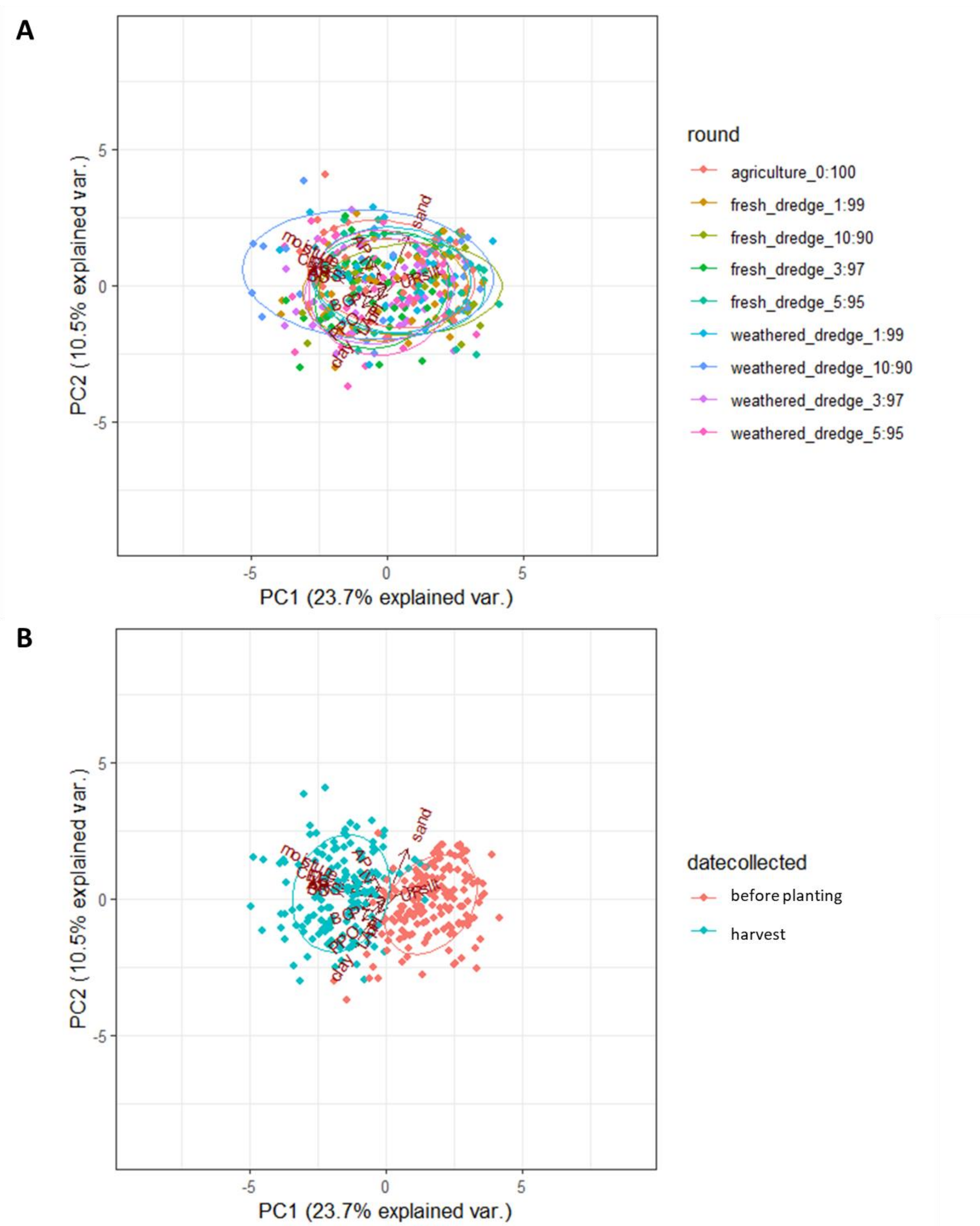

Fig 3.4. Both (**A**) soil type and application ratio combinations (100:0 agricultural soils = light pink, 1:99 fresh dredged sediments = tan, 1:99 weathered dredged sediments = olive, 3:97 fresh dredged sediments = green, 3:97 weathered dredged sediments = teal, 5:95 fresh dredged sediments = light blue, 5:95 weathered dredged sediments = indigo, 10:90 fresh dredged sediments = purple, and 10:90 weathered dredged sediments = hot pink; P = 0.001, $R^2 = 0.26$) and (**B)** time (before planting = blue, harvest = pink; P = 0.001, $R^2 = 0.67$) were significant when accounting for differences in soil enzyme

activities. Ellipses represent 95% confidence intervals, and points represent individual soil samples (n = 20 per soil type and application ratio combinations 1:99, 3:97, 5:95, 0:100, n = 10 per soil type and application ratio combination 10:90).

Unamended Agricultural Soil compared to 100% Dredged Sediments

Plant Responses to 100% soil types

For corn germination rates within the 100% soil types, day since planting was significant ($F_{1, 957} = 1070.56$, $P < 0.0001$) but not soil type ($P = 0.4276$). Additionally, for corn grown in the 100% treatments, RGR was not significantly changed by the interaction of soil type and time ($P = 0.2587$) or soil type independently ($P = 0.3451$), nor was plant height different across the 100% soil treatments ($P = 0.4245$).

Plant stage development was significantly different across the 100% soil types ($F_{36, 948} = 4.22$, $R^2 = 0.85$, $P < 0.0001$; **Fig 3.5A**). Corn grown in the fresh dredged sediments was consistently slower than weathered dredged sediments to reach plant stages V5 - V7 and was also slower to reach V6 than the corn grown in both agricultural soils (**Fig 3.5A**). Corn grown in the weathered dredged sediments was faster to reach V8 than the corn grown in the agricultural soil and faster to reach V9 overall (**Fig 3.5A**). Additionally, all corn in the fresh dredged sediments failed to reach any plant stage past V9, including any subsequent reproductive stages (**Fig 3.5A**). Additionally, minimal differences were found in leaf count for corn grown in the 100% soil types ($F_{2, 974} = 326.53$, $R^2 = 0.49$, $P = 0.0139$; **Fig S3.9**), such that the number of leaves for corn grown in fresh dredged sediments was significantly less than the number of leaves for corn grown in both agricultural soils and weathered dredged sediments during Week 4, however, no other weeks resulted in significant leaf count differences (**Fig S3.9**).

In the 100% soil treatments, the above ground ($F_{2, 43} = 6.69$, $R^2 = 0.24$, $P = 0.0173$; **Fig 3.5B**), below ground ($F_{2, 39} = 44.52$, $R^2 = 0.69$, $P < 0.0001$; **Fig 3.5C**), and total biomass ($F_{2, 43} = 11.45$, $R^2 = 0.35$, $P = 0.0001$; **Fig 3.5D**) for corn grown were all significantly different. The above ground biomass for corn grown in weathered dredged sediments was ~6% higher than for corn in agricultural soils and ~13% higher than for corn in fresh dredged sediments (**Fig 3.5B**). The same patterns were found for the below ground corn biomass in weathered dredged sediments, which was ~44% higher than in agricultural soils and ~64% higher than in fresh dredged sediments (**Fig 3.5C**). Altogether, total biomass for corn grown in the weathered dredged sediments was ~8% higher than in agricultural soils, and ~16% less in the fresh dredged sediments (**Fig 3.5D**). Additionally, the root to shoot ratio was significant for corn grown in the 100% soil types ($F_{2, 43} = 32.19$, $R^2 = 0.60$, $P < 0.0001$; **Fig 3.5E**), such that corn grown in the weathered dredged sediments had a higher root to shoot ratio, compared to the corn grown in either agricultural soils or fresh dredged sediments (**Fig 3.5E**).

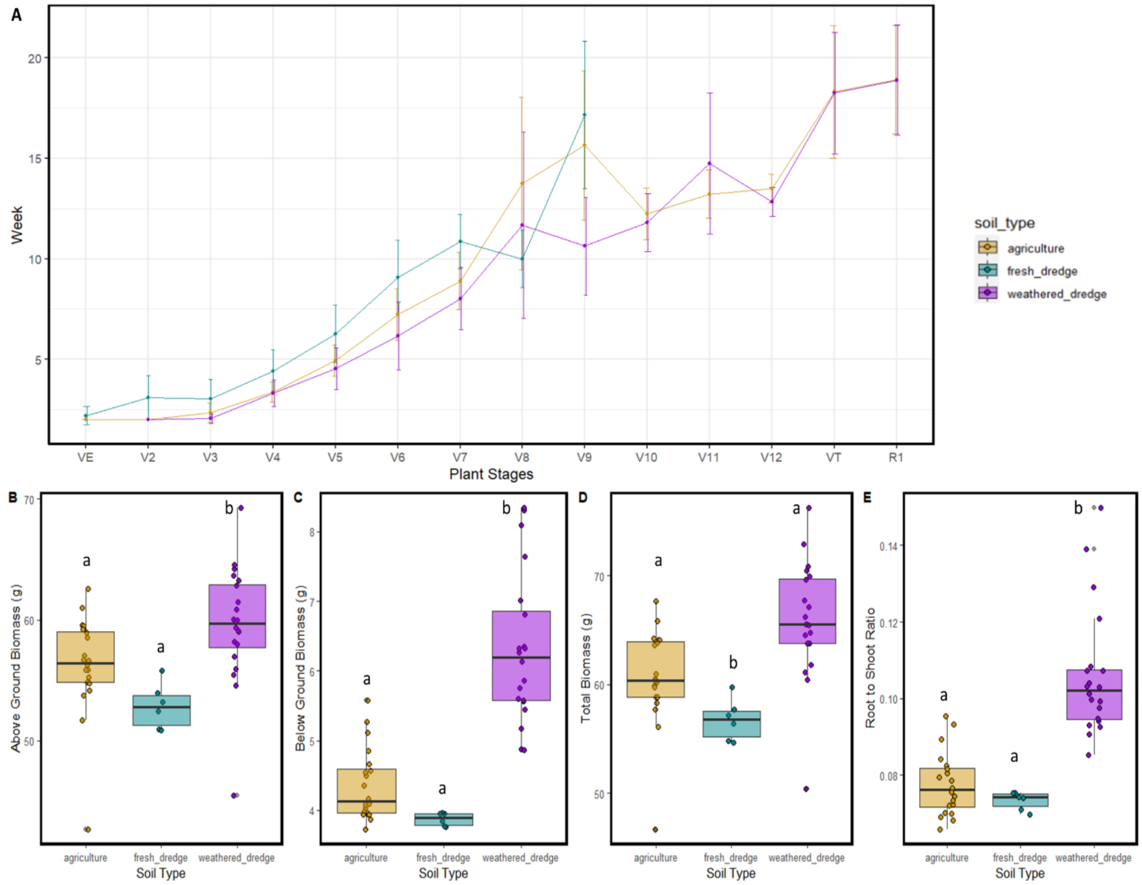

Fig 3.5. (A) Plant stage development differed between the 100% soil types, such that the corn grown in fresh dredged sediments never developed past the V9 stage, while corn grown in both weathered dredged sediments and agricultural soils reached the beginning of reproduction (VT; $F_{36, 948} = 4.22$, $R^2 = 0.85$, $P < 0.0001$). **(B)** Above ground biomass ($F_{2, 43} = 6.69$, $R^2 = 0.24$, $P = 0.0173$) and **(C)** below ground biomass ($F_{2, 39} = 44.52$, $R^2 = 0.69$, $P < 0.0001$) and**(D)** Total biomass ($F_{2, 43} = 11.45$, $R^2 = 0.35$, $P = 0.0001$) for corn grown in both the fresh dredged sediments and agricultural soils followed the same pattern such that biomass was significantly lower than the biomass of corn grown in weathered dredged sediment. **(E)** The root to shoot ratio was significantly higher for corn grown in the weathered dredged sediments than corn grown in fresh dredged sediments or agricultural soils ($F_{2, 43} = 32.19$, $R^2 = 0.60$, $P < 0.0001$).). For all graphs **(A-E)** colors represent soil type: agricultural soils are brown, fresh dredged sediments are turquoise, and weathered dredged sediments are purple. For the graphs **(B-E)** boxes are 50% quartiles, with the thick black line indicating the median value. Each point represents one corn plant (n = 40 for weathered dredged sediments, n = 19 for agricultural soil, n = 5 for fresh dredged sediments). Letters indicate significant differences based on Tukey's post hoc analyses ($P < 0.05$).

Within the 100% treatments, soil type significantly influenced the probability of plant survival ($X^2_{2, 60} = 571$, $P < 0.0001$; **Fig S3.10**) such that only corn grown on the weathered dredged sediments had 100% survival until harvest. Corn growing in the agricultural soils had minimal loss of only four plants, while corn grown in the fresh dredged sediments did not survive, with all but one plant dying before Week 14 of the growing season.

Plant Response and Soil Property Relationships

For corn grown in the 100% soil treatments, the constituents of soil texture drove changes in total biomass. As the percentage of sand increased, total biomass decreased for corn grown in agricultural soils, increased for the corn grown in fresh dredged sediments, and remained the same for corn grown in weathered dredged sediments ($P < 0.0001$, $F_{5, 53} = 21.12$, $R^2 = 0.67$; **Fig 3.6A**). This was opposite of the pattern found with the percentage of silt, such that as silt increased, total biomass increased in agricultural soils and decreased in fresh and weathered sediments ($P < 0.0001$, $F_{5, 53} = 20.60$, $R^2 = 0.66$; **Fig 3.6B**). The increase in the percentage of clay resulted in decreases in total biomass in all 100% soil treatments ($P < 0.0001$, $F_{5, 53} = 21.80$, $R^2 = 0.67$; **Fig 3.6C**). However, neither moisture ($P = 0.2360$) or BD ($P = 0.8569$) influenced changes in total biomass across any 100% soil type.

Several chemical properties significantly influenced the total biomass for corn grown in the 100% soil types. As soil pH increased, total biomass for corn grown in all the 100% soil treatments decreased ($P < 0.0001$, $F_{5, 53} = 20.89$, $R^2 = 0.66$; **Fig 3.6D**). Total biomass decreased in agricultural soils and fresh dredged sediments but increased in weathered dredged sediments with increasing EC ($P < 0.0001$, $F_{5, 48} = 20.35$, $R^2 = 0.66$; **Fig 3.6E**). Increases in CEC levels ($P < 0.0001$, $F_{5, 48} = 18.95$, $R^2 = 0.66$; **Fig 3.6F**), Mg^+ ($P < 0.0001$, $F_{5, 48} = 19.25$, $R^2 = 0.67$; **Fig 3.6G**) and Ca^+ ($P < 0.0001$, $F_{5, 48} = 18.10$,

$R^2 = 0.65$; **Fig 3.6H**) did not change total biomass for corn grown in agricultural soils but increased the total biomass for corn grown in both fresh and weathered dredged sediments. Total biomass decreased for corn grown in agricultural soils and weathered dredged sediments and increased for corn grown in fresh dredged sediments with increasing K ($P < 0.0001$, $F_{5, 48} = 23.61$, $R^2 = 0.71$; **Fig 3.5I**) and OM content ($P < 0.0001$, $F_{5, 48} = 28.59$, $R^2 = 0.75$; **Fig 3.6J**). As P increased, total biomass decreased for corn grown in agricultural soils, increased for corn grown in fresh dredged sediments, and remained constant for corn grown in weathered dredged sediments ($P < 0.0001$, $F_{5, 48} = 19.40$, $R^2 = 0.67$; **Fig 3.3.6K**).

Nutrient cycling enzyme activities significantly influenced total biomass for corn grown in the 100% soil types. With increasing AKP ($P < 0.0001$, $F_{5, 53} = 22.28$, $R^2 = 0.68$; **Fig 3.6L**) and BG ($P < 0.0001$, $F_{5, 53} = 21.86$, $R^2 = 0.67$; **Fig 3.6M**) activities, total biomass increased for corn grown in both agricultural soils and weathered dredged sediments; however, this was opposite for corn grown in fresh dredged sediments, where increasing AKP and BG activities resulted in decreases in total biomass. Increasing ARS activity resulted in decreases in total biomass for corn grown in all 100% soil types ($P < 0.0001$, $F_{5, 53} = 20.32$, $R^2 = 0.66$; **Fig 3.6N**). This relationship was the opposite for PPO activity, where increases in enzyme activity led to increases in total biomass for corn grown in all the 100% soil types ($P < 0.0001$, $F_{5, 53} = 35.55$, $R^2 = 0.77$; **Fig 3.6O**). As IV activity increased, total corn biomass increased in fresh dredged sediments but decreased for corn grown in agricultural soils and weathered dredged sediments ($P < 0.0001$, $F_{5, 53} = 24.49$, $R^2 = 0.70$; **Fig 3.6Q**). With increasing LAP activity, total corn biomass remained unchanged in agricultural soils and weathered dredged sediments but increased in fresh dredged sediments ($P < 0.0001$, $F_{5, 53} = 21.15$, $R^2 = 0.67$; **Fig 3.6R**). Total corn biomass grown in agricultural soils and fresh dredged sediments remained unchanged with increasing PER activity; however, total corn biomass decreased with increasing PER activity in weathered dredged sediments ($P < 0.0001$, $F_{5, 53} = 41.16$, $R^2 = 0.80$; **Fig 3.6S**). As UR activity increased, total corn biomass increased in fresh dredged sediments, remained unchanged in agricultural soils and decreased in weathered dredged sediments ($P < 0.0001$, $F_{5, 53} = 21.02$, $R^2 = 0.66$; **Fig 3.6T**).

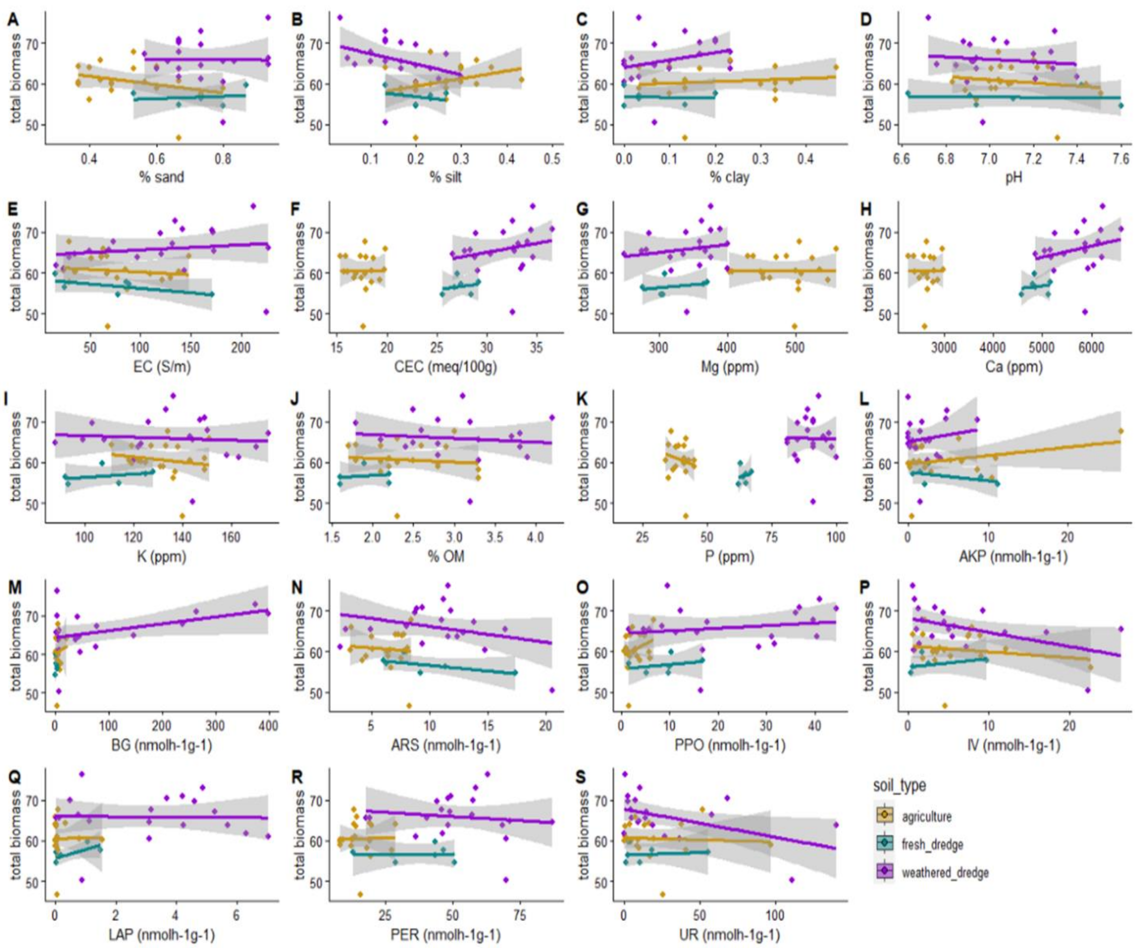

Fig 3.6. Total biomass for corn grown in the 100% soil treatments were significantly influenced by % sand (**A;** $P < 0.0001$, $F_{5, 53} = 21.12$, $R^2 = 0.67$), % silt (**B**; $P < 0.0001$, $F_{5, 53} = 20.60$, $R^2 = 0.66$), % clay (**C**; $P < 0.0001$, $F_{5, 53} = 21.80$, $R^2 = 0.67$), pH (D; $P < 0.0001$, $F_{5, 53} = 20.89$, $R^2 = 0.66$), electrical conductivity (EC; **E**; $P < 0.0001$, $F_{5, 48} = 20.35$, $R^2 = 0.66$), cation exchange capacity (CEC; **F**; $P < 0.0001$, $F_{5, 48} = 18.95$, $R^2 =$

0.66), Magnesium (Mg^+) content (**G**; $P < 0.0001$, $F_{5, 48} = 19.25$, $R^2 = 0.67$), Calcium (Ca^+) content (**H**; $P < 0.0001$, $F_{5, 48} = 18.10$, $R^2 = 0.65$), Potassium (K) content (**I**; $P < 0.0001$, $F_{5, 48} = 23.61$, $R^2 = 0.71$), organic matter (OM) content (**J**; $P < 0.0001$, $F_{5, 48} = 28.59$, $R^2 = 0.75$), phosphorus (P) content (**K**; $P < 0.0001$, $F_{5, 48} = 19.40$, $R^2 = 0.67$), alkaline phosphatase (AP) activity (**L**; $P < 0.0001$, $F_{5, 53} = 22.28$, $R^2 = 0.68$), β-glucosidase (BG) activity (**M**; $P < 0.0001$, $F_{5, 53} = 21.86$, $R^2 = 0.67$), arylsulfatase (ARS) activity (**N**; $P < 0.0001$, $F_{5, 53} = 20.32$, $R^2 = 0.66$), polyphenol oxidase (PPO) activity (**O**; $P < 0.0001$, $F_{5, 53} = 35.55$, $R^2 = 0.77$), invertase (IV) activity (**P**; $P < 0.0001$, $F_{5, 53} = 24.49$, $R^2 = 0.70$), leucine aminopeptidase (LAP) activity (**Q**; $P < 0.0001$, $F_{5, 53} = 21.15$, $R^2 = 0.67$), peroxidase (PER) activity (**R**; $P < 0.0001$, $F_{5, 53} = 41.16$, $R^2 = 0.80$), and urease (UR) activity (**S**; $P < 0.0001$, $F_{5, 53} = 21.02$, $R^2 = 0.66$). Colors represent soil type: agricultural soils are brown, fresh dredged sediments are turquoise, and weathered dredged sediments are purple. Shaded regions represent 95% confidence intervals; each point represents one plant (n = 20 for weathered dredged sediments, n = 19 for agricultural soils, n = 5 for fresh dredged sediments).

Soil properties and plant response associations within 100% Soil Types

All soil properties and the plant responses of total biomass, height and leaf count for the 100% soil types were placed into two matrices to assess their associations through the dimensionality of a canonical correlation analysis (**Fig 3.7A**). For the 100% soil types, only one of the three canonical dimensions were statistically significant from each other (**Table S3.2A**; $P < 0.05$). The first dimension was strongly influenced by the soil properties % silt (1.24) and % clay (-1.14) and also influenced by the plant response height (-0.09; **Table S3.2B**).

Soil physical, chemical and biological properties were condensed into two principal components for the 100% soil type data, which explained 25.5% and 19.6% of the variation within the data. The analysis of similarity of the two linear principal components ascertained differences in the soil properties based on the interaction of soil type and time, but separation was minimal ($P = 0.019$, $R^2 = 0.02$; **Fig 3.7B**).

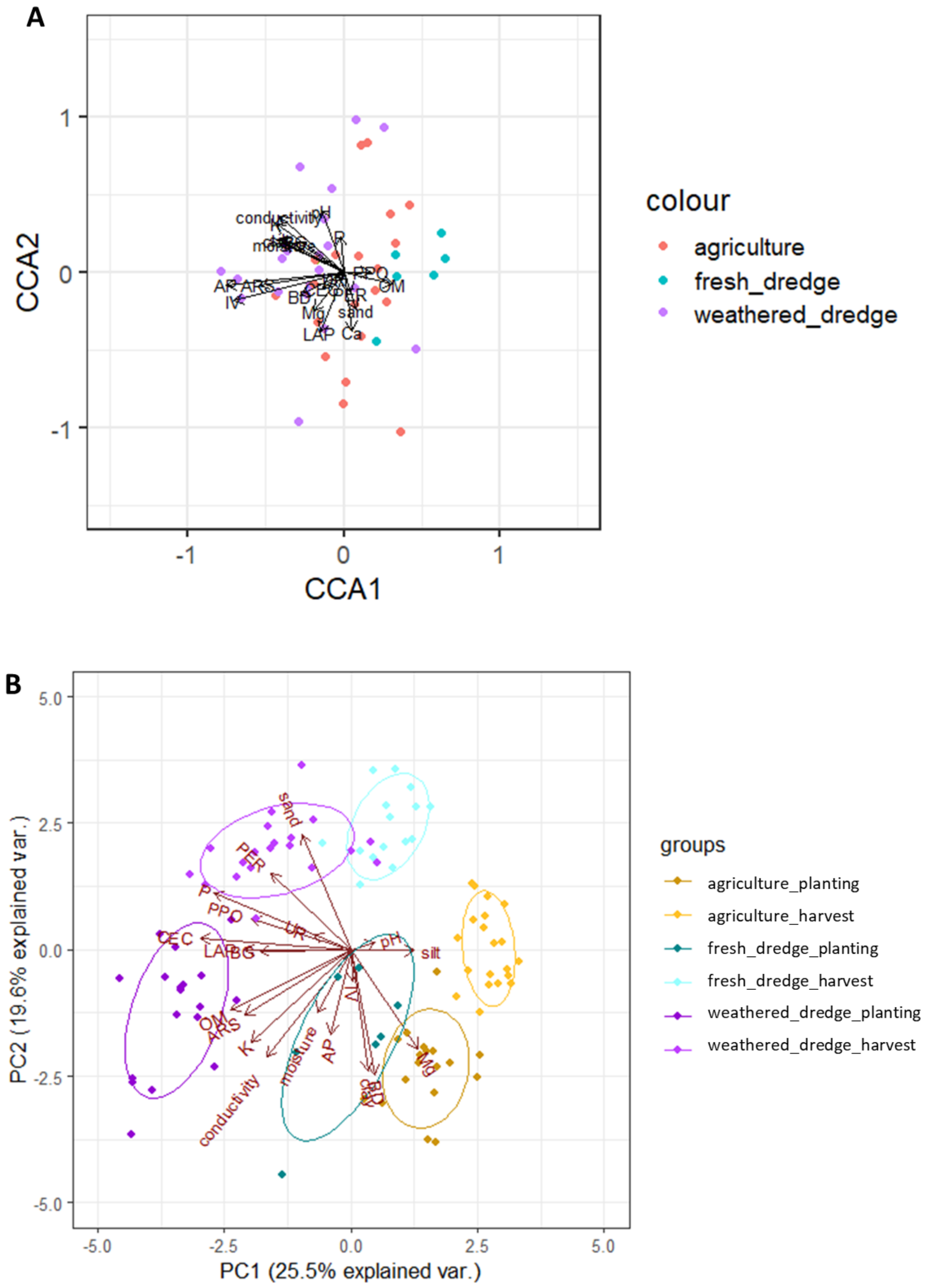

Fig 3.7. (A) Soil properties and plant responses were separated into two matrices to ascertain their associations influenced by the 100% soil types through a canonical correlation analysis (CCA; $P < 0.05$). Colors represent agricultural soil in pink, fresh dredged sediments in blue, and weathered dredged sediments in purple. **(B)** The

interaction of soil type and time was significant when accounting for differences in soil properties of the 100% soil treatments ($P = 0.019$, $R^2 = 0.02$). Ellipses represent 95% confidence intervals, and points represent individual soil samples (n = 20 per soil type per date collected). Colors represent soil type and shading represents time (darker colors are prior to planting while lighter colors are following harvest): agricultural soils are brown, fresh dredged sediments are turquoise, and weathered dredged sediments are purple.

Soil Physical Property Responses to 100% soil types

For the 100% soil types, moisture content changed over time and was dependent on soil type ($F_{2,57} = 23.64$, $R^2 = 0.59$, $P < 0.0001$; **Fig 3.8A**), such that before planting, moisture content was ~2.7x greater in the fresh dredged sediments than either weathered dredged sediments or agricultural soils (**Fig 3.8A**). However, this difference was lost at harvest, when moisture content decreased in the agricultural soils and fresh dredged sediments, so that both contained ~45% less moisture than weathered dredged sediments (**Fig 3.8A**). BD also changed over time and was dependent on 100% soil type ($F_{2,57} = 53.66$, $R^2 = 0.89$, $P < 0.0001$; **Fig 3.8B**). Prior to planting, BD in fresh and weathered dredged sediments was 6% and 10% lower than in agricultural soils, respectively (**Fig 3.8B**). At harvest, BD was 24% lower in fresh dredged sediments than in either weathered dredged sediments or agricultural soils, with both fresh and weathered dredged sediments falling below the USDA recommended lower threshold for BD (1.0 -1.4 g/ml^{-1}; **Fig 3.8B**).

The constituents of soil texture changed over time by 100% soil type. The percentage of sand prior to planting was ~23% higher in weathered dredged sediments than fresh dredged sediments or agricultural soils ($F_{1,114} = 10.42$, $R^2 = 0.43$, $P < 0.0001$; **Fig S3.11A**). At harvest, the percentage of sand increased in all 100% treatments resulting in no differences between dredged sediment types, but dredged sediments had a higher percentage of sand than agricultural soils (**Fig S3.11A**). Contrastingly, the percentage of clay significantly decreased over time in all 100% soil types ($F_{2, 110}$ = 5.300, R^2 = 0.41, P = 0.0018; **Fig S3.11B**). Before planting, there was a significantly higher percentage of clay in both dredged sediment types compared to agricultural soils, however, at harvest, this percentage of clay difference from agricultural soils was lost (**Fig S3.11B**). There was no significant difference in silt content with respect to soil type, time, or their combination (P < 0.05).

Soil compaction differed between the 100% soil types over time ($R^2_{4, 200}$ = 20.91, P = 0.0003; **Fig S3.3**). Before planting, all 100% soil types had soil compaction classified as 'low' within the pots (**Fig S3.3**). However, at harvest, agricultural soils had increases to 'moderate' soil compaction in a few pots while fresh dredged sediments increased to 'high' compaction in most of the pots, whereas weathered dredged sediment applications maintained 'low' compaction (**Fig S3.3)**.

<u>Soil Chemical Property Responses to 100% soil types</u>

Both Soil pH and EC were altered by 100% soil type over time. Fresh dredged sediments had a higher soil pH compared to both weathered dredged sediments and agricultural soils before planting ($F_{2, 57}$ = 23.64, R^2 = 0.59, P < 0.0001; **Fig S3.12**). By harvest, soil pH decreased in the fresh dredged sediments, resulting in no differences between 100% soil types within the USDA recommended range (6.5-7.2; **Fig S3.12**). Additionally, there were initially no differences in EC across the 100% soil types ($F_{2, 110}$ = 4.864, R^2 = 0.66, P = 0.0095; **Fig 3.8C**). However, with a significant overall decrease in EC at harvest, weathered dredged sediments had ~10% higher EC than fresh dredged sediments or agricultural soils (**Fig 3.8C**).

Cation content changed by 100% soil type over time. Specifically pattern established for CEC continued for Ca^+ content, such that CEC levels and Ca^+ content in 100% weathered dredged sediments were ~46% higher than in fresh dredged sediments, with fresh dredged sediments having ~25% higher CEC levels and Ca^+ content than agricultural soils before planting (CEC: $F_{2, 88} = 15.08$, $R^2 = 0.92$, $P < 0.0001$, **Fig 3.8D**; Ca^+: $F_{2, 88} = 15.08$, $R^2 = 0.92$, $P < 0.0001$, **Fig S3.13**). With a decrease in CEC levels and Ca^+ content in both agricultural soils and weathered dredged sediments, there were no differences between dredged sediments, however, CEC levels and Ca^+ content were ~39% lower in the agricultural soils (**Fig 8D**, **Fig S13.3**). Additionally, before planting, Mg^+ content was~37% higher in agricultural soils compared to weathered dredged sediments, which had ~29% higher Mg^+ content than fresh dredged sediments ($F_{2, 88} = 15.08$, $R^2 = 0.92$, $P < 0.0001$; **Fig 3.8E**). At harvest, there was a decrease in Mg^+ content in both agricultural soils and weathered dredged sediments, resulting in agricultural soils maintaining higher Mg^+ content than either dredged sediment type, which had the same Mg^+ content (**Fig 3.8E**). Lastly, K content was 7% higher in weathered dredged sediments than in agricultural soils, which had 30% higher K content than fresh dredged sediments prior to planting ($F_{2, 52} = 30.31$, $R^2 = 0.81$, $P < 0.0001$; **Fig 3.8F**). With a significant decrease in K content in both agricultural soils and weathered dredged sediments and no change in the K content of fresh dredged sediments, this resulted in no difference in K content between the 100% soil types (**Fig 3.8F**).

Soil nutrient content varied in response to the 100% soil types. Soil type alone significantly changed P content within the 100% treatments ($F_{2,53} = 268.7$, $P < 0.0001$; **Fig 3.8G**), such that weathered dredged sediments had 124% higher P content than agricultural soils, and fresh dredged sediments had 64% higher P content than agricultural soils, regardless of time ($P > 0.05$). However, the interaction of 100% soil type and time significantly influenced OM content ($F_{2, 88} = 9.601$, $R^2 = 0.73$, $P = 0.0002$; **Fig 3.8H**). Before planting, weathered dredged sediments had 57% higher OM content compared to agricultural soils, which had 30% higher OM content than fresh dredged sediments (**Fig 3.8H**). While OM content decreased in both agricultural soils and weathered dredged sediments at harvest, weathered dredged sediments still maintained higher OM content than fresh dredged sediments or agricultural soils, which were not different (**Fig 3.8H**).

<u>Soil Biological Property Responses to 100% soil types</u>

The interaction of 100% soil type and time significantly changed BG activity ($F_{2,54} = 4.362$, $R^2 = 0.53$, $P = 0.0175$; **Fig 3.8I**) such that there was ~61% higher BG activity in fresh dredged sediments compared to agricultural soils, but this higher activity was lost by harvest. Additionally, weathered dredged sediments had ~1500% greater BG activity than agricultural soils and ~292% greater activity than fresh dredged sediments. This high BG activity was maintained through harvest **(Fig 3.8I**). Meanwhile, soil type ($F_{2,109} = 69.55$, $P < 0.0001$; **Fig 3.8J**) and time ($F_{1,109} = 7.83$, $P = 0.0061$; **Fig S3.14**) independently changed PPO activity in the 100% soil types. PPO activity was ~315% higher in fresh and ~450% in weathered dredged sediments compared to agricultural soils (**Fig 3.8J**). Regardless of soil type, PPO activity was significantly lower at harvest compared to before planting (**Fig S3.14**).

In the 100% soil type treatments, several enzyme activities were influenced by soil type alone. Weathered dredged sediments had ~77% higher ARS activity than any other soil type ($F_{2,57}$ = 4.596, P = 0.0141; **Fig 3.8K**). IV activity in fresh dredged sediments was ~310% less than either agricultural soils or weathered dredged sediments ($F_{2,54}$ = 16.18, P < 0.0001, R^2 = 0.40; **Fig** 3.**8L**). Compared to agricultural soils, LAP activity was ~276% higher in fresh dredged sediments and ~1186% higher in weathered dredged sediments ($F_{2,108}$ = 45.41, P < 0.0001; **Fig 3.8M**). PER activity was ~176% higher in both fresh and weathered dredged sediments compared to agricultural soils but were not different between each other ($F_{2,108}$ = 46.73, P < 0.0001; **Fig 3.8N**). UR activity was 1.5x higher in weathered dredged sediments compared to fresh dredged sediments, but neither were different compared to agricultural soils ($F_{2, 113}$ = 4.60, P = 0.0120; **Fig 3.8O**). However, neither time (ARS: P = 0.0630, IV: P = 0.8748, LAP: P = 0.2405, PER: P = 0.1643, UR: P = 0.4612) nor the interaction between soil type and time was significant (ARS: P = 0.4294, IV: P = 0.6661, LAP: P = 0.7831, PER: P = 0.2044, UR: P = 0.7700). There was no significance among the 100% treatments regarding soil type (P = 0.0810), time (P = 0.3447), or their interaction (P = 0.6760) for AKP activity.

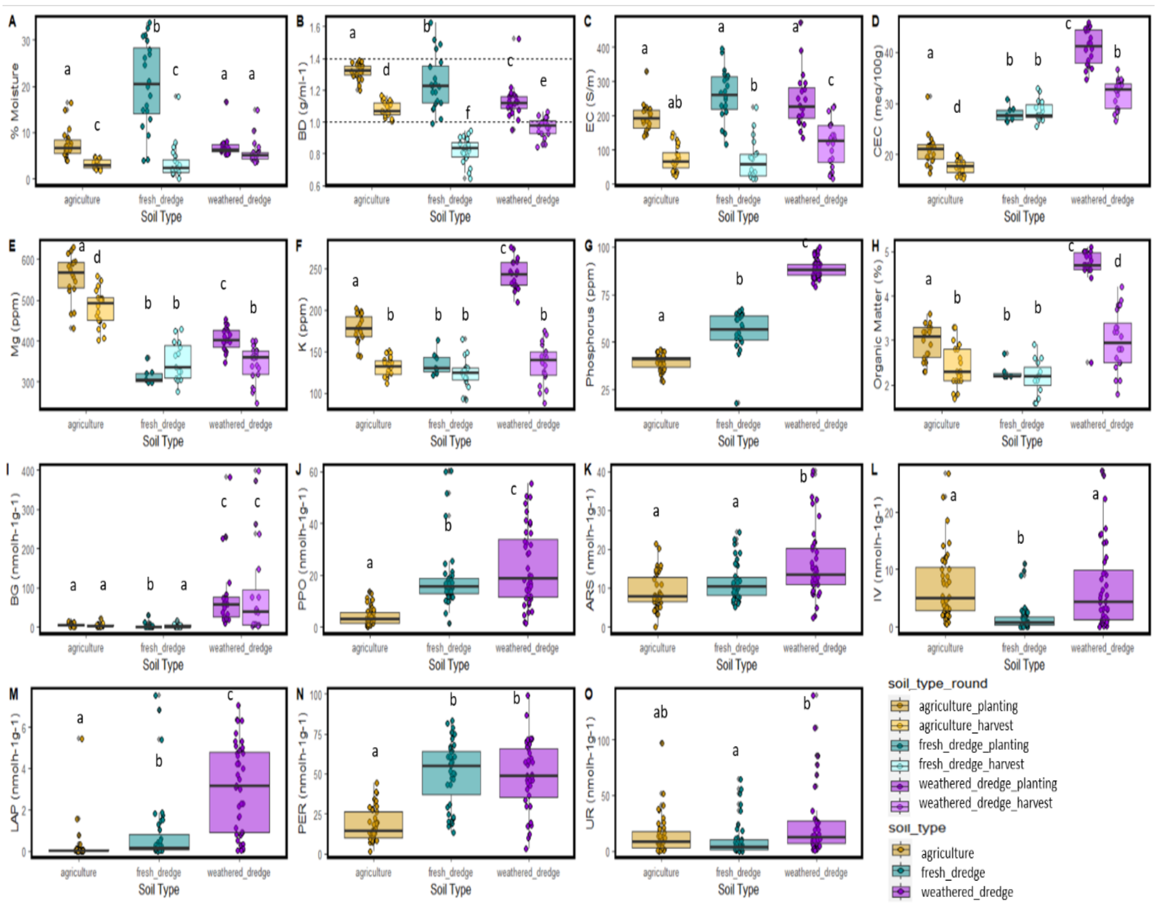

Fig 3.8. (A) Moisture content was higher in the fresh dredged sediments than either weathered dredged sediments or agricultural soils before planting. However, this advantage was lost through the growing season as fresh dredged sediments lost moisture content along with agricultural soils, while weathered dredged sediments maintained moisture content throughout the growing season ($F_{2, 57} = 23.64$, $R^2 = 0.59$, $P < 0.0001$). **(B)** Bulk Density (BD) was higher in the agricultural soils compared to fresh dredged sediments followed by weathered dredged sediments; however, BD decreased overall, with only the agricultural soils maintaining levels within the USDA recommended range (dashed black line; 1.0 -1.4 g/ml^{-1}), while both fresh and weathered dredged sediments fell below the lower threshold ($F_{2, 57} = 53.66$, $R^2 = 0.89$, $P < 0.0001$). **(C)** Electrical conductivity (EC) was the same among the soil types before planting, but decreased at harvest, with weathered dredged sediments maintaining higher EC than fresh dredged sediments, but neither were different from agricultural soils ($F_{2, 110} = 4.864$, $R^2 = 0.66$, $P = 0.0095$). **(D)** Cation exchange capacity (CEC) was highest in weathered dredged sediments, followed by fresh dredged sediments compared to agricultural soils before planting. However, at harvest, CEC levels in weathered dredged sediments decreased to match that found in fresh dredged sediments, which were both higher than agricultural soils ($F_{2, 88} = 15.08$, $R^2 = 0.92$, $P < 0.0001$). **(E)** Magnesium (Mg^+) content was highest overall in agricultural soils, followed by weathered dredged sediments compared to fresh dredged sediments before planting; however, at harvest there was a decrease in Mg^+ in both weathered dredged sediments and agricultural soils that removed any difference between dredged sediments, with agricultural soils maintaining higher Mg^+ content than

either fresh or weathered dredged sediments ($F_{2,\,88}$ = 15.08, R^2 = 0.92, P < 0.0001). **(F)** Potassium (K) content was highest in weathered dredged sediments, followed by agricultural soils when compared to fresh dredged sediments, however, these differences were lost over the growing season ($F_{2,\,52}$ = 30.31, R^2 = 0.81, P < 0.0001). **(G)** Phosphorus (P) content was highest in the weathered dredged sediments and high in the fresh dredged sediments compared to the P content in agricultural soils ($F_{2,53}$ = 268.7, P < 0.0001). **(H)** Organic matter (OM) content was highest in the weathered dredged sediments, followed by agricultural soils compared to fresh dredged sediments before planting; however, both weathered dredged sediments and agricultural soils lost OM content such that OM content in agricultural soils matched that found in fresh dredged sediments and weathered dredged sediments still maintained the highest OM content overall ($F_{2,\,88}$ = 9.601, R^2 = 0.73, P = 0.0002). **(I)** β-glucoside (BG) activity was higher in fresh dredged sediments than agricultural soils before planting but that was lost at harvest, while BG activity maintained significantly higher throughout the growing season ($F_{2,54}$ = 4.362, R^2 = 0.53, P = 0.0175). **(J)** Polyphenol oxidase (PPO) activity was highest in the weathered dredged sediments, followed by fresh dredged sediments, and PPO activity was lowest in agricultural soils ($F_{2,109}$ = 69.55, P < 0.0001). **(K)** Arylsulfatase (ARS) activity was higher in the weathered dredged sediments than either fresh dredged sediments or agricultural soils ($F_{2,57}$ = 4.596, P = 0.0141). **(L)** Invertase (IV) activity was lower in the fresh dredged sediments than either weathered dredged sediments or agricultural soils ($F_{2,54}$ = 16.18, P < 0.0001, R^2 = 0.40). **(M)** Leucine aminopeptidase (LAP) activity was higher in the weathered than fresh dredged sediments, which were both higher than LAP activity in agricultural soils ($F_{2,108}$ = 45.41, P < 0.0001). **(N)** Peroxidase (PER) activity was equal between fresh and weathered dredged sediments but was higher than PER activity in agricultural soils ($F_{2,108}$ = 46.73, P < 0.0001). **(O)** Urease (UR) activity was higher in weathered dredged sediments compared to UR activity in fresh dredged sediments, however, neither were different than UR activity in agricultural soils ($F_{2,\,113}$ = 4.60, P = 0.0120). For all graphs **(A-O)** colors represent soil type and shading represents time (darker colors are prior to planting while lighter colors are following harvest): agricultural soils are brown, fresh dredged sediments are turquoise, and weathered dredged sediments are purple and boxes are 50% quartiles, with the thick black line indicating the median value. Each point represents one soil sample (soil type: n = 40; soil type, and time combinations: n = 20). Letters indicate significant differences based on Tukey’s post hoc analyses (P < 0.05).

DISCUSSION

Dredged sediments are increasingly used an amendment to restore degraded soils (Daniels et al. 2007; Darmody and Marlin 2008; Roddy et al. *in press*; Julian et al. *in review*), however, the rate of application necessary to get the greatest benefits from their application is largely unknown. Furthermore, the degree to which the application rate changes based on whether the sediments are weathered or sourced directly from the dredging barge (‘fresh’) is also unknown. We found that while there was variability in the responses of soil properties among the application ratios determined using the nutrient recovery ratio, this did not translate to any changes in corn growth or production. Additionally, it was only in the 100% applications that changes in soil properties due to dredged sediment condition drove changes in corn production. Specifically, weathered dredged sediments provided greater benefits to both soil properties and corn responses when compared to the agricultural soils, while the fresh dredged sediments created an inhospitable environment for corn growth. This research expands our current understanding of the application ratios of different dredged sediment conditions to agricultural soils.

Agricultural Soil compared to Application Ratios of Dredged Sediments

Application ratios of dredged sediments to agricultural soils as a function of soil type significantly increased germination rates but this failed to translate to significant changes in plant growth compared to the agricultural control. Germination rates for the higher application ratios (5:95, 10:90) of weathered dredged sediments to agricultural soils and the highest and lowest application ratios of fresh dredged sediments to agricultural soils (10:90, 1:99) reached 100% germination rates faster compared to any other soil type and application ratio combination. Despite these differences, we failed to identify significant differences in growth metrics (*i.e.*, plant stage, leaf count, RGR, height) or production (*i.e.*, biomass) for any application ratio of either fresh or weathered dredged sediments to agricultural soils when compared to the unamended agricultural soils. This does not mean that dredged sediments themselves are not a good amendment for increasing plant performance. Indeed, other greenhouse studies using dredged sediments found that their application as a soil amendment increased plant germination rates, growth and production for a variety of agriculturally important plants including both corn and soybean (*Glycine maximum*; Woodard 1999; Canet et al. 2003; Darmody et al. 2004; Ebbs et al. 2006; Benson 2017; Brigham et al. 2021). Instead, our results suggest that the nutrient recovery ratio does not provide adequate guidance for the application rates of dredged sediments to agricultural soils if improving plant growth is the overarching goal. Consequently, to better understand the ideal application rate for applying dredged sediments to agricultural soils, further research is needed.

While we didn't identify changes in plant performance, the soil physical environment did significantly vary in several ways due to the different soil type and application ratio combinations. Specifically, BD within all application ratio and soil type combinations was either equal to or below the levels of unamended agricultural soils. However, at harvest the higher application ratios of fresh (5:95 and 10:90) and weathered (10:90) dredged sediments to agricultural soils fell below the USDA recommended range for agricultural systems with clayey soils (Stott 2019), suggesting that despite decreasing BD, these ratios are not ideal for improving soil porosity in agricultural soils. These findings align with previous dredged sediment application research in a greenhouse where BD decreased when weathered dredged sediments were applied (Brigham et al. 2021). It also aligns with other soil amendment research where BD decreased with the application of biosolids compared to unamended agricultural soils in both greenhouse and field experiments (García-Orenes et al. 2005; Spargo et al. 2008). Another measure of soil porosity, soil compaction, also changed over the growing season, shifting slightly from 'low' to 'moderate' for all combinations of soil type and application ratios except the highest application rate of weathered dredged sediments to agricultural soil (10:90). As 'moderate' compaction still falls within an acceptable range for successful crop production (Stott 2019), these results suggest that dredged sediment applications following guidelines from the nutrient recovery ratio successfully alter compaction in beneficial ways, supporting previous research demonstrating dredged sediments can hold soil compaction within the acceptable range when applied in a field setting (Darmody and Diaz 2017). Taken together, changes in BD and compaction due to the application of dredged sediments to agricultural soils are beneficial for soil porosity.

Changes to physical properties due to the application of dredged sediments to agricultural soils also occurred throughout the growing season. We identified changes in soil moisture content over the growing season as a function of the interaction of soil type and application ratio. Despite initial differences in soil moisture content where there was less moisture in the 3:97 ratios of both weathered and fresh dredged sediments to agricultural soils and higher moisture content in the 10:90 weathered dredged sediments to agricultural soil ratio, at harvest, all differences in moisture content were lost, resulting in all application ratio and soil type combinations containing the same moisture content. These changes to soil moisture were perhaps the most surprising as dredged sediments are known to have high water holding capacities (Averett et al. 1990); however these results could have been an artifact of the controlled greenhouse environment as both temperature and water application which are highly linked to soil moisture were controlled.

Finally, the addition of dredged sediments to agricultural soils, regardless of condition, shifted soil texture from a sand-clay-loam texture to a clay-sand-loam texture, bringing the texture of amended agricultural soils more in line with row-cropping agricultural standards (Stott 2019). These findings were expected given previous research where clay loam sediments applied to sandy soils shifted soil texture to a more stable physical soil environment (Darmody and Diaz 2017). Overall, the soil physical environment was only marginally altered by different applications of dredged sediments, regardless of condition, which suggests that application rates for dredged sediments based on the nutrient recovery ratio may be successful for mitigating the degradation of soil physical properties in agricultural systems (Hellerstein et al. 2019). Additionally, there is evidence to suggest that the benefits quantified within our study, such as lower BD and compaction and a shift in soil texture, would be even greater with subsequent growing seasons (Darmody and Diaz 2017; Kiani et al. 2023).

Several biochemical cycles that are vital for crop growth changed as a function of soil type and application ratio. Compared to initial values, P content decreased at harvest across all soil types and application ratios; however, all soil type and application ratio combinations contained higher P content than unamended agricultural soils. This aligns with previous research that found dredged sediments contain higher levels of P than agricultural soils (Canet et al. 2003; Darmody and Diaz 2017; Brigham et al. 2021; Kiani et al. 2021). Additionally, AKP activity, an enzyme necessary for P mineralization, also decreased at harvest compared to before planting, regardless of soil type or application rate. These findings were surprising as mineralized P is typically found to be limited in agricultural soils (Ringeval et al. 2017; Hallama et al. 2019); however, this could indicate that the available P is sufficient within the dredged sediments without microbially-driven mineralization (Grzyb et al. 2020).

We did not measure soil N content; however, we found evidence that several enzymes associated with N cycling were altered as a function of soil type and application ratio of dredged sediment to agricultural soil. LAP activity, an enzyme associated with both C and N mineralization (Greenfield et al. 2021), was initially higher in the 5:95 and 10:90 weathered dredged sediment to agricultural soil ratios, and increased overall over the growing season. However, both IV and UR activity, two enzymes associated with only N mineralization, were either significantly lower in the highest fresh dredged sediment application compared to any other soil type and application ratio, regardless of time (IV), or did not change over the growing season for any soil type or application ratio combination (UR). These findings suggest N mineralization by microbially-driven enzyme activities vary within the calculated application rates by specific enzyme, likely as a result of the available forms of N within dredged sediments. These results align with other soil amendment research which demonstrates that when N is already present in high concentrations, the need for N mineralization following the application of soil amendments is reduced (Clark et al. 2019). However, since we did not measure soil N content, we do not know how these changes in activity translate to differences in overall N dynamics.

While both N and P cycling appear to change due to the addition of dredged sediments, constituents of C cycling were not influenced by any soil type or application rate combination. Typically, dredged sediments are high in OM when compared to agricultural soils (Sigua 2005; Oliveira et al. 2017; Kiani et al. 2021), but in this study, OM content did not differ across application ratios as a function of soil type compared to the unamended agricultural soils and instead decreased over the growing season in all but the 3:97 dredged sediment to agricultural soil application. Additionally, the enzymes associated with the breakdown of OM (BG, PPO, IV, PER) either decreased in activity over the growing season or were not significantly altered by soil type and application ratio combination or time. Taken together, this suggests that the calculated application rates for the different dredged sediments may contain enough OM content to negate the need for C mineralization through enzyme activities (Aon and Colaneri 2001) and aligns with findings from biosolids research (Fernández et al. 2009).

Previous assessments of the dredged sediments used in this study reported higher S content than typically found in agricultural soils (Hull & Associates 2018). While we did not quantify S content within this study, we did assess ARS activity and found that it decreased over the growing season independent of soil type or application ratio. Our findings were not surprising, given that S mineralization through microbially-driven enzyme activities happens quickly in soils (Grzyb et al. 2020; Kumar et al. 2022) but also suggests changes in S content due to the application of dredged sediments, even if they are high in S, are unlikely.

Unamended Agricultural Soil compared to 100% Dredged Sediments

Within our study, we included 100% applications of fresh and weathered dredged sediments to agricultural soils as there are some instances, such as land reclamation or the removal of topsoil, where applications of 100% dredged sediments are desirable (Daniels et al. 2007; Darmody and Marlin 2008). Our results demonstrate that under these conditions, weathered dredged sediments outperform agricultural soils in creating a more hospitable environment for crop growth and production, while fresh dredged sediments proved detrimental to overall crop growth.

Biomass for corn grown in both agricultural soil and weathered dredged sediments had a positive relationship with the activity of the C cycling enzymes BG, PER and PPO, indicating that weathered dredged sediments and agricultural soils rely heavily on microbial-driven C mineralization. This may reflect limitations in available C common in agricultural systems (Clark et al. 2019; Hellerstein et al. 2019; Stott 2019). While the biomass for corn grown in fresh dredged sediments had a positive relationship with OM content, corn biomass decreased with increasing OM content in agricultural soil and weathered dredged sediments. As S cycling is often linked to OM content (Kumar et al. 2022), corn biomass decreased increasing ARS activity in all three 100% soil types. These negative relationships found together for both the agricultural soil and weathered dredged sediments may indicate that there is a threshold for non-mineralized C forms within agricultural systems beyond which crop production is reduced (Aon and Colaneri 2001; Stott 2019). Furthermore, these relationships may indicate that nutrient mineralization of the S forms present in both the agricultural soils and dredged sediments, regardless of condition, are not necessary for corn production (Schoenau and Malhi 2008; Scherer 2009).

A positive relationship was found between AKP, the P mineralizing enzyme, and corn biomass when grown in agricultural soil and weathered dredged sediments but not in fresh dredged sediments. This positive relationship suggests these soil types can provide greater microbially-driven P availability which seems to be lacking in fresh dredged sediments (Ringeval et al. 2017; Hellerstein et al. 2019; Stott 2019). Contrastingly, we found a negative relationship between corn biomass and P content in both agricultural soils and weathered dredged sediments, which may indicate that there is a threshold for non-mineralized P within agricultural soils beyond which is detrimental to crop production (Stott 2019; Durrer et al. 2021).

The relationships between corn biomass and the N cycling enzymes (IV, LAP and UR) varied across soil types. Biomass for corn grown in agricultural soils and weathered dredged sediments decreased with increasing IV and UR activity while corn biomass increased with increasing IV and UR activity in fresh dredged sediments. Additionally, total biomass for corn grown in weathered dredged sediments decreased with increasing LAP activity compared to agricultural soils and fresh dredged sediments, where increasing LAP activity led to increasing corn biomass. These varied relationships between biomass and N mineralizing enzymes within the different soil types may indicate that either there are larger amounts of N than is needed, and therefore nutrient mineralization is not necessary, or mineralization of the N forms present in agricultural soils are not necessary for corn production due to sufficient availability (Canet et al. 2003; Sigua 2005).

Corn biomass varied in response to changes in several soil chemical properties of the 100% soil types. Biomass for corn grown in unamended agricultural soil and weathered dredged sediments had a negative relationship with soil pH, while corn biomass increased with increasing pH in fresh dredged sediments. Corn biomass grown in the 100% agricultural soils and weathered dredged sediments decreasing with increasing pH was expected given As weathered dredged sediments often have higher pH than agricultural soils (Tarkalson et al. 2006), finding a negative relationship between corn biomass and increasing soil pH was expected in the weathered dredged sediments to align with that in agricultural soils as a balance in soil chemistry within agricultural soils is essential for crop development (Russell et al. 2006; Gaspar 2019). The positive relationship found in fresh dredged sediments between corn biomass and soil pH was unexpected and may be a positive benefit of the weathering process these sediments were undergoing. As EC is often connected to the soil's cation content (NRCS USDA 2017), the positive relationships between corn biomass and EC, K, Mg^+ and Ca^+ content found in both weathered and fresh dredged sediments is not surprising. With these relationships in contrast to what we found for biomass grown in agricultural soils, this suggests dredged sediments, regardless of condition, provide levels of cations necessary for plant growth often lacking in agricultural soils (Canet et al. 2003; Daniels et al. 2007; Darmody and Diaz 2017).

Corn biomass increased as a function of increases in the soil texture constituent clay in both the unamended agricultural soils and 100% weathered dredged sediments. However, biomass for corn grown in the 100% fresh dredged sediments decreased with increasing clay content. As a result, corn biomass had the opposite relationship with sand where the biomass for corn grown in 100% agricultural soils decreased but remained unchanged in 100% weathered dredged sediments, while the biomass for corn grown in 100% fresh dredged sediments increased. Additionally, the biomass for corn grown in both 100% weathered and fresh dredged sediments decreased with increasing silt, whereas the biomass for corn grown in 100% agricultural soils increased. These results likely reflect nuances of the experiment as Toledo soils are often high in clay and low in sand and silt (USDA 2012) and the seed variety chosen grows optimally in high clay soils.

While germination rates were not different among the 100% soil types, once emerged, corn varied in plant development and production by soil type. Most of the differences found in plant stage development for corn grown in the 100% soil types were between corn vegetation stages V5 – V8, but those differences resolved by V9. However, these results likely reflects differences in survival as corn grown in the fresh dredged sediments failed to develop past V9 stage. Despite the differences in plant stage development, we found no differences in plant height or the associated RGR for corn grown in any of the 100% soil types. This could have been due to greenhouse conditions including the infestation of pests during week 12 of the experiment when corn was reaching vegetative stages V9-V11, as these findings do not align with previous greenhouse research which found that plants grown in 100% dredged sediments grew better than those plants grown in agricultural soils (Canet et al. 2003; Ebbs et al. 2006; Brigham et al. 2021).

While corn production was better overall when grown in 100% weathered dredged sediments, 100% fresh sediments were detrimental to corn production as above, below and total biomass were significantly lower when compared to 100% agricultural soils. The biggest difference in corn production between corn grown in weathered and fresh dredged sediments is allocation. Plants in the fresh dredged sediments were much smaller with less root biomass than those grown in unamended agricultural soils or weathered dredged sediments. This suggests that fresh dredged sediments cannot provide the proper soil environment for corn growth and production compared to unamended agricultural soils. In total, plant performance results support the idea that weathered dredged sediments provide a better soil environment for corn growth and development than either unamended agricultural soils or fresh dredged sediments, which aligns with previous research (Daniels et al. 2007; Darmody and Marlin 2008; Darmody and Diaz 2017; Julian et al. *in review*).

In addition to differences in plant performance, we also quantified differences in soil physical properties among the 100% soil types reflective of the weathering process undergone by fresh dredged sediments. Specifically, initial moisture content was drastically higher in the 100% fresh dredged sediments compared to the 100% weathered dredged sediments and agricultural soils. Additionally, soil compaction increased from ‘low’ to ‘high’ over the growing season in most of the fresh dredged sediment pots, after the growing season, compared to the ‘low’ to ‘moderate’ of both the 100% weathered dredged sediments and agricultural soils. Our findings align with observations from field studies using fresh dredged sediments for reclamation (Daniels et al. 2007; Darmody and Marlin 2008), rapid dewatering and soil compaction due to the weathering processes (Vermeulen et al. 2005). In contrast, by the end of the growing season, weathered dredged sediments had higher moisture content than either of the other two 100% soil types. Similarly, soil compaction remained ‘low’ in the 100% weathered dredged sediments compared to agricultural soils. These results support previous research that weathered dredged sediments can maintain moisture content regardless of environmental conditions (Julian et al. *in review*) and aligns with previous research demonstrating low compaction for weathered dredged sediments (Darmody and Diaz 2017). Consequently, 100% weathered sediments may provide a suitable growth media in environments where soil moisture is advantageous.

Dredged sediments had similar responses for both BD and soil texture when compared to unamended agricultural soils. In both 100% dredged sediments conditions, BD started lower than the agricultural soils, and decreased at harvest to fall just below USDA recommended range (Stott 2019). This has been seen in previous research with 100% dredged sediments (Sigua 2005; Benson 2017; Julian et al. *in review*), and suggests soil porosity is overall better in dredged sediments than unamended agricultural soils. Over the course of the experiment, soil texture shifted from a clay-sand-silt ratio to a sand-clay-silt ratio for both fresh and weathered dredged sediments compared to the unamended agricultural soils, supporting previous research that found soil texture shifts to create a more stable texture for plant growth with dredged sediment use (Fonseca et al. 1998; Sigua 2005; Daniels et al. 2007; Darmody and Diaz 2017). Together, our findings suggest that while fresh dredged sediments can provide some stability to physical properties, overall, weathered dredged sediments provide the most beneficial physical environment for plant growth.

Soil chemistry also changed over the growing season for the 100% fresh and weathered dredged sediments compared to the unamended agricultural soils. By the end of the growing season, both soil pH and EC fell within the USDA recommended range despite starting out of those ranges for fresh sediments (Stott 2019). These findings align with previous research which suggest that both the pH and EC of dredged sediments provide a better and more stable chemical environment when compared to unamended agricultural soils (Ebbs et al. 2006; Daniels et al. 2007; Darmody and Diaz 2017; Benson 2017; Brigham et al. 2021; Julian et al. *in review*). Similarly, both 100% dredged sediment types maintained steady levels of CEC, Ca^{+}, and Mg^{+} content over the growing season which is also consistent with previous research and suggests either dredged sediment source are adequate sources for these cations (Canet et al. 2003; Daniels et al. 2007; Darmody and Diaz 2017). In contrast, K content started out higher in the weathered dredged sediments compared to agricultural soils, and fresh dredged sediments had the lowest K content overall, but following a decrease at harvest, there was no difference between the 100% soil types. These results suggests that dredged sediments, regardless of condition, provides the same available K content adequate for growing crops in agricultural soils (Rhem 1994; Darmody and Diaz 2017). Altogether, these changes in soil chemical properties in the 100% soil types suggest that dredged sediments provide a beneficial chemical environment for plant growth regardless of sediment type.

Biogeochemical cycles also differed for 100% dredged sediments compared to agricultural soils such that weathered dredged sediments had higher constituents of the C, S and P cycle compared to 100% fresh dredged sediments and agricultural soils. Weathered dredged sediments had increased constituents of the C cycle, including OM content and the C mineralizing enzymes BG, PPO, and PER, compared to either 100% fresh dredged sediments or agricultural soils. With the breakdown of OM being an important process in soils (Gougoulias et al. 2014), our findings suggest weathered dredged sediments increase C cycling in agricultural systems and support previous research in this area (Averett et al. 1990; Sigua 2005; Vermeulen et al. 2005). Similarly, weathered sediments also had higher enzyme activity for the S mineralizing enzyme ARS compared to either fresh dredged sediments or agricultural soils. As S content is often linked to OM content (Kumar et al. 2022), this may suggest that weathered dredged sediments contain elevated S content, resulting in greater mineralization, benefiting the agricultural system overall by contributing to microbial biomass and amino acid production in crops (Schoenau and Malhi 2008; Scherer 2009; Kumar et al. 2022). Finally, P content was higher in the 100% weathered dredged sediments than fresh dredged sediments, which was higher than agricultural soils. However, despite these differences, there was no difference in AKP activity, an enzyme responsible for P mineralization. This may suggest that dredged sediments contain enough available P that mineralization is not necessary, although this differs from previous 100% dredged sediments research which found evidence for increasing P mineralization in weathered sediments (Julian et al. *in review*).

In contrast, variation in the enzymes responsible for N mineralization, including IV, LAP and UR, was more complicated. Both IV and UR activities were not different between the 100% weathered dredged sediments or agricultural soils but were lower in the fresh dredged sediments; however, LAP activity was higher in the weathered dredged sediments than in fresh dredged sediments followed by agricultural soils, suggesting weathered and fresh dredged sediments may contain different forms of N available for mineralization compared to agricultural soils. These different forms may promote the diversification and function of the microbial communities (Ouyang and Norton 2020), however, neither microbial community function or N content were measured in this study. Together, these findings suggest that weathered dredged sediments, used at 100%, provide adequate nutrients to influence biogeochemical cycling that can improve the agricultural system when compared to fresh dredged sediments.

Weathering process as a driver of change of dredged sediments soil properties

The overall purpose of our study was to assess the differences in soil property and crop responses to the condition of dredged sediments applied at different rates. While we failed to find significant differences in soil property or crop responses between dredged sediment conditions when applied in rates calculated based on the nutrient recovery ratio, we found varied responses soil properties, such as nutrient content, enzyme activities, and even physical properties between dredged sediment conditions of the 100% soil types that have not been previously documented. Not only does weathering alter the physical properties, shifting towards soils from sediments (Cappuyns et al. 2004; Vermeulen et al. 2003, 2005, 2007; Daniels et al. 2007; Oliveira et al. 2017), but weathering can also increase the biogeochemical processes within the soils (Cappuyns et al. 2004; Vermeulen et al. 2003, 2005, 2007; Daniels et al. 2007; Oliveira et al. 2017) when compared to fresh dredged sediments. This suggests that the weathering process is important when considering the use of dredged sediments in agricultural systems. Our research also suggests that exposure to actual weather is not necessary to incur the benefits of the weathering process since our experiment took place in the controlled environment of a greenhouse, with a constant temperature, moisture (humidity) and air flow, but further research is needed to understand differences in these two weathering environments.

Conclusion

Ratios of dredged sediments to agricultural soil based on the nutrient recovery ratio failed to drive differences in soil properties or plant performance for both weathered and fresh dredged sediments. Despite this failure, we did find an overall effect of dredged sediments such that corn grown in 100% weathered sediments grew larger as a function of changes in soil physical and chemical, and biogeochemical properties of weathered sediments which make them an extremely viable option as a growth compared to unamended agricultural soils and fresh dredged sediments. However, fresh sediments must be able to go through the weathering process to be viable for plant growth. This is particularly relevant in environments looking to start agricultural production or restoration from scratch (Daniels et al. 2007; Darmody and Diaz 2017; Roddy et al. *in press*; Julian et al. *in review*). Overall, this study expands our understanding of the viability of different dredged sediment conditions and application ratios as a soil amendment to agricultural soils.

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CHAPTER 4

A SECOND GROWING SEASON, REGARDLESS OF THE CROP SPECIES GROWN, INCREASES THE BENEFITS OF DREDGED SEDIMENTS AS A SOIL AMENDMENT

ABSTRACT

Crop rotation and soil amendments are field management practices farmers often utilize to increase crop yields and minimize soil degradation. Rotating crops provides many benefits including a reduced need for fertilizer applications and an increase in both microbial diversity and yields. In addition to crop rotation, amending soils with organic materials can also provide benefits such as improved soil structure and nutrients as well as increased yields. Research concerning their combined use indicates crop rotation can enhance the benefits of soil amendments. Recently dredged sediments have been identified as a potentially important soil amendment, however, research examining their effectiveness in conjunction with crop rotation is lacking. Additionally, there is no research to date investigating whether the fresh or weathered conditions of dredged sediments alters their benefits. Here, we factorially manipulated amendments of each dredged sediment condition combined with either crop rotation (corn / soybean) or continuous cropping (corn / corn) strategies in a greenhouse experiment to quantify changes in soil properties and crop responses. We found that while crop rotation does not drive changes in either soil property responses or plant responses, a second growing season of either soybean or corn increased the overall benefits to soil properties when using dredged sediments. Additionally, weathered dredged sediments improved soil properties and crop responses more than fresh dredged sediment compared to pure agricultural soil. Our study demonstrates that the application of dredged sediments,

especially weathered dredged sediments, across multiple growing seasons can enhance soil quality and promote increased crop yield, which are vital for mitigating soil degradation in agricultural soils.

INTRODUCTION

Row cropping agriculture is the dominant form of agriculture used in the US, encompassing approximately 300 million hectares of land (FOA 2016; Hellerstein et al. 2019); however, this technique has detrimental impacts on agricultural soils including the loss of nutrients, soil instability, and reduced microbiota (Wallander 2013; Baumhardt et al. 2015; Richardson and Dooley 2017; Scherr and Yadav 2020). To help mitigate and reverse the degradation of agricultural soils used in row cropping, field management practices like rotating crops and applications of soil amendments have gained traction. Crop rotation, or the growing of different crops over alternating growing seasons, is by far the most common field management practice used in the US and is used for 82 - 94% of all US row-cropping agricultural systems (Wallander 2013; Hellerstein et al. 2019; NRCS USDA 2021). Despite the success of crop rotation, the use of organic soil amendments like manure and biosolids which supplement degraded soils with organic nutrients faster than crop rotations has also gained popularity (Antonious 2016; Oliveira et al. 2017; Sharma et al. 2017; Hellerstein et al. 2019) and is often used in combination with crop rotation (Bronick and Lal 2005; Barbarick et al. 2012; Tian et al. 2015; Darmody and Diaz 2017; Ozlu et al. 2019). Dredged sediments, which are the mechanically removed sediments from waterways, have gained popularity for their ability to provide nutrients and improve the soil's structure and microbiota (Canet et al. 2003; Sigua 2005; Daniels et al. 2007; Darmody and Diaz 2017; Brigham et al. 2021; Kiani et al. 2021; Rúa et al. 2023; Roddy et al. *in press*; Julian et al. *in review* Chapter 2). Dredged sediments can be applied in two different conditions: fresh from the water source ('fresh') or following a period of dewatering on land ('weathered'). Both conditions are effective for restoring degraded soil properties and enhancing plant performance (Julian et al. *in prep* Chapter 3), but their ability to continue to be effective in combination with field management techniques like crop rotation is largely unknown. Here, we factorially manipulated dredged sediment condition (weathered vs fresh) and crop rotation strategy (corn / soybean vs corn / corn) in a greenhouse experiment to assess the combined benefits of these field techniques compared to unamended agricultural soils.

Within row-cropping agricultural systems, rotating crops each growing season can help restore soil nutrients and increase both soil stability and microorganism diversity (Jiang and Thelen 2004; Jayasundara et al. 2007; Karlen et al. 2013; Wallander 2013; McDaniel et al. 2014; Ashworth et al. 2017). Rotating crops also decreases the need for fertilizer application by approximately 43-92% (UCS 2017). Additionally, crop yields increase 4-20% with crop rotation by when compared to a continuous cropping strategy (Crookston et al. 1991; Wallander 2013; UCS 2017). By capitalizing on the demonstrated benefits of crop rotation, the incorporation of different organic soil amendments into combined field management practices presents potential for further enhancing the sustainability and productivity of agricultural systems.

The benefits of combining organic soil amendments (*i.e.*, manure, biosolids, biochar) with crop rotation have been well documented (Major et al. 2010; Tian et al. 2015; Yucel et al. 2015; Hoover et al. 2019; Ozlu et al. 2019; Song et al. 2019). These benefits include improvements to the soil physical environment, soil chemistry and the soil microbiota. For example, crop rotation coupled with organic soil amendments can reduce BD and compaction while increasing moisture content compared to crop rotation in unamended agricultural soils (Tian et al. 2015; Yucel et al. 2015). Rotation crops each growing season coupled with organic soil amendments can stabilize pH and electrical conductivity as well as increase nutrient content when compared to crop rotations in unamended agricultural soils (Major et al. 2010; Tian et al. 2015; Yucel et al. 2015; Hoover et al. 2019; Ozlu et al. 2019; Song et al. 2019). Additionally, crop rotations can drive increases in soil microorganism community function such as nutrient mineralization (Ozlu et al. 2019; Song et al. 2019), as well as increases in crop production (Major et al. 2010; Song et al. 2019; Hoover et al. 2019) that are minimal or not found in unamended agricultural soils. While there is a breadth of knowledge regarding the combination of the established soil amendments and crop rotation, the potential benefits of combing crop rotation with dredged sediments are less understood.

Dredged sediments alone can improve the soil environment when applied to an agricultural system (Fonseca et al. 1998; Darmody and Marlin 2002; Canet et al. 2003; Sigua et al. 2004; Sigua 2005; Daniels et al. 2007; Darmody and Diaz 2017; Rúa et al. 2023). For example, dredged sediments can improve the soil physical environment by providing better soil structure in the way of lowering bulk density, increasing moisture content, and lowering compaction for better crop production (Canet et al. 2003; Sigua et al. 2004; Daniels et al. 2007; Gabriel and Quemada 2011; Steele et al. 2012; Darmody and Diaz 2017). Additionally, applying dredged sediments to agricultural soils can improve the soil chemistry by stabilizing soil pH and increasing the nutrients needed for crop production (Fonseca et al. 1998; Darmody and Marlin 2002; Sigua et al. 2004). Lastly, dredged sediments have been shown to contain microorganisms beneficial for crop growth (Rúa et al. 2023) and these microorganisms can increase nutrient mineralization leading to increases in crop yields (Julian et al. *in review* Chapter 2). Despite these documented benefits from dredged sediment use in agriculture, research is just now emerging into understanding of the effects of dredged condition on these benefits to agricultural systems (Julian et al. *in prep* Chapter 3).

Research investigating the combination of dredged sediments and crop rotation is limited. Across different plant species, in both a greenhouse and field setting, the rotation of crops have shown to increase crop yields, when grown in 100% dredged sediments (Canet et al. 2003; Darmody and Diaz 2017). However, to date, there has been no research investigating the combination of different conditions of dredged sediments (weathered vs fresh) and a crop rotation strategy on soil properties and plant performance. Here, we sought to quantify changes in soil properties and crop responses with the combination of different dredged sediment conditions (weathered vs fresh) and a crop rotation strategy typical of Midwest agricultural production (corn / soybean) compared to a continuous cropping strategy (corn / corn). Based on the known benefits of rotating crops and the use of dredged sediments as a soil amendment, we hypothesized that these benefits would be further enhanced with the combination of dredged sediments and crop rotation, compared to a single crop grown continuously. Specifically, we expect to see an improved soil physical and chemical environment as well as increased microbial-associated enzymatic activities, which would all lead to increased crop yields.

METHODS

Experimental Design

We used a greenhouse study at Wright State University in Dayton, Ohio, USA to investigate how crop rotation alters crop responses and soil properties of agricultural soil amended with two different dredged sediment conditions. We factorially manipulated dredged sediment condition (weathered vs. fresh) and evaluated plant performance over two growing seasons: the first with corn only ('monocrop'), and the second with half of the pots receiving corn and half receiving soybean ('rotation'). Application rates of dredged sediments (1:99, 3:97 and 5:95) were applied according to the nutrient recovery ratio for corn and soybeans based on the known nutrient content (N and P) of the sediments and the agricultural soils (Julian et al. *in prep* Chapter 3) as well as the US EPA guidelines for amendment application (2013). There were no differences in plant growth, soil properties or their interaction among application rates during the first growing season (Julian et al. *in prep* Chapter 3), therefore, data was pooled across application rate by dredged sediment condition for all subsequent analyses. Additionally, we initially included three experimental controls: 100% unamended agricultural soil (0:100) to reflect unamended agricultural soil conditions, 100% fresh dredged sediments (100:0) and 100% weathered dredged sediments (100:0); however, during the first growing season, 75% of the corn grown in the 100:0 fresh dredged sediments died during the first growing season (Julian et al. *in prep* Chapter 3), so this control was excluded from the rotation growing season.

Dredged sediments and agricultural soils were collected as previously described (Julian et al. *in prep* Chapter 3). Briefly, weathered dredged sediments were collected in

January 2019 from the Great Lakes Dredged Material Center for Innovation in Toledo, Ohio, USA (41.6700354ºN, -83.5029711ºW; Rúa et al. 2023; Julian et al. *in review* Chapter 2) and fresh dredged sediments were collected in November and December 2019 by Luedtke Engineering during annual dredging of the Toledo Harbor (41.729301 ºN, -83.395612 ºW). Additionally, in January 2019 and January 2020, we collected agricultural soil from a conventionally farmed field of continuous corn in Oregon, OH (41.6751180 ºN, -83.4272100 ºW). This field is also in the same soil series as the dredged sediments (Toledo soil series).

All sediments and soils were transported to Wright State University campus following collection and stored in the greenhouse (weathered dredged sediments and agricultural soils) or the cold room (fresh dredged sediments) for up to 1.5 years until initial use for the first growing season. All soil and sediment sources were hand processed to remove plants, rocks and mollusk shells prior to being homogenized by hand. Each treatment combination was hand-mixed in an individual batch and cut with 20% sand (Garick, LLC, Cleveland, OH, USA) to improve drainage. Weathered dredged sediments were replicated 40 times for corn /corn 'monocropping' and 40 times for corn / soybean 'rotation', fresh dredged sediments were replicated 30 times for corn /corn 'monocropping' and 30 times for corn / soybean 'rotation' with an additional 10 replicates for both 'monocropping' and 'rotation' for 100% unamended agricultural soils, totaling 160 pots [(2 cropping strategies x 40 replicates x 2 soil types) = 160 pots]. We potted all treatment combinations into 1-gallon pots, weighing ~2.5 kg each.

During the first growing season, corn was planted in all pots on 20 August 2020 with two seeds obtained from Pioneer® (P33T60; Corteva Agriscience™, Wilmington, DE, USA) and allowed to grow an entire growing season until harvest (25 January 2021). Following the first growing season, corn and soil samples were collected, and pots were supplemented with additional treatment material to restore soil loss and simulate re-application between growing seasons. Treatment material was stored between 4 – 6 ^oC for approximately six months prior to re-application. Approximately one month following the end of the first growing season (28 February 2021), all pots were planted for the second growing season with half the pots planted with corn again (‘continuous corn’) and half planted with soybeans (‘rotation’). Since these soils had never been planted in soybean, the seeds obtained from Pioneer® (C442; Corteva Agriscience™, Wilmington, DE, USA) were first inoculated with a mixture of *Bradyrhizobium* sp. (Vigna), *Bradyrhizobium japonicum*, *Rhizobium leguminosarum biovar phaseoli* and *Rhizobium leguminosarum biovar viceae* prior to planting, using the slurry method instructions provided for Guard-N inoculate (Verdesian Life Sciences, LLC., Cary, NC, USA).

In the greenhouse, pots were randomly distributed with an equal number of each treatment combination across five blocks (A-E). All blocks were rotated every four weeks to mitigate any effects from greenhouse placement. The greenhouse environment was maintained with an average temperature of 23.9 ^oC and an average of 29.8% humidity.

After germination, all pots were thinned to a single seedling (4 weeks) by removing the seedling at a lower vegetative stage (Nafziger 2017; Ciampitti 2017). If there were two plants of the same plant stage and height, then we removed the center-most plant. If no seeds germinated, then a seedling from the same soil treatment combination was transplanted so that each pot contained a single plant by experiment day 29, following the protocol outlined in Julian et al. (*in prep* Chapter 3). Halfway through the growing season (24 May 2021), all plants were fertilized with 100 ml of the Miracle-Gro solution made following the Miracle-Gro application directions for potted plants, supplying each plant with a 24- 8-16 ratio of N, P, and K (Scotts Miracle-Gro Products, LLC, Marysville, OH, USA) as done in the previous growing season (Julian et al. *in prep* Chapter 3). All plants were then allowed to grow until senescence (21 June 2021 for corn, 2 July 2021 for soybean).

Sample Collection

Soil samples were collected from each pot at first harvest (25 January 2021, 'before planting') and again at rotation harvest (21 June 2021 for corn grown pots, 2 July 2021 for soybean grown pots, 'harvest') to measure soil properties. For physical and chemical properties, ~750 g of soil was collected from the center of each pot with a scoopula and stored at -20 ^{o}C for up to four months for the first harvest samples and up to two months for the second harvest samples until analysis. To quantify bulk soil enzyme activities, two 2 ml tubes from the center of each pot were collected, held on dry ice in the greenhouse, and stored for 10-12 months for both harvest samples at -20 ^{o}C until processing.

Soil Physical and Chemical Properties

We analyzed soil samples to assess the soil physical properties: soil texture, bulk density, soil compaction, and water content. Soil texture (% sand, % silt and % clay) was analyzed following the protocol provided for the LaMotte soil texture kit (LaMotte, Chestertown, MD, USA; Julian et al. *in review* Chapter 2). We quantified gravimetric water content (moisture) using the mass ratio protocol where we recorded the initial 'wet' mass (g) of a ~20 g soil sample, dried the sample for 48 hours at 105 ^{o}C, then recorded the final 'dry' mass (Julian et al. *in review* Chapter 2). Moisture (%) was then calculated by subtracting the 'dry' mass from the 'wet' mass, then dividing by the 'wet' mass, and finally multiplying by 100 (**Equation 1**).

$$\% = \left(\frac{wet\ mass\ (g) - \ dry\ mass\ (g)}{wet\ mass\ (g)}\right) * 100$$

Equation 1. Moisture content (%) calculation.

We measured bulk density (BD) following established protocols and using the 1-gallon pots as a soil 'core' (Julian et al. *in prep* Chapter 3). First, the volume of each pot was calculated as the 'field moist volume', then we measured the weight of the pot containing soil as 'field moist mass,' and finally we used the moisture content values as the 'subsample' values to calculate the bulk density of each sample (**Equation 2**).

$$g/ml^{-1} = \left(\frac{field\ moist\ mass\ (g)}{field\ moist\ volume\ (mL)}\right) * \left(\frac{subsample\ 'dry'\ mass\ (g)}{subsample\ 'wet'\ mass\ (g)}\right)$$

Equation 2. Soil bulk density calculation.

Soil compaction was measured following the protocol provided for the Dickey-john penetrometer (Churchill Industries, Minneapolis, MN, USA). We first added 100 ml of water to each pot to ensure the soil was moist before using the ½ inch tip recommended for firm soil types to insert the penetrometer into the pot and then recorded the pressure range (green < 200 psi, yellow = 200 – 300 psi, red >300 psi) at a depth of 7.2 cm (3 inches).

We assessed soil chemical properties including pH, conductivity (EC), cation exchange capacity (CEC), organic matter (OM) and nutrient content [phosphorus (P), potassium (K), calcium (Ca^+), magnesium (Mg^+)] to quantify nutrient differences among the treatments. We measured soil pH by following the 2:1 water to soil preparation method where 10 g of soil was mixed with 20 ml of water and shook by hand for 30 seconds (Woods et al. 2019; Julian et al. *in review*). Following a 30-minute settling period, we recorded pH using a Fisherbrand^{TM} accumet^{TM} AB15 Basic and BioBasic^{TM} pH / mV / ^{o}C meter (ThermoFisher, Waltham, MA, USA). EC analysis was assessed using the same samples prepared for soil pH and followed the provided protocol for the Traceable Conductivity/Total Dissolved Solids Meter (Cole-Palmer, Vernon Hills, IL, USA). Soil samples for each growing season were also sent to A & L Great Lakes Laboratory (Fort Wayne, IN, USA) for CEC, P (Mehlich), K, Mg^+, Ca^+, and OM analysis following EPA guided laboratory protocols.

Extracellular Enzyme Activities

We assayed bulk soil enzyme activity for four specific fluorometric enzymes [alkaline phosphatase (AKP), arylsulfatase (ARS), β-glucosidase (BG) and leucine aminopeptidase (LAP)] and four colorimetric enzymes [invertase (IV), peroxidase (PER), polyphenol oxidase (PPO) and urease (UR)]. To do so, we used a soil homogenate made of a mixture of 0.25 g dry weight of soil sample and 31.5 mL of 50 mM sodium acetate buffer (pH 5.6) for all enzyme assays except AKP which instead used 31.5 mL of NaOH Tris buffer (pH 11.0). Soil homogenates were stored in the dark at 4 ^{o}C for up to one week prior to analysis. Enzyme assays were performed in triplicate using 96-well plates consisting of a buffer blank, substrate blank, soil homogenate blank, soil homogenate and substrate mix. Fluorometric enzymes also included a standard curve for each sample

(Sinsabaugh et al. 2000; Brockett et al. 2012; Hoehn 2016; Woods et al. 2019; Wan et al. 2020; Julian et al. *in prep* Chapter 3).

For the fluorometric enzymes AKP, ARS and BG, we used the fluorescence indicator Methylumbelliferone in the form of 50 μL of substrates 4-Methylumbelliferyl phosphate, 4- Methylumbelliferyl sulfate, and Metheylumbelliferyl beta-D-glycopyranoside. For LAP, we used the fluorescence indicator 7-Amino-4-methylcoumarin in the form of 50 μL of substrate L-Leucine-7-amid-4-methylcoumarin hydrochloride. After samples were plated and prepared, the plates were incubated at room temperature (22 ^{o}C) in the dark for the following times: AKP, four to six hours; ARS, eight hours; BG, 24 to 72 hours; and LAP, 96 hours. After incubation, plates were read at 360 nm excitation and 450 nm emission using a Synergy H1 BioTek microplate reader (BioTek, Agilent, Santa Clara, CA, USA). We calculated all fluorometric enzyme activities in µmol h^{-1} g^{-1} as shown in **Equation 3** using the dilution factor (DF = mL volume of buffer / g of soil sample), extinction coefficient (Ɛ; the slope of the sample's standard curves) and the calculated net fluorescence unit (NFU; **Equation 4**).

$$\text{µmol } h^{-1}g^{-1} = \frac{\left(\frac{\text{NFU}}{\frac{\mathcal{E}}{0.25}\text{mL}}\right)}{\left(incubation\ time * \left(\frac{1}{DF}\right) * 0.200\ \ mL\right)}$$

Equation 3. Fluorometric enzymatic activity calculation for alkaline phosphatase, arylsulfatase, β-glucosidase and leucine aminopeptidase using the Net Fluorescence Unit (NFU) and Dilution Factor (DF).

$$NFU = SH - SHB - B * \left(\frac{slope\ of\ standard\ with\ sample}{slope\ of\ standard\ with\ buffer}\right)$$

Equation 4. Net Fluorescence Unit (NFU) calculation for fluorometric enzymatic activities where 'SH' is the soil homogenate + substrate fluorescence, 'SHB' is the soil homogenate blank fluorescence and 'B' is the substrate blank fluorescence.

The colorimetric enzyme assays for PER and PPO were performed using 50 µl of 25 µM 3,4-Dihydroxyphenylalanine with 10 µl of 3% hydrogen peroxide added for PER only. Once samples were plated, the plates were incubated at room temperature (22 ^{o}C) in the dark between 16 and 24 hours. Following incubation, we read plates at 450 nm emission on a Synergy H1 BioTek microplate reader (BioTek, Agilent, Santa Clara, CA, USA). We calculated the activities for PER and PPO in µmol h^{-1} g^{-1} following **Equation 5** using net absorbance units (NAU = soil homogenate absorbance – soil homogenate blank absorbance – substrate blank absorbance) and the extinction coefficient (Ɛ) 1.8446 for PER and 2.4942 for PPO.

$$\mu\text{mol } h^{-1}g^{-1} = \frac{\text{NAU} * Buffer\ volume\ (ml)}{(\text{Ɛ} * 0.200\ \text{mL} * Incubation\ Time\ (hr) * sample\ (g)}$$

Equation 5. Colorimetric enzyme activity calculation for invertase, peroxidase, polyphenol oxidase and urease using the Net Absorbance Unit calculation (NAU).

We quantified IV activity using 50 µl of the substrate 50 mM sucrose. Samples were plated and incubated at room temperature (22 ^{o}C) in the dark for four hours until being read at 340 nm emission on a Synergy H1 BioTek microplate reader (BioTek, Agilent, Santa Clara, CA, USA). We then calculated IV activity in µmol g^{-1} h^{-1} using the net absorbance units (NAU) and the extinction coefficient (Ɛ) of 6.3 (**Equation 5**).

UR activity was assayed using 10 µl of a 400 mM Urea substrate and incubated in the dark at room temperature (22 ^{o}C) for 24 hours followed by the addition of 40 µl of both salicylate and cyanurate acid that was incubated for an additional one-hour. We read UR enzyme plates at 610 nm using a Synergy H1 BioTek microplate reader (BioTek, Agilent, Santa Clara, CA, USA) and calculated activity in µmol NH4 g^{-1} h^{-1} following

Equation 5 by using the net absorbance units (NAU) and an extinction coefficient (Ɛ) of 0.2403.

Plant Responses

We assessed plant responses throughout the second growing season and at harvest to determine the effects of crop rotation with dredged sediment amended agricultural soil. Emergence was documented during a 28-day period for both corn and soybeans and germination rates were calculated (# emerged / # planted). Growth indicators were measured following the Illinois Agronomy Handbook, Chapter 2: Corn for corn (Nafziger 2017) and the Kansas State University's Soybean Growth and Development Guide for soybean (Ciampitti 2017) by taking weekly measurements of leaf count and plant height. Leaf count was used to indicate growth stages of vegetation (*i.e.*, for V6, soybeans had five fully developed leaf triplicates and the 2-leaf cotyledon and corn had 6 fully developed leaves) and reproduction status (*i.e.*, R1 for flowering, R2 for pod development, R3-R8 for bean development for soybeans; VT for tassel and R1 for silk production for corn).

Throughout the second growing season, we assessed plant survivorship. At the time of plant death, final measurements and plant stage were documented, the plant was harvested and categorized as dead. All remaining corn plants were harvested on 21 June 2021, and soybean plants were harvested on 2 July 2021. At harvest, each plant (corn / soybean) was removed from the pot and separated into above and below ground material. Above ground plant material was placed into a labeled paper bag, and below ground root material rinsed with water until free of any soil and placed into a separate labeled paper bag. Bags containing plant material were dried at 60 ^{o}C for 48 hours and final dry weights (g) were recorded. Flower / pod / bean / ear count was documented for each plant, if applicable; however, there were no viable kernels produced.

Data Analysis

To determine how applied dredged sediments of different conditions (weathered vs. fresh) and crop rotation (corn / soybean) may influence agricultural soil properties and crop responses, we performed both univariate and multivariate statistical analyses using the statistical programming environment R, version 4.3.0 (R Core Team 2023). We normalized data as needed with log transformation to match model assumptions, then used a variety of linear models, generalized linear mixed effects models, and linear mixed effects models for univariate analyses. All linear models were tested with a Type III ANOVA using the *anova()* function in base R, and any significant interactions were evaluated using the *emmeans()* function from the *emmeans* package (Lenth et al. 2023), where all pair-wise comparisons used a Tukey's HSD test. We visualized the data for all linear models using the *ggplot2* package (Wickham et al. 2023).

Separate linear mixed effects models were created for soil properties using either the *lme()* function in the *nlme* package (Pinheiro et al. 2023) or the *lmer()* function in the *lme4* package (Bates et al. 2023), such that each soil property metric [physical (sand, silt, clay, BD, moisture), chemical (pH, EC, OM, P, CEC, Ca^{+}, K, and Mg^{+}), biological (AKP, ARS, BG, IV, LAP, PER, PPO, UR)] was a function of the interaction between soil type (agricultural soil, fresh dredged sediments, weathered dredged sediments), cropping strategy (corn / soybean, corn / corn), and date collected [before planting (20 August 2020), harvest (25 January 2021)] with a random effect in each model for unique plant ID.

We used a principal component analysis (PCA) created with the *prcomp()* function in base R to determine if soil property responses differed between groupings of soil type and cropping strategy, by condensing changes in the soil properties into two linear principal components, excluding OM, Ca^+ and PER due to their high correlation with other properties. We then performed an analysis of similarity (ANOSIM) for all PCAs using the *adonis2()* function in the *vegan* package (Oksanen et al. 2022) and visualized using the *ggbiplot* package (Vu et al. 2011).

To evaluate soil compaction as a function of the different soil types, we performed the chi-squared test using the *chisq.test()* function in base R on the relative frequency of each level of compaction (*i.e.*, ‘low’, ‘moderate’, ‘high’) to determine significance with regards to the interaction of soil type, cropping strategy and time.

Germination rates for plants grown in the different soil types by cropping strategy were calculated by dividing the number of seeds germinated by the total number of seeds planted for each day germination was recorded. We then created a linear mixed effects models using the *lme()* function in the *nlme* package (Pinheiro et al. 2023), where germination rate was a function of the interaction of soil type, cropping strategy and day of data collection, with unique plant ID as the random effect.

To quantify changes in plant development, we created separate linear models for each plant species (corn, soybean) using the *lm()* function in base R, using week of plant data collection as a function of the interaction between soil type and plant stage (corn: VE – R1, soybean: VE-R8). We created separate generalized linear models with a Poisson distribution using the *glm()* function in base R to evaluate leaf count as a function of the interaction between soil type and week of data collection. To assess differences in plant height over time, we created separate linear mixed effects model using the *lme()* function in the *nlme* package (Pinheiro et al. 2023), where height was a function of the interaction of soil type and the week of data collection with the random effect as unique plant ID.

Relative growth rate (RGR) was calculated by week for each plant species by taking the height of the current week, subtracting it from the height in the previous week, and dividing that value by the height in the previous week. We evaluated changes in the weekly RGR using separate linear mixed effects models for each plant species using the *lme()* function in the *nlme* package (Pinheiro et al. 2023), where RGR was a function of the interaction between soil type and week, with a random effect of individual plant ID.

Plant production was captured with above ground, below ground, and total plant biomass measurements for both corn and soybeans. We then created separate linear mixed effects models for each plant species using the *lme()* function in the *nlme* package (Pinheiro et al. 2023), where biomass (above ground, below ground, or total) was a function of soil type, with a random effect of unique plant ID. Furthermore, to quantify changes in plant matter allocation, we calculated the ratio of roots (below ground biomass) to shoots (above ground biomass) for each plant species and then created separate linear models using the *lm()* function in base R, where the root to shoot ratio was a function of soil type. Additionally, we assessed reproduction for soybean as a function of soil type by creating separate linear models using the *lm()* function in base R for number of flowers, number of beans and bean weight (g). There was no reproduction to analyze for corn.

The probability of survival over the growing season was assessed using the *survfit()* function in the *survival* package (Therneau et al. 2023), with the categories alive (status = 1) and dead (status = 2), in response to the different soil type and cropping strategy. We then visualized the data using the *ggsurvplot()* function in the *survminer* package (Kassambara et al. 2021).

Finally, the relationship of plant production and individual soil properties was assessed by creating linear models using the *lm()* function in base R, where total biomass was a function of a single soil property. To determine associations among plant responses (total biomass, leaf count, height) and soil properties for each plant species, we performed separate canonical correlation analysis (CCA) using the *CCA()* function in the *CCA* package (González and Déjean 2021) to condense changes in soil properties and plant responses into two matrices. We then determined significance between the canonical covariates for each plant species based on soil type were assessed using the 'Wilkes' test in the *p.asym()* function in the *CCP* package (Menzel 2022).

RESULTS

Plant responses to dredged sediment conditions combined with crop rotation strategies

Germination rates varied by soil type and crop rotation strategy ($P = 0.0002$, $R^2 = 0.71$, $F_{2, 4474} = 8.665$; **Fig 4.1**). Agricultural soils generally produced higher germination rates for both soybean and corn compared to either the fresh or weathered dredged sediments such that germination rates were consistently lower in the dredged sediments from Day 9 until the pots were thinned on Day 29 (**Fig 4.1**).

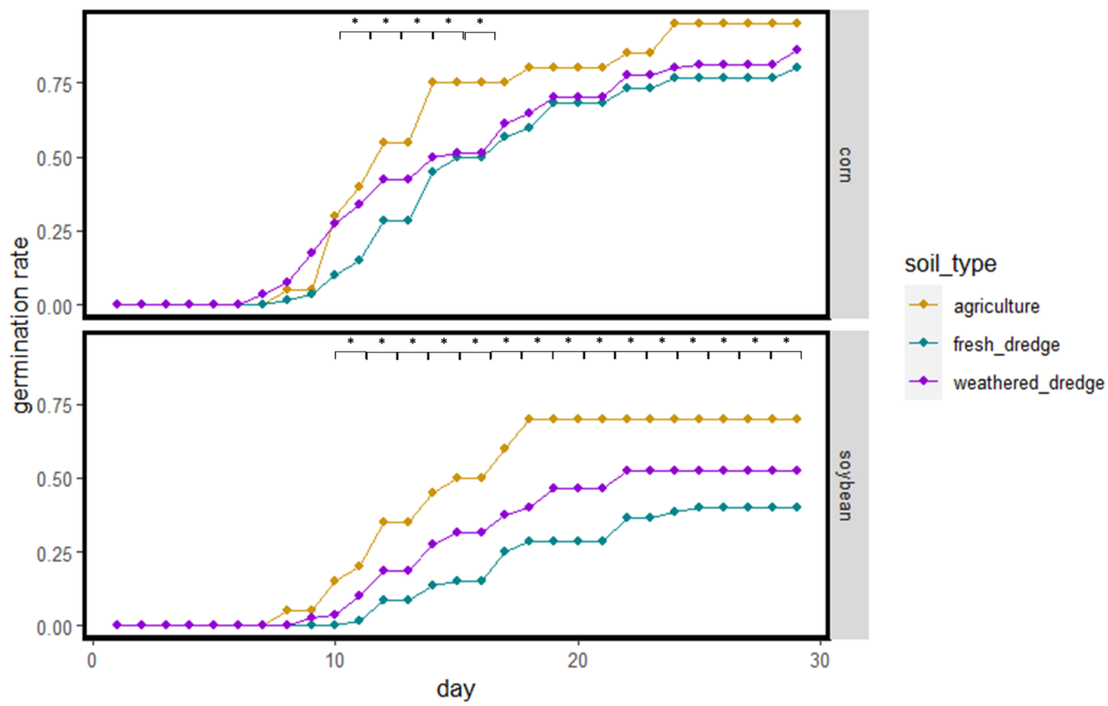

Fig 4.1. Germination rates differed based on soil type for both corn and soybeans ($P = 0.0002$, $R^2 = 0.71$, and $F_{2, 4474} = 8.665$). When grown in agricultural soils, both soybean and corn had higher germination rates compared to fresh or weathered dredged sediments. Additionally, corn had higher overall germination rates than soybeans. Colors represent soil type such that agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple. Each point represents the average germination rate per day for each soil type.

Plant stage development varied by soil type for corn ($P < 0.0001$, $R^2 = 0.84$, $F_{24, 1038} = 2.7114$; **Fig S4.1**). Corn grown in the weathered dredged sediments reached plant stages V7 and V8 faster than its fresh dredged sediment counterpart (**Fig S4.1**).

Additionally, only one corn plant grown in the agricultural soils reached R1 stage (**Fig S4.1**). Despite this variation, soil type did not influence leaf count for corn, which is used to determine plant stage ($P = 0.7464$). Neither plant stage development ($P = 0.2406$) nor leaf count ($P = 0.2482$) significantly varied with soil type for soybean.

There were no differences in corn height over time across all soil types ($P = 0.5322$). However, soybeans grown in agricultural soils were shorter compared to those in fresh and weathered dredged sediments from Week 9 until harvest ($P = 0.0178$, $R^2 = 0.24$, $F_{2,\,907} = 4.981$; **Fig 4.2**). Additionally, when grown in the different soil types, there was no significant difference in RGR for corn ($P = 0.0822$) and soybean ($P = 0.1332$).

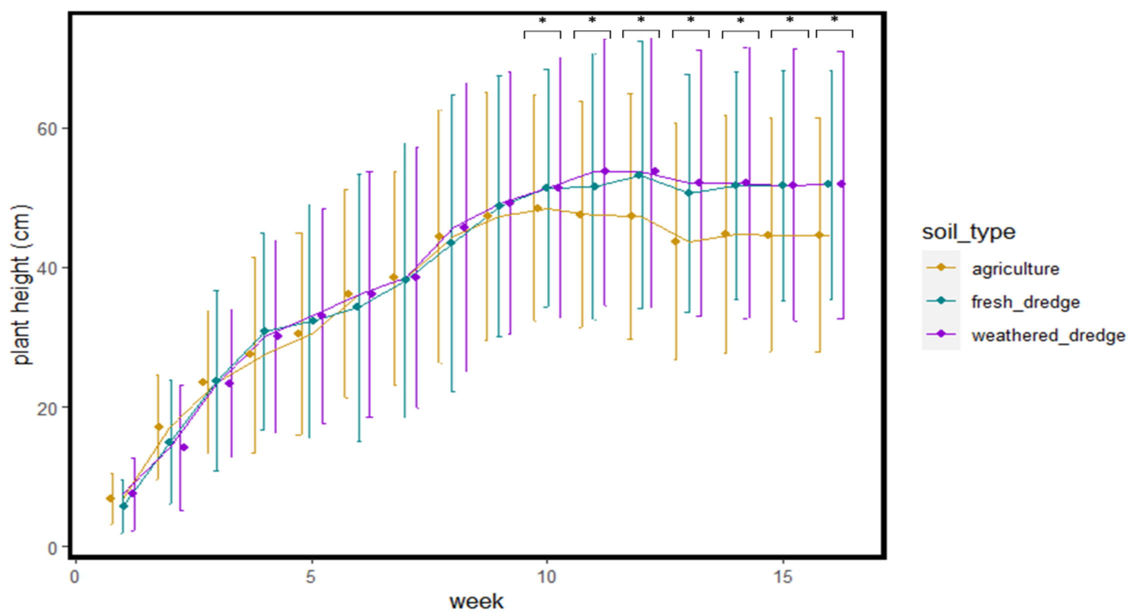

Fig 4.2. Plant height varied for soybean when grown in different soil types ($P = 0.0178$, $R^2 = 0.24$, $F_{2,\,907} = 4.981$). Soybeans grown in agricultural soils were shorter than their counterparts in fresh and weathered dredged sediments. Colors represent soil type: agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple. Each point with corresponding standard error bars represents average weekly plant height. Asterisks (*) indicate significant differences based on Tukey's HSD post hoc analyses ($P < 0.05$).

Plant biomass, including above ground (corn: $P = 0.3547$, soybean: $P = 0.6034$), below ground (corn: $P = 0.8591$, soybean: $P = 0.1168$) and total (corn: $P = 0.2609$, soybean: P = 0.5668), did not differ between soil types for either crop rotation or continuous corn. However, root to shoot ratio significantly differed based on soil type for the crop rotation strategy but not for continuous corn. Soybeans grown in agricultural soils had a higher root to shoot ratio than the ones grown in either dredged sediment condition ($P = 0.0040$, $R^2 = 0.15$, $F_{2, 67} = 6.003$; **Fig 4.3**), whereas corn did not show any difference when grown in different soil types ($P = 0.9392$). There were no differences in the number of flowers ($P = 0.4988$), bean count ($P = 0.1331$) or bean weight ($P = 0.1006$) for soybeans grown in the different soil types; however, there were no viable corn kernels produced for analysis.

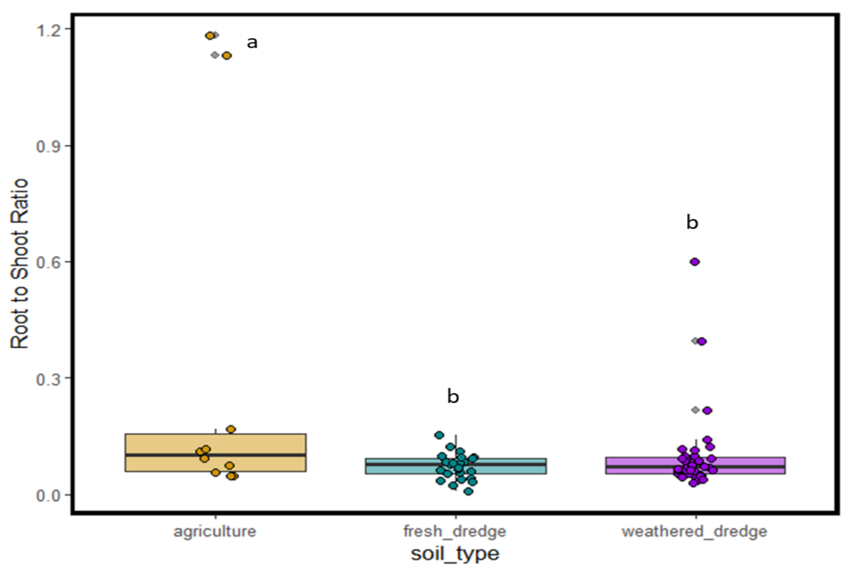

Fig 4.3. The root to shoot ratio at harvest varied for the soybeans grown in different soil types ($P = 0.0054$, $R^2 = 0.18$, $F_{5, 144} = 6.492$). Soybeans grown in agricultural soils had a higher root to shoot ratio than either fresh or weathered dredged sediments. Colors represent soil type: agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple. Boxes are 50% quartiles, with the thick black line indicating the median value and gray points outliers. Colored points represent independent samples (n = 20 for agricultural soil, n = 60 for fresh dredged sediments, and n = 80 for weathered dredged sediments). Letters indicate significant differences based on Tukey's HSD post hoc analyses ($P < 0.05$).

Plant survival probability varied between soil types for soybean and corn ($X^2_{5, 155} = 27.8$, $P < 0.0001$; **Fig 4.4**). For corn, prior to an infection of spider mites in Week 10, there was one plant death in each of the dredged sediment types but none in the agricultural soils (**Fig 4.4)**. However, by Week 16, half of all corn plants grown in fresh dredged sediments and agricultural soils and two-thirds of the corn plants grown in weathered dredged sediments had died (**Fig 4.4**). By Week 18, all corn plants died (**Fig 4.4**). Soybean survival also varied with soil type, such that prior to natural senescence, approximately half of the plants grown in agricultural soils, approximately one third of the plants grown in weathered dredged sediments and one third of those grown in fresh dredged sediments had died (**Fig 4.4**).

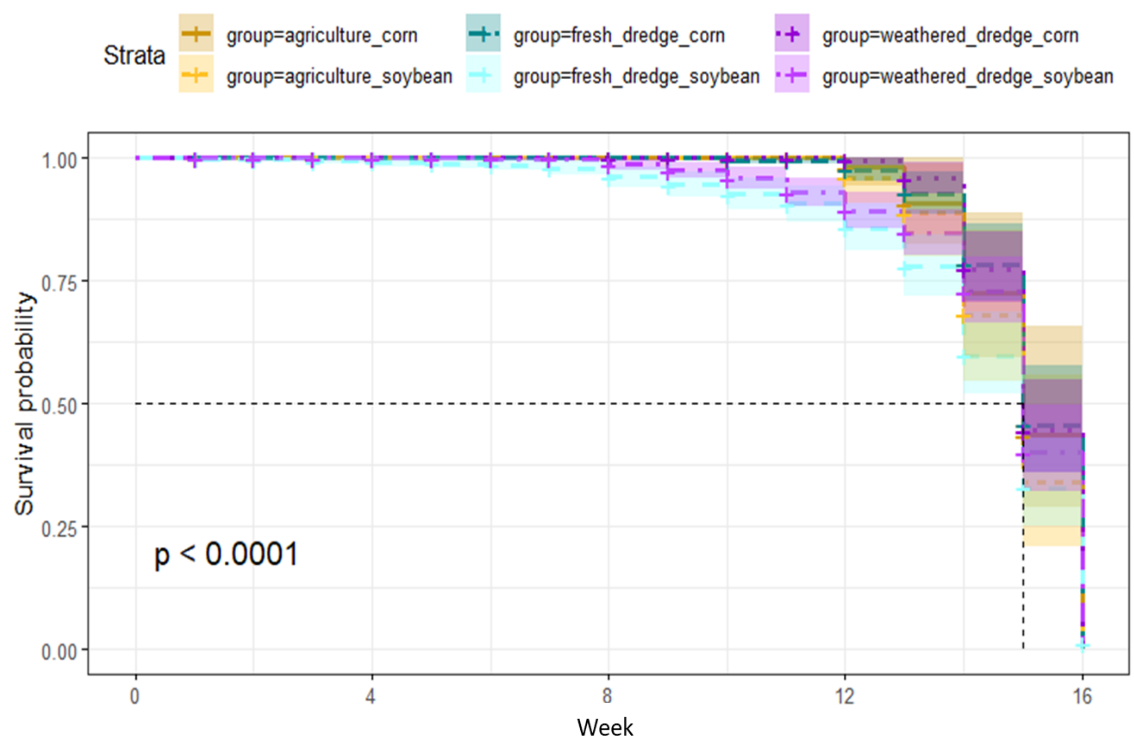

Fig 4.4. Plant survival probability varied when grown in different soil types ($X^2_{5, 155}$ = 27.8, P < 0.0001). Colors represent the different soil type and plant type combinations as follows: agricultural soils and corn = brown, agricultural soil and soybean = tan, fresh dredged sediments and corn = turquoise, fresh dredged sediments and soybean = teal, weathered dredged sediments and corn = purple, weathered dredged sediments = lavender, with shaded areas representing 95% confidence intervals.

Plant and soil property relationships with combination of dredged sediment conditions and crop rotation strategies

Two soil properties influenced plant biomass based on the interaction of soil type and rotation strategy. As P increased ($P = 0.0491$, $R^2 = 0.98$, $F_{2, 143} = 3.078$), biomass of both corn (**Fig 4.5A**) and soybeans (**Fig 4.5B**) grown in the agricultural soils and weathered dredged sediments increased, while biomass for corn and soybeans grown in fresh dredged sediments decreased. Additionally, when BD increased ($P = 0.0477$, $R^2 = 0.98$, $F_{2, 146} = 3.108$), the biomass of corn grown in fresh dredged sediments increased, while the biomass of corn grown in the weathered dredged sediments decreased and agricultural soils remained unchanged (**Fig 4.5C**). However, soybean biomass decreased as BD increased (**Fig 4.5D**) in all soil types.

Rotation strategy altered several soil properties which influenced plant biomass. For example, both corn and soybean biomass increased as the percentage of sand ($P < 0.0001$, $R^2 = 0.98$, $F_{1, 162} = 7.1366$; **Fig 4.5E, F**), moisture content ($P = 0.0073$, $R^2 = 0.98$, $F_{1, 166} = 7.4022$; **Fig 4.5G, H**), CEC ($P < 0.0001$, $R^2 = 0.98$, $F_{3, 149} = 17.96$; **Fig 4.5I, J**) and Ca^+ ($P < 0.0001$, $R^2 = 0.98$, $F_{3, 151} = 20.503$; **Fig 4.5K, L**) increased regardless of soil type. In contrast, corn and soybean biomass decreased as the percentage of clay ($P = 0.0005$, $R^2 = 0.98$, $F_{3, 152} = 12.57$; **Fig 4.5M, N**), Mg^+ ($P = 0.0084$, $R^2 = 0.98$, $F_{3, 151} = 7.1426$; **Fig 4.5O, P**), K ($P = 0.0038$, $R^2 = 0.98$, $F_{3, 151} = 8.624$; **Fig 4.5Q, R**), and PER activity ($P = 0.0001$, $R^2 = 0.97$, $F_{3, 152} = 6.1461$; **Fig 4.5S, T**) increased across rotation strategies. Additionally, as OM content ($P = 0.0008$, $R^2 = 0.98$, $F_{3, 151} = 11.69$; **Fig 4.5U, V**) and LAP activity ($P = 0.0290$, $R^2 = 0.97$, $F_{3, 152} = 4.862$; **Fig 4.5W, X**) increased across rotation strategies, corn biomass increased while soybean biomass decreased.

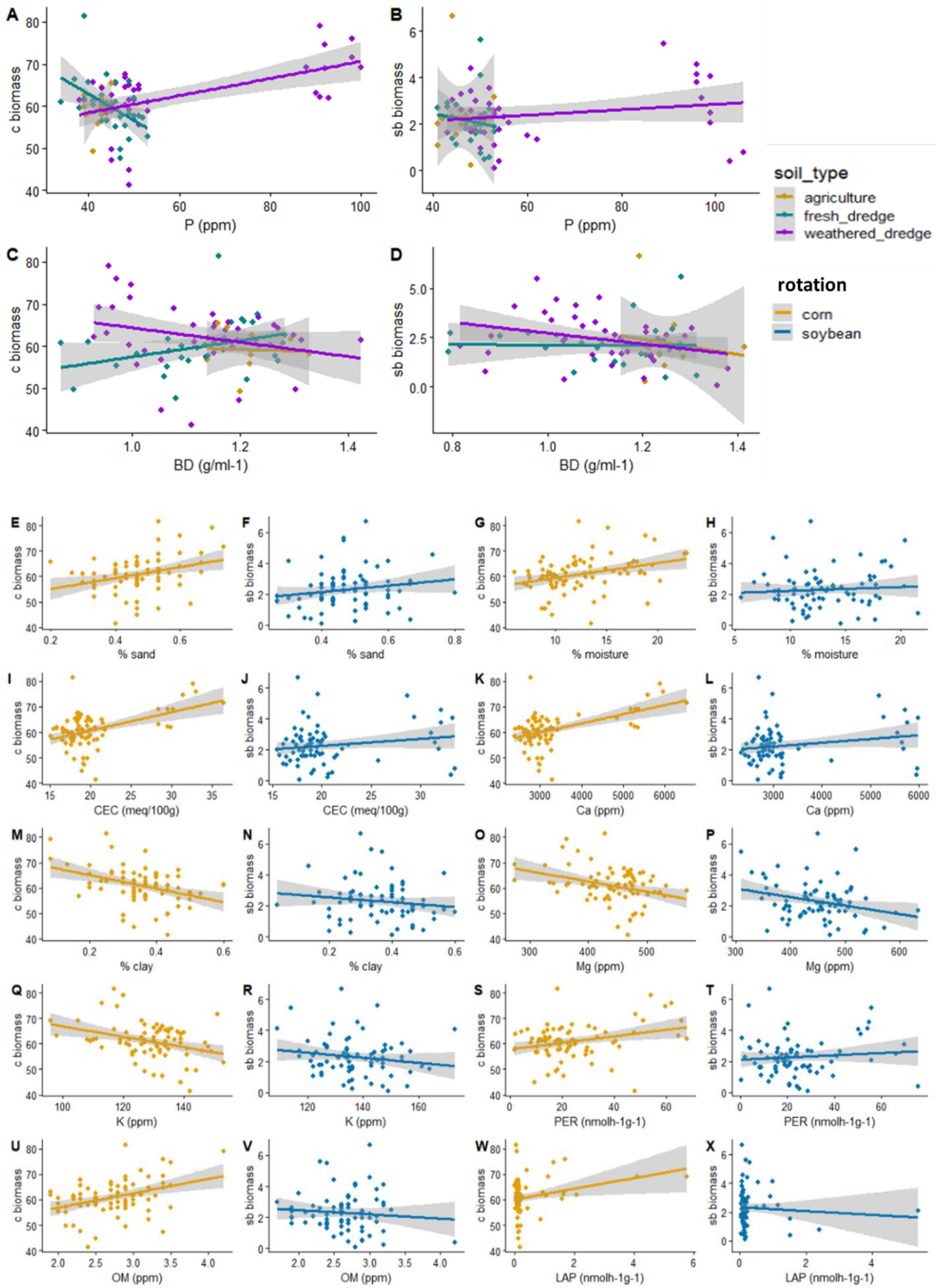

Fig 4.5. Total plant biomass for both corn and soybean changed with several soil parameters. As P increased in the different soil types, plant biomass for both corn (**A**) and soybeans (**B**) grown in agricultural soils and weathered dredged sediments increased, while the biomass for both plant types decreased in fresh dredged sediments decreased (P

= 0.0491, R^2 = 0.98, $F_{2, 143}$ = 3.078). As BD increased, corn biomass (**C**) increased in the fresh dredged sediments, decreased in the weathered dredged sediments, and remained unchanged in the agricultural soils; however, soybean biomass (**D**) decreased with increasing BD within all soil types (P = 0.0477, R^2 = 0.98, $F_{2, 146}$ = 3.108). Total plant biomass of both corn and soybean increased as the percentage of sand (P < 0.0001, R^2 = 0.98, $F_{1, 162}$ = 7.1366; **E, F**), moisture content (P = 0.0073, R^2 = 0.98, $F_{1, 166}$ = 7.4022; **G, H**), CEC (P < 0.0001, R^2 = 0.98, $F_{3, 149}$ = 17.96; **I, J**) and Ca^+ (P < 0.0001, R^2 = 0.98, $F_{3, 151}$ = 20.503; **K, L**) increased across rotation strategies. The biomass for both corn and soybean decreased as the percentage of clay (P = 0.0005, R^2 = 0.98, $F_{3, 152}$ = 12.57; **M, N**), Mg^+ (P = 0.0084, R^2 = 0.98, $F_{3, 151}$ = 7.1426; **O, P**), K (P = 0.0038, R^2 = 0.98, $F_{3, 151}$ = 8.624; **Q, R**), and PER activity (P = 0.0001, R^2 = 0.97, $F_{3, 152}$ = 6.1461; **S, T**) increased for both rotation strategies. As OM content (P = 0.0008, R^2 = 0.98, $F_{3, 151}$ = 11.69; **U, V**) and LAP activity (P = 0.0290, R^2 = 0.97, $F_{3, 152}$ = 4.862; **W, X**) increased across rotation strategies, biomass for corn increased while soybean decreased. Colors for figures (**A-D)** represent soil type: agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple and each point represents one plant (n = 20 for agricultural soils, n = 60 for fresh dredged sediments, and n = 80 for weathered dredged sediments). Colors (**E-X**) represent rotation strategy such that continuous corn is gold and corn / soybean rotation is blue. Each point represents one plant (n = 80 for continuous corn, n = 80 for rotation).

Plant responses of total biomass, height and leaf count and all soil properties, excluding Ca^+ and % clay for high correlation, were placed into two matrices to assess their associations through the dimensionality of a canonical correlation analysis (**Fig 4.6**). For crop rotation, zero of the three canonical dimensions were statistically significant from each other (**Table S4.1A**; P > 0.05). However, for continuous corn, one of the three canonical dimensions was significant (**Table S4.1B**; P = 0.003, F = 1.75) and was influenced by the soil property BD (-0.18) and plant responses of leaf count (0.09), biomass (0.08) and height (-0.08; **Table S4.1C**).

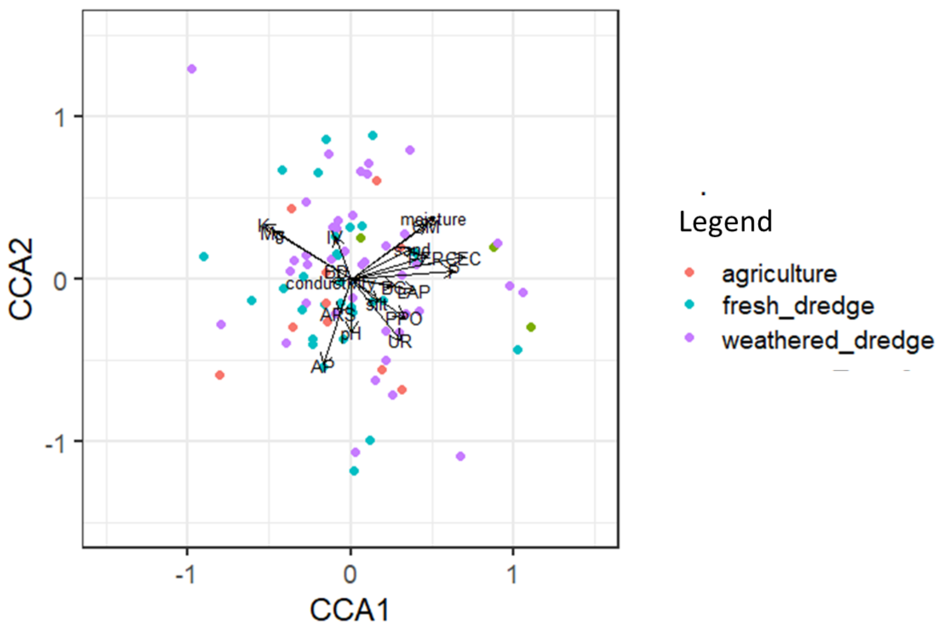

Fig 4.6. Soil properties and plant responses for corn only were separated into two matrices to ascertain their associations influenced by soil type through a canonical correlation analysis (CCA). Colors represent agricultural soil in pink, fresh dredged sediments in blue, and weathered dredged sediments in purple.

Soil property responses to dredged sediment conditions combined with crop rotation strategies

BD significantly increased over time differently across the three soil types regardless of rotation strategy (P = 0.0252, R^2 = 0.12, $F_{2,\ 154}$ = 3.771; **Fig 4.7**). Before planting, BD was similar across all soil types, but at the time of harvest, BD measured ~7% higher in weathered dredged sediment amendments, 4% higher in fresh dredged sediment amendments, and approximately 2% higher in unamended agricultural soil (**Fig 4.7**). Additionally, BD within all three soil types remained within the USDA recommended range (1.0 -1.4 g/ml^{-1}; Stott 2019). However, BD was not influenced by the interaction between soil type, rotation strategy and time (P = 0.8410) or rotation strategy alone (P = 0.8977). Soil compaction, which is directly linked to BD, was not influenced by the interaction of soil type, rotation strategy or time (**Fig S4.3**; P > 0.05), such that all soil types and rotation strategies maintained either ‘low’ or ‘moderate’ compaction from before planting to harvest.

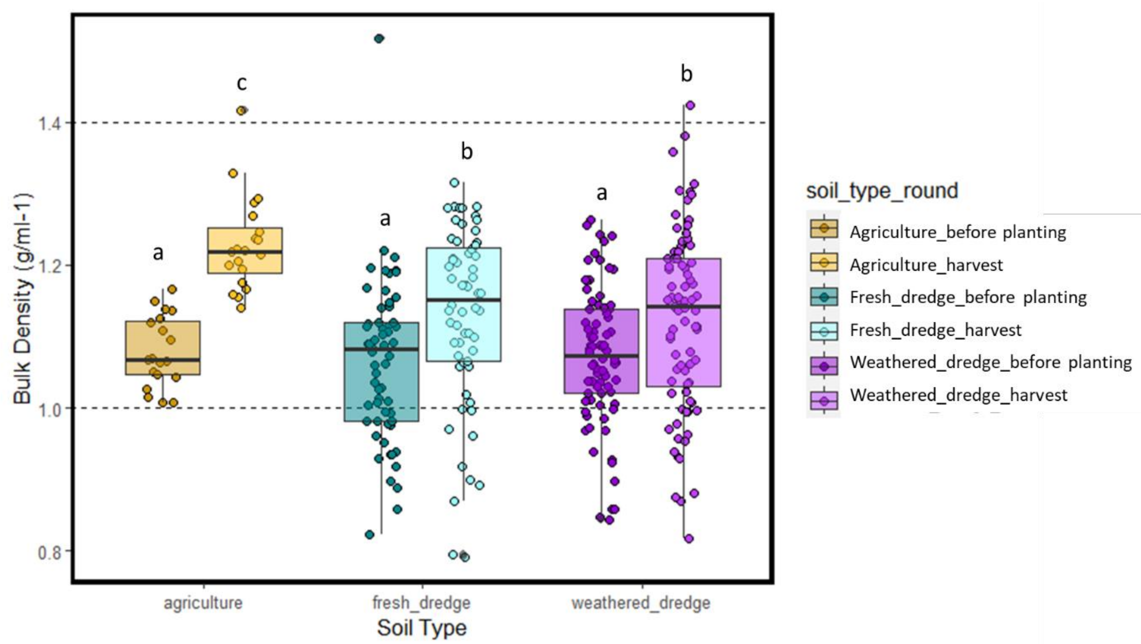

Fig 4.7. Bulk Density (BD) was uniform across all soil types before planting but increased following the second growing season ($P = 0.0252$, $R^2 = 0.12$, $F_{2, 154} = 3.771$). At harvest, BD was higher across all soil types, wherein BD in the unamended agricultural soils was highest overall. Colors represent soil type and shading represents time (darker colors are prior to planting while lighter colors are following harvest): agricultural soils are brown, fresh dredged sediments are turquoise, and weathered dredged sediments are purple. Boxes are 50% quartiles, with the thick black line indicating the median value. Each point represents one soil sample (n = 20 for agricultural soil, n = 60 for fresh dredged sediments, and n = 80 for weathered dredged sediments). Letters indicate significant differences based on Tukey’s HSD post hoc analyses ($P < 0.05$).

Moisture content was ~29% higher in the fresh dredged sediment amendments than weathered dredged sediment amendments, but neither were different from the unamended agricultural soils ($P = 0.0223$, $R^2 = 0.83$, $F_{2, 150} = 3.901$; **Fig 4.8A**). Additionally, moisture content was higher at harvest than before planting ($P < 0.0001$, $F_{1, 154} = 986.9$; **Fig S4.2A**) but was not influenced by the interaction of soil type, rotation strategy or time ($P = 0.5034$) or by rotation strategy alone ($P = 0.6266$).

The constituents of soil texture (sand, silt, clay) were influenced by soil type and time independently, but unaffected by rotation strategy. Specifically, the percentage of sand was influenced by soil type ($P = 0.0114$, $F_{2, 154} = 4.608$; **Fig 4.8B**) such that the percentage of sand was lower in the fresh dredged sediment amendments than weathered dredged sediment amendments or unamended agricultural soil. Contrastingly, there was a higher percentage of clay in the fresh dredged sediments than either weathered dredged sediments or the unamended agricultural soils ($P = 0.0317$, $F_{2, 150} = 3.532$; **Fig 4.8C**). Additionally, regardless of soil type, the percentage of sand ($P = 0.0015$, $F_{1, 154} = 10.42$; **Fig S4.2B**) and silt ($P < 0.0001$, $R^2 = 0.25$, $F_{1, 154} = 97.25$; **Fig S4.2C**) decreased at harvest while the percentage of clay increased ($P < 0.0001$, $F_{1, 154} = 76.6333$; **Fig S4.2D**). However, neither the interaction of soil type, time and crop rotation strategy nor rotation strategy alone influenced any constituent of soil texture ($P > 0.05$).

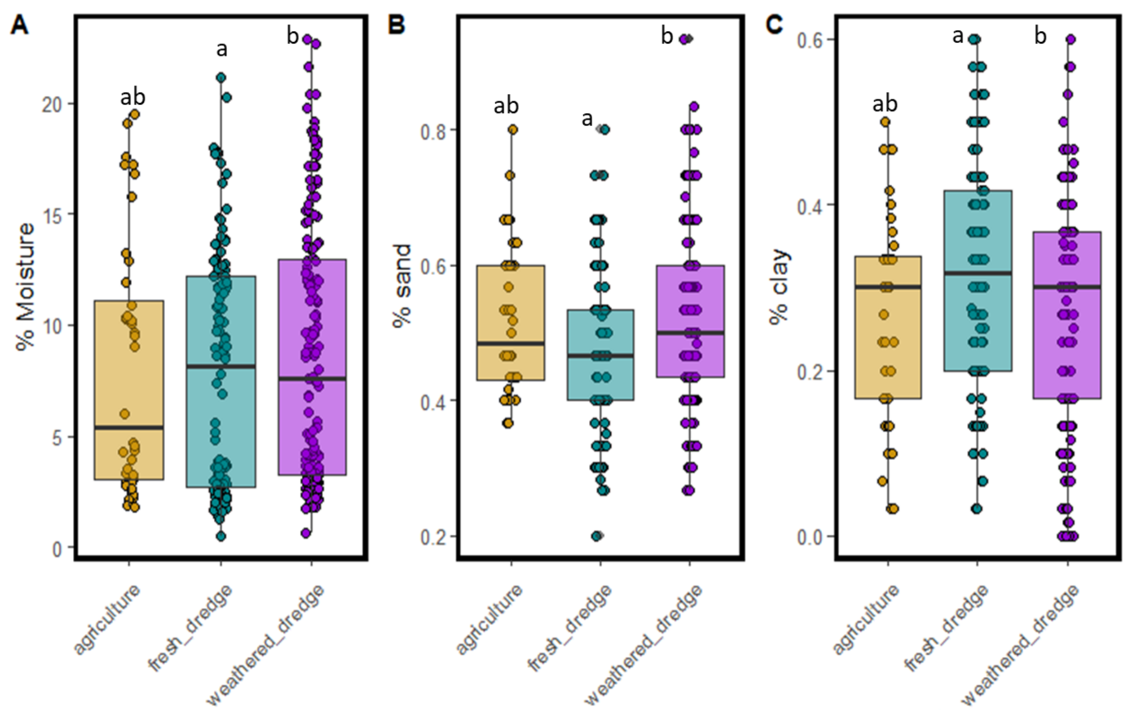

Fig 4.8. (**A**) Moisture content was higher in weathered dredged sediments than fresh dredged sediments, but neither were different from moisture content in the

agricultural soils ($P = 0.0223$, $R^2 = 0.83$, $F_{2, 150} = 3.901$). Soil texture varied among the soil types, such that the fresh dredged sediments had less sand (**B**; $P = 0.0114$, $F_{2, 154} = 4.608$) and more clay (**C**; $P = 0.0317$, $F_{2, 150} = 3.532$) than either the weathered dredged sediments or agricultural soils. Colors represent soil type: agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple. Boxes are 50% quartiles with the thick black line indicating the median value. Each point represents one soil sample (n = 40 for agricultural soil, n = 120 for fresh dredged sediments, and n = 160 for weathered dredged sediments). Letters indicate significant differences based on Tukey's HSD post hoc analyses ($P < 0.05$).

<u>Soil chemical property responses to dredged sediment conditions combined with crop rotation strategies</u>

Both the interactions of soil type and time ($P = 0.0214$, $R^2 = 0.67$, $F_{2, 154} = 3.944$; **Fig 4.9A**) and crop rotation strategy and time ($P = 0.0077$, $R^2 = 0.67$, $F_{2, 154} = 7.289$; **Fig 4.9B**) significantly influenced EC. Before planting, EC was similar across all soil types but at harvest there was a ~1590% increase in EC for both weathered dredged sediments and agricultural soils and an additional ~30% increase in EC in fresh dredged sediments (**Fig 4.9A**). Similarly, before planting, EC was the same between crop rotation strategies, but at harvest, there was an overall ~1750% increase, with rotation having ~25% more EC than continuous corn (**Fig 4.9B**). The interaction between soil type, time and rotation strategy did not affect EC ($P = 0.0845$).

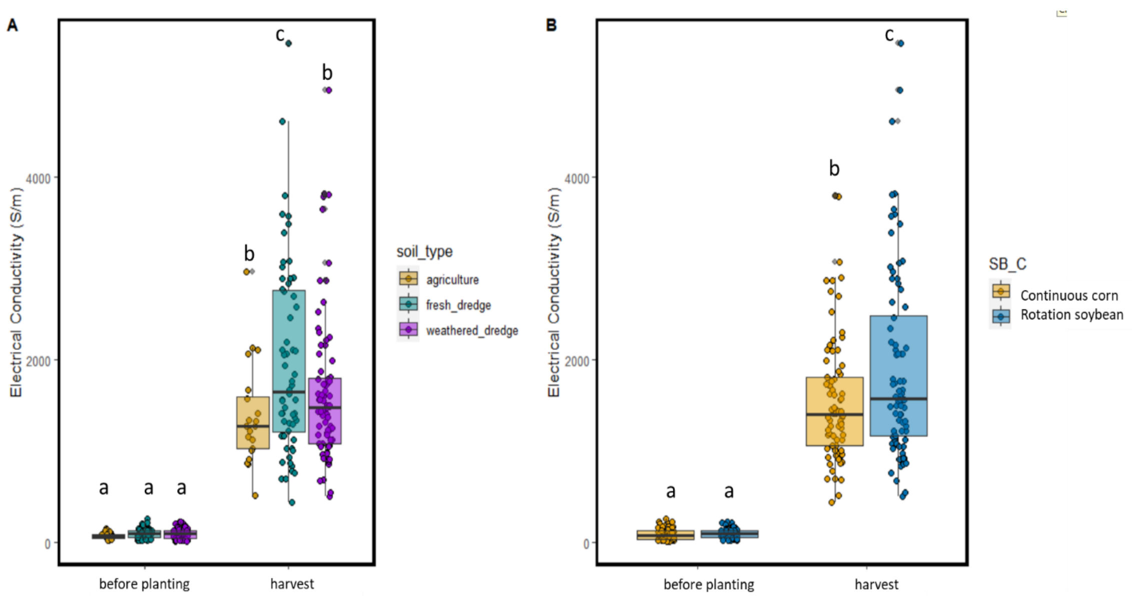

Fig 4.9. Electrical conductivity (EC) increased over time in agricultural soils and weathered dredged sediments as function of soil type (**A;** $P = 0.0214$, $R^2 = 0.67$, $F_{2, 154} = 3.944$) and rotation (**B**; $P = 0.0077$, $R^2 = 0.67$, $F_{2, 154} = 7.289$). Colors represent agricultural soils (brown), fresh dredged sediments (turquoise), weathered dredged sediments (purple), 'continuous' corn (gold) and 'rotation' (blue). Boxes are 50% quartiles, with the thick black line indicating the median value. Each point represents one soil sample (n = 20 for agricultural soil, n = 60 for fresh dredged sediments, and n = 80 for weathered dredged sediments; n = 80 for each crop species). Letters indicate significant differences based on Tukey's HSD post hoc analyses ($P < 0.05$).

Soil type alone altered CEC levels ($P < 0.0001$, $R^2 = 0.18$, $F_{2, 154} = 18.16$; **Fig 4.10A**), Ca^+ content (P < 0.0001, R2 = 0.20, F2, 154 = 20.01; **Fig 4.10B**), and Mg^+ content ($P = 0.0003$, $R^2 = 0.08$, $F_{2, 153} = 8.468$; **Fig 4.10C**). Weathered dredged sediments had higher CEC and Ca^+ content (~21%) than fresh dredged sediments and agricultural soils, with the latter soil types having no difference (**Fig 4.10A,B)**. Mg^+ content was 5% higher in the agricultural soils than in fresh dredged sediments and 9% higher than in the weathered dredged sediments (**Fig 4.10C)**. Despite these differences, there was no influence of crop rotation strategy (CEC: $P = 0.7214$, Ca^+: $P = 0.8619$, Mg^+: $P = 0.5274$) time (CEC: $P = 0.2919$, Ca^+: $P = 0.0676$, Mg^+: $P = 0.3237$) or their interaction with soil type (CEC: $P = 0.9346$, Ca^+: $P = 0.5440$, Mg^+: $P = 0.5226$) for either CEC levels, Ca^+ content or Mg^+ content.

OM content in soil was influenced by both soil type ($P = 0.0035$, $R^2 = 0.11$, $F_{2, 152} = 5.876$; **Fig 4.10D**) and time ($P = 0.0001$, $R^2 = 0.11$, $F_{1, 151} = 15.39$; **Fig S4.4B**). Weathered dredged sediments had ~7% higher OM content than agricultural soil and fresh dredged sediments which did not differ from one another (**Fig 4.10D**). Additionally, there was an overall higher OM content at harvest than before planting (**Fig S4.4B**). However, neither crop rotation strategy ($P = 0.6521$) nor the interaction of soil type, time and rotation strategy ($P = 0.1809$) altered OM content.

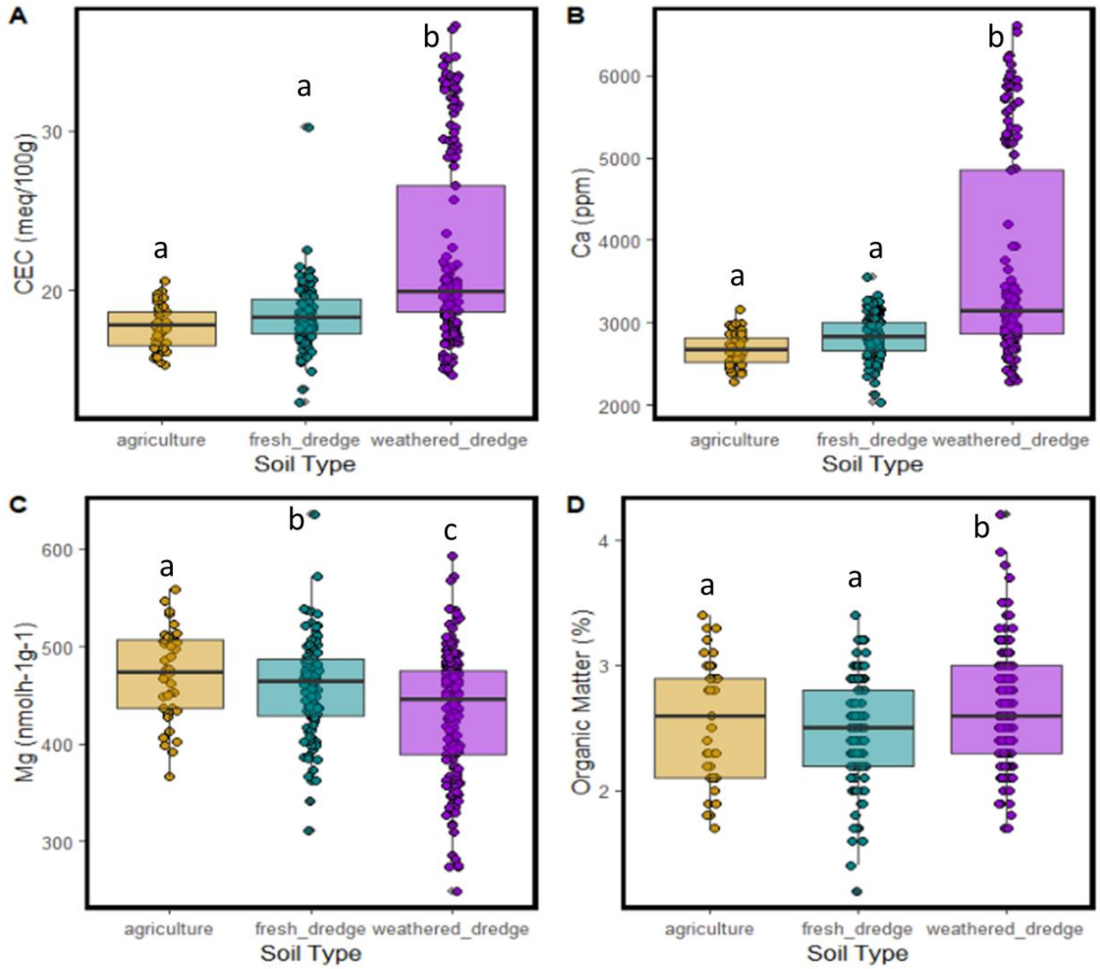

Fig 4.10. (**A**) Cation exchange capacity (CEC; $P < 0.0001$, $R^2 = 0.18$, $F_{2, 154} = 18.1614$), (**B**) calcium (Ca^+) content ($P < 0.0001$, R2 = 0.20, F2, 154 = 20.01) and (**D**) Organic matter (OM) content ($P = 0.0035$, $R^2 = 0.11$, $F_{2, 152} = 5.876$) were all higher in the weathered dredged sediments than either the fresh dredged sediments or agricultural soils, which were not different from each other. (**C**) Magnesium (Mg^+) content was higher in the agricultural soils than in the fresh dredged sediments, which was higher than the Mg^+ content in weathered dredged sediments ($P = 0.0003$, $R^2 = 0.08$, $F_{2, 153} = 8.4684$). Colors represent agricultural soils (brown), fresh dredged sediments (turquoise) and weathered dredged sediments (purple). Boxes are 50% quartiles, with the thick black line indicating the median value. Each point represents one soil sample (n = 40 for agricultural soil, n = 120 for fresh dredged sediments, and n = 160 for weathered dredged sediments). Letters indicate significant differences based on Tukey's HSD post hoc analyses ($P < 0.05$).

Crop rotation strategy alone influenced K content (P = 0.0203, R2 = 0.06, F1, 150 = 5.507; **Fig 4.11**), where K content was ~3% higher with crop rotation compared to continuous corn. There was no difference between soil type ($P = 0.2345$), time ($P = 0.6177$) or their interaction with crop rotation strategy ($P = 0.5525$).

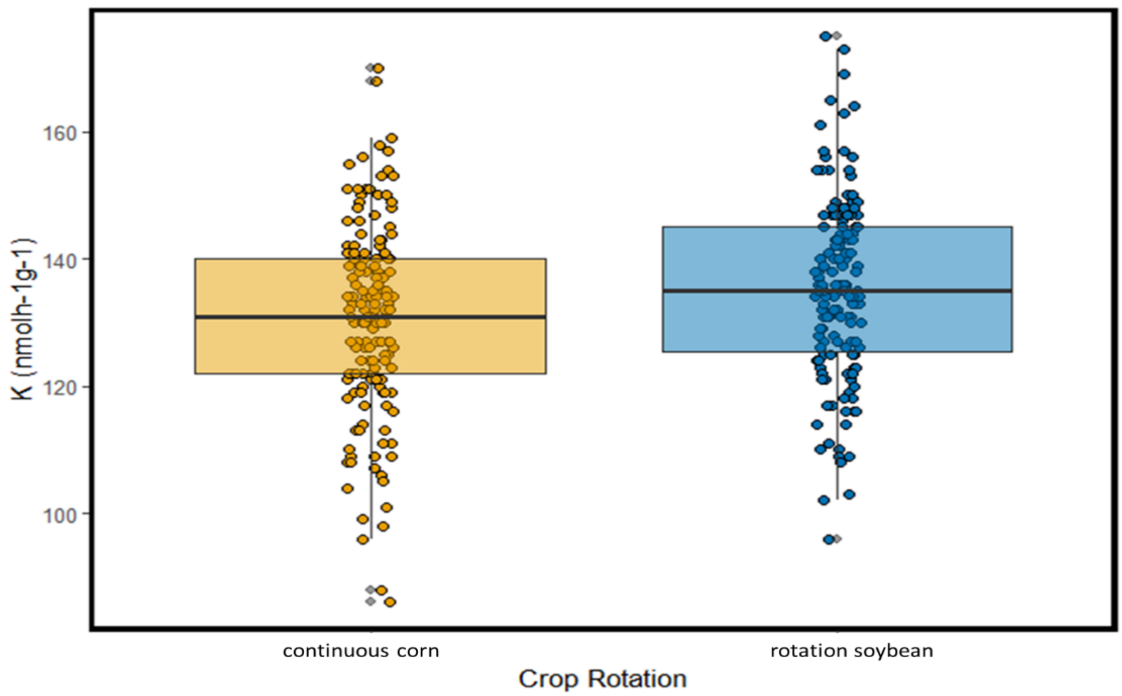

Fig 4.11. Potassium (K) content was higher in pots with soybeans compared to pots with corn ($P = 0.0203$, $R^2 = 0.06$, $F_{1,\ 150} = 5.507$). Colors represent continuous corn (gold) and rotation soybean (blue). Boxes are 50% quartiles, with a thick black line indicating the median value. Each point represents one soil sample (n = 80 per rotation strategy).

The interaction between time and crop rotation strategy altered P content such that P content was ~9% higher with rotation than continuous corn at harvest compared to before planting ($P < 0.0001$, R2 = 0.22, $F_{1,\ 149} = 16.35$; **Fig 4.12A**). Additionally, P content was ~35% higher in the weathered dredged sediments compared to either the fresh dredged sediments or agricultural soil ($P < 0.0001$, $F_{2,\ 154} = 21.22$; **Fig 4.12B**). The interaction between soil type, time, and crop rotation strategy had no significant influence on P content ($P = 0.9166$).

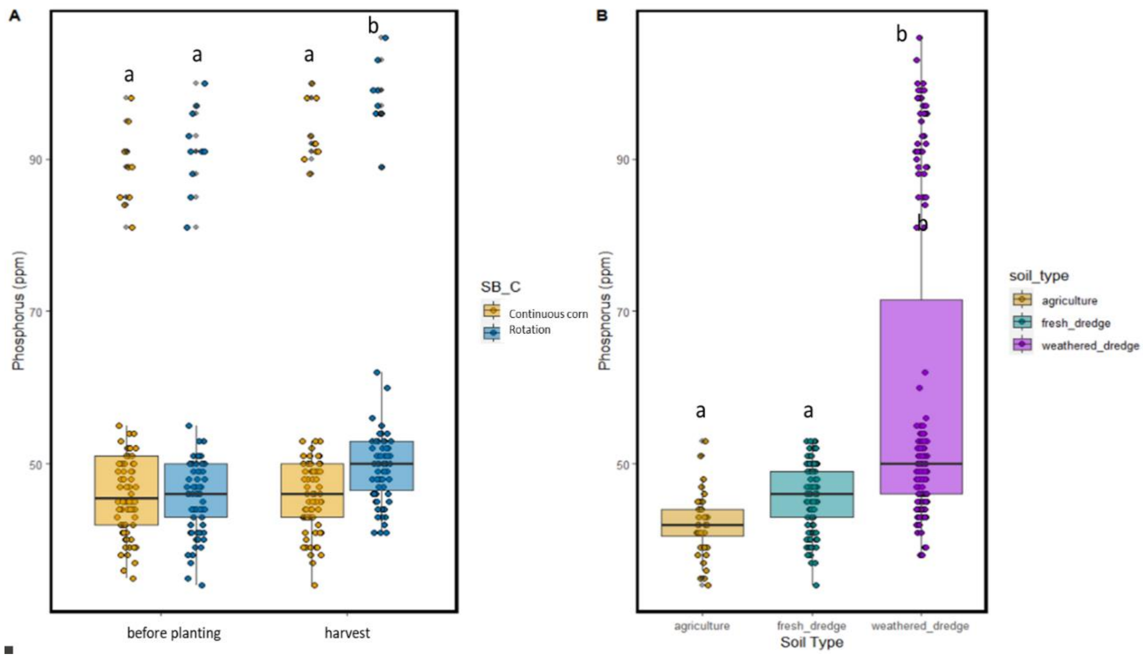

Fig 4.12. Phosphorus (P) content was higher with crop rotation than continuous corn at final harvest compared to before planting (**A**; P < 0.0001, R2 = 0.22, $F_{1, 149}$ = 16.35). Additionally, there was higher P content in the weathered dredged sediments than either the fresh dredged sediments or agricultural soil (**B**; P < 0.0001, $F_{2, 154}$ = 21.22). Colors represent agricultural soils (brown), fresh dredged sediments (turquoise), weathered dredged sediments (purple), 'continuous' corn (gold) and 'rotation' soybean (blue). Boxes are 50% quartiles, with the thick black line indicating the median value. Each point represents one soil sample (n = 160 per rotation strategy; n = 40 for agricultural soil, n = 120 for fresh dredged sediments, and n = 160 for weathered dredged sediments). Letters indicate significant differences based on Tukey's HSD post hoc analyses (P < 0.05).

Soil pH significantly declined with time (P < 0.0001, R^2 = 0.25, $F_{1, 154}$ = 89.38; **Fig S4.4A**) but all pH values remained within the USDA recommended range (6.5-7.2; Stott 2019). Nevertheless, soil pH remained unaffected by soil type (P = 0.8140), crop rotation strategy (P = 0.3995), or the interaction among soil type, time, and rotation strategy (P = 0.5738).

<u>Soil biological property responses to dredged sediment conditions combined with crop rotation strategies</u>

LAP activity changed based on soil type over time (P = 0.0068, R^2 = 0.19, $F_{2, 154}$ = 5.155; **Fig 4.13A**). Before planting, LAP activity in weathered dredged sediments was

~440% higher than that in fresh dredged sediments or agricultural soil. However, LAP activity increased in fresh dredged sediments and agricultural soils and decreased in weathered dredged sediments, so this initial difference was lost. (**Fig 4.13A**). Neither crop rotation (P = 0.7424) nor its interaction with soil type and time (P = 0.4213) influenced LAP activity.

PPO activity was also altered by soil type over time (P = 0.0430, R^2 = 0.06, $F_{2, 154}$ = 3.211; **Fig 4.13B**). Before harvest, there was ~170% higher PPO activity in the weathered dredged sediments compared to both the fresh dredged sediments and agricultural soils, but this difference was lost at harvest through a decrease in activity in weathered dredged sediments and an increase in fresh dredged sediments (**Fig 4.13B**). Neither crop rotation (P = 0.1940) nor the interaction of soil type, time or rotation (P = 0.2391) changed PPO activity.

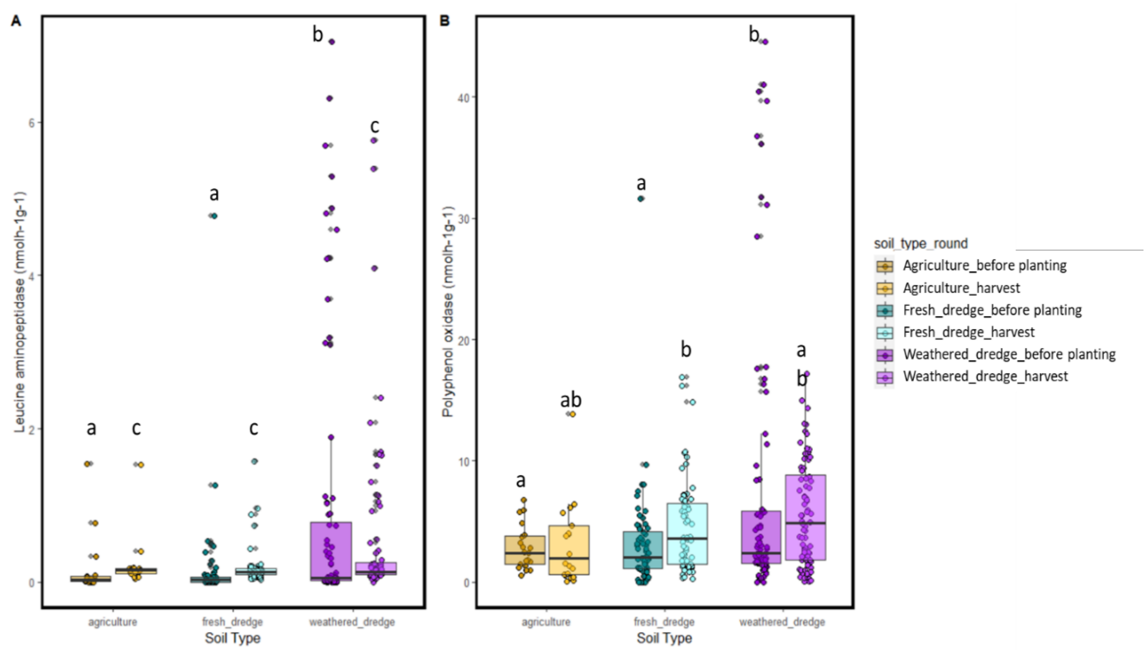

Fig 4.13. (**A**) Leucine aminopeptidase (LAP) activity before planting was higher in the weathered dredged sediments than either the fresh dredged sediments or agricultural soil, but this difference was lost at harvest so that LAP activity was the same across all soil types (P = 0.0068, R^2 = 0.19, $F_{2, 154}$ = 5.155). (**B**) Polyphenol oxidase (PPO) activity before planting was higher in the weathered dredged sediments than either

the fresh dredged sediments or agricultural soil, but with a decrease in the weathered dredged sediments, this difference was lost at harvest, so that PPO activity was the same across all soil types ($P = 0.0430$, $R^2 = 0.06$, $F_{2,\,154} = 3.211$). Colors represent agricultural soils (brown), fresh dredged sediments (turquoise) and weathered dredged sediments (purple) and shading represents time with darker shades before planting and lighter shades at harvest. Boxes are 50% quartiles with the thick black line indicating the median value. Each point represents one soil sample (n = 20 for agricultural soil, n = 60 for fresh dredged sediments, and n = 80 for weathered dredged sediments). Letters indicate significant differences based on Tukey's HSD post hoc analyses ($P < 0.05$).

Soil type ($P = 0.360$, $R^2 = 0.12$, $F_{2,\,154} = 3.398$; **Fig 4.14A**) and time ($P < 0.0001$, $R^2 = 0.12$, $F_{1,\,154} = 29.69$; **Fig S4.5C**) independently changed BG activity. There was ~170% higher BG activity in the weathered dredged sediments compared to either the fresh dredged sediments or agricultural soil (**Fig 4.14A**). BG activity was higher overall at harvest compared to before planting (**Fig S4.5C**). Despite these differences, there was no influence on BG activity from crop rotation strategy ($P = 0.5449$) or the interaction between soil type, time and rotation strategy ($P = 0.4621$).

Soil type alone altered PER activity such that there was ~43% higher PER activity in the weathered dredged sediments than either the fresh dredged sediments or agricultural soil ($P = 0.0162$, $R^2 = 0.6$, $F_{2,\,154} = 4.232$; **Fig 4.14B**). There were no differences in PER activity based on time ($P = 0.3600$), crop rotation strategy ($P = 0.4623$) or their interaction with soil type ($P = 0.6867$).

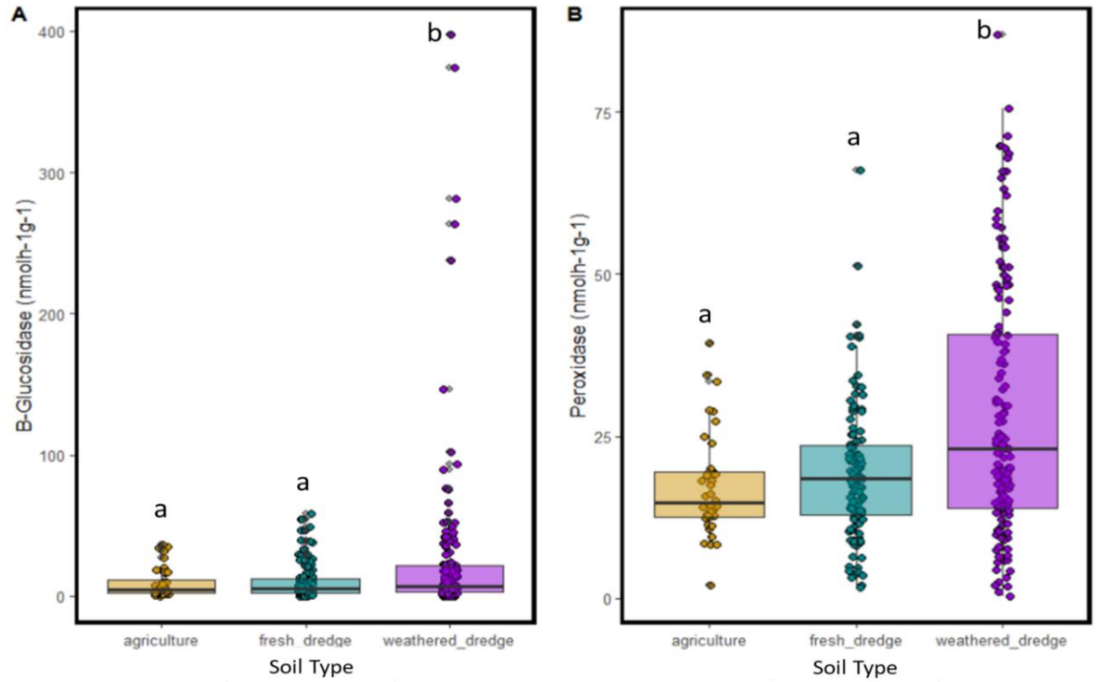

Fig 4.14. (**A**) β-glucosidase (BG; P = 0.360, R^2 = 0.12, $F_{2, 154}$ = 3.398) and (**B**) peroxidase (PER; P = 0.0162, R^2 = 0.6, $F_{2, 154}$ = 4.232) activity were higher in the weathered dredged sediments than either the fresh dredged sediments or agricultural soils. Colors represent agricultural soils (brown), fresh dredged sediments (turquoise) and weathered dredged sediments (purple). Boxes are 50% quartiles, with the thick black line indicating the median value. Each point represents one soil sample (n = 40 for agricultural soil, n = 120 for fresh dredged sediments, and n = 160 for weathered dredged sediments). Letters indicate significant differences based on Tukey's HSD post hoc analyses (P < 0.05).

Time alone altered several enzyme activities with higher overall activity at harvest than before planting for AKP (P < 0.0001, R^2 = 0.33, $F_{1, 154}$ = 108.24; **Fig S4.5A**) and ARS (P < 0.0001, R^2 = 0.59, $F_{1, 154}$ = 350.0; **Fig S4.5B**) activity, while IV activity was higher before planting than at harvest (P < 0.0001, R^2 = 0.16, $F_{1, 154}$ = 46.57; **Fig S4.5D**). However, there were no differences in these enzyme activities by soil type (AKP: P = 0.6263, ARS: P = 0.3357, IV: P = 0.7378), crop rotation strategy (AKP: P = 0.0934, ARS: P = 0.3506, IV: P = 0.4813) or their interaction with time (AKP: P = 0.6730, ARS:

P = 0.7865, IV: P =0.6919). UR activity was not influenced by soil type (P = 0.8105), time (P =0.1793), crop rotation strategy (P = 0.0964) or their interaction (P = 0.2430).

All soil physical, chemical and biological properties, excluding PER, ARS and UR due to high correlation with one another, were condensed into two principal components that explained 14.0% and 27.6% variation within the data. An analysis of similarity on the resulting two linear principal components discerned significant differences in soil properties based on the interaction between soil type and rotation strategy (P = 0.048, R^2 = 0.03, **Fig 4.15**), but separation was minimal.

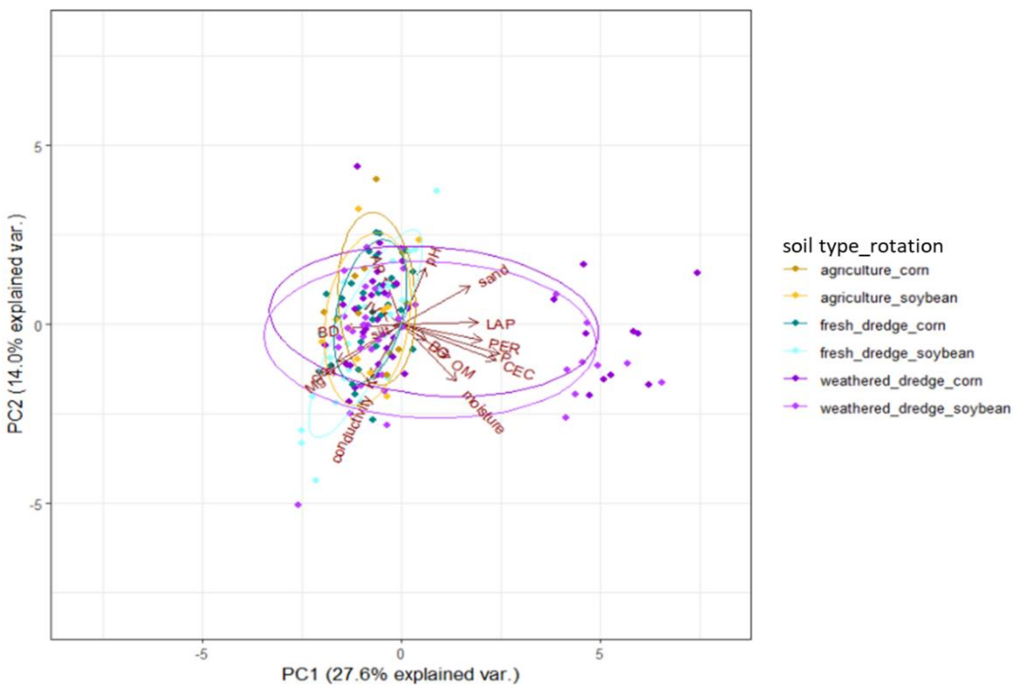

Fig 4.15. The interaction of soil type and rotation strategy was significant when accounting for differences in soil properties (P = 0.048, R^2 = 0.03). Colors represent agricultural soils (brown), fresh dredged sediments (turquoise), and weathered dredged sediments (purple) with continuous corn in darker tones and rotation in lighter tones. Ellipses represent 95% confidence intervals, and points represent individual soil samples (n = 10 for agricultural soil for each rotation strategy, n = 30 for fresh dredged sediments for each rotation strategy and n = 40 for weathered dredged sediments for each rotation strategy)

DISCUSSION

Crop rotation is a field management practice that is widely used in the US for the many benefits provided to agricultural systems, including improved soil stability and soil chemistry as well as increases in crop yield (Wallander 2013; Hellerstein et al. 2019; NRCS USDA 2021). When coupled with the use of organic soil amendments, the benefits of rotating crops are increased (Major et al. 2010; Tian et al. 2015; Yucel et al. 2015; Hoover et al. 2019; Ozlu et al. 2019; Song et al. 2019). When used as a soil amendment, dredged sediments are also known to provide benefits to agricultural soils similar to other organic soil amendments, however, research investigating the combination of dredged sediments and crop rotation is limited despite the widespread nature of both practices (Canet et al. 2003; Darmody and Diaz 2017). In the study described here, we found that crop rotation was unnecessary to acquire the benefits of dredged sediments as an amendment but instead a second growing season, regardless of plant species identity, was necessary to improve the physical, chemical, and biological properties of the soil. Additionally, weathered sediments outperformed their fresh counterpart when used as an amendment.

Plant biomass and soil property relationships influenced by soil type and rotation strategy

Changes in two soil properties significantly altered plant production (biomass) following a second growing season (regardless of crop rotation status) as a function of dredged sediment type. This was particularly true for properties reflective of P cycling. The biomass for both soybeans and corn increased as P content increased in agricultural soils or weathered dredged sediments increased, which is what we expected to see given the importance of P in plant growth and development (Hellerstein et al. 2019; Stott 2019).

However, the biomass for both corn and soybeans grown in fresh dredged sediments decreased with increasing P content. This may suggest that the P content within fresh dredged sediments is not in biologically available forms, but is instead bound in the sediments in forms such as iron phosphate (Durrer et al. 2021). Regardless of the differences found in relationships between plant biomass and P content within the different soil types, we failed to find any relationship between plant biomass and AKP activity, an enzyme responsible for P mineralization. One possible explanations is that plants within this system may be using other mechanisms of obtaining P, such as directly from the soils (Mori et al. 2023).

Similarly, several physical properties changed as a function of soil type and cropping strategy to alter plant biomass. As BD increased, the biomass of corn grown in agricultural soils remained unchanged while the biomass increased for corn grown in fresh dredged sediments and decreased for corn grown in weathered dredged sediments. These results suggest BD is limiting for corn biomass in agricultural soils but is stimulated by weathered dredged sediments and impaired by fresh dredged sediments. However, total biomass for soybeans grown in all three soil types decreased with increasing BD, suggesting that increases in BD did not support plant growth for soybeans regardless of soil type. High BD leads to lower soil porosity resulting in a lower ability to conduct water, air and nutrients through the soil (Stott 2019) making these results consistent with those of soil texture and moisture content. Specifically, plant biomass for both species increased with the increasing percentage of sand and moisture content, as well as a with decreasing percentage of clay regardless of soil type. As a result, the relationships found here between plant biomass and BD for both corn in fresh dredged sediments and soybean in all treatments reflect these trends and align with previous findings in agricultural systems with and without dredged sediments (Munkholm et al. 2001; Darmody and Diaz 2017; Ashworth et al. 2017; Brigham et al. 2021). Consequently, the application of weathered dredged sediments is particularly important in systems like continuous corn to stave off declines in soil porosity following several growing seasons but may be less important in systems using a soybean crop rotation.

Constituents of the C cycle had variable effects in changing plant biomass such that soybean and corn often differed in their responses independent of dredged sediment type. There were opposite relationships with C mineralizing and C associated enzymes and plant biomass, where both corn and soybean biomass decreased with increasing PER activity; however, as both LAP activity, which is associated with both C and N mineralization (Greenfield et al. 2021), and OM content increased, corn biomass also increased but soybean biomass decreased. Taken together, these findings suggest that cropping strategy may dictate both the mineralization of different forms of C needed for crop production as well as the amount of non-mineralized C forms within agricultural systems (Aon and Colaneri 2001; Stott 2019; Mori et al. 2023) but this problem is not mitigated with the use of dredged sediments as a soil amendment. Specifically, the lack of soil type influence indicates that soybeans following corn may not be the most optimal rotation sequence, which may suggest the need for an alternative order of the plant species in the rotation when using dredged sediments as an amendment (Chamberlain et al. 2020).

Several additional chemical properties changed over the course of the growing season in ways which altered plant biomass regardless of dredged sediment type. As both

CEC and the Ca^+ cation content increased, biomass for soybean and corn increased. These results were anticipated, as higher cation exchange capacity in soils is necessary for crop production (Ochoa-Hueso et al. 2023), and findings from previous dredged sediments research have found increases in CEC and Ca^+ content when using dredged sediments (Canet et al. 2003; Tarkalson et al. 2006; Daniels et al. 2007; Darmody and Diaz 2017; Julian et al. *in review* Chapter 2, Chapter 3). In contrast, for the cations Mg^+ and K, as their content increased, biomass for both corn and soybean decreased. While this relationship is not typically seen between Mg^+ and plant biomass, where higher Mg^+ content has been found with increased crop production (Canet et al. 2003; Daniels et al. 2007; Darmody and Diaz 2017), this was the expected relationship between K content and plant biomass (Rhem 1994; Darmody and Diaz 2017). Taken together, this suggests that soybean crop rotation elicits similar relationships between the cations and plant biomass as found with continuous corn, making a second growing season of any crop viable with dredged sediments.

<u>Soil properties changed by soil type and rotation strategy</u>

There were several soil properties that changed due to crop rotation or dredged sediment application that did not significantly influence plant biomass. Crop rotation had minimal influence on the soil physiochemical properties, significantly influencing changes in only EC and K content. Regardless of soil type, K content was higher with crop rotation than with continuous corn, which is opposite of what we would expect to see, given that soybeans typically take up more K than corn, especially during reproduction (Mallarino et al. 2013). Additionally, EC was higher with crop rotation than continuous corn, as well as higher in fresh dredged sediments than either weathered dredged sediments or unamended agricultural soils, but there was no interactive effect of rotation and soil type on EC. These findings do not follow established patterns, as previous crop rotation research found no difference in EC between crop rotation and continuous corn in agricultural soils (Karlen et al. 2013) or between dredged sediment condition compared to agricultural soils following a single growing season (Chapter 3). Here, crop rotation and dredged sediment condition changed EC and K content in ways different than found in traditional agricultural systems, suggesting the combination may provide a beneficial chemical environment for agricultural soils.

Of the remaining physical properties, including moisture, BD, and soil texture, none were influenced by crop rotation, but instead all were altered by soil type alone. Weathered dredged sediments provided a better physical environment for crop growth and production, with increased moisture content, decreased BD, and more balanced soil texture than unamended agricultural soils, aligning with previous research which showed the same patterns (Canet et al. 2003; Sigua et al. 2004; Karlen et al. 2006; Daniels et al. 2007; Gabriel and Quemada 2011; Steele et al. 2012; Darmody and Diaz 2017). Additionally, these improvements to the soil's physical environment were better in weathered dredged sediments than fresh, following the same trends found in previous research on dredged sediments condition following a single growing season (Chapter 3). As previous crop rotation research has found that rotating crops can improve the soil physical environment (Ball et al. 2005; Yuan et al. 2022), the lack of significant influence of rotation strategy found here may indicate that dredged sediments, specifically weathered, are more influential than plant species in creating a stable soil physical environment.

The remaining chemical properties CEC, Ca^+ and Mg^+ content varied in their responses to soil type but were not influenced by crop rotation, and soil pH was only influenced by time. Both CEC levels and Ca^+ content were higher in weathered dredged sediments than either fresh dredged sediments or unamended agricultural soils, which supports findings from previous research that weathered dredged sediments provide higher cation content than unamended agricultural soils (Canet et al. 2003; Daniels et al. 2007; Darmody and Diaz 2017). However, Mg^+ content was higher in unamended agricultural soils than either dredged sediment condition, which contradicts previous dredged sediment research where Mg^+ content increased following the application of dredged sediments (Canet et al. 2003; Daniels et al. 2007; Darmody and Diaz 2017). Despite this contradiction, fresh dredged sediments had higher Mg^+ content than weathered, which does align with previous dredged sediment condition research following a single growing season (Chapter 3), suggesting the weathering process alter Mg^+ content in such a way as to make it inaccessible when applied to agricultural soils as weathered sediments (Vermeulen et al. 2003). Finally, soil pH was higher at harvest than before planting, regardless of soil type or cropping strategy, but still fell within the USDA recommended range (Stott 2019). Combined, these findings suggest that while crop rotation has a limited influence on soil physiochemical properties of the different soil types, dredged sediments, particularly weathered sediments, can maintain a beneficial physiochemical environment needed in agricultural systems for crop growth and production through a second crop growing season (Tarkalson et al. 2006; Russell et al. 2006; Oliveira et al. 2017; Darmody and Diaz 2017; Gaspar 2019; Julian et al. *in review*).

The biochemical properties of the different soil types varied in response to crop rotation compared to continuous corn independent of their effects on plant biomass. Specifically, P content was higher for crop rotation than continuous corn at harvest, which is surprising given that soybean typically demands more P from soils, especially during reproduction, leaving soils depleted in P following soybean growth (Vance et al. 2003). Additionally, P content was higher in weathered dredged sediments than either fresh or unamended agricultural soils, regardless of time, which likely reflects the high P content typically found in dredged sediments (Canet et al. 2003; Darmody and Diaz 2017; Brigham et al. 2021; Kiani et al. 2021). These results together suggest that dredged sediments may provide enough P content to support the plant reproduction needs of soybean during crop rotation. Regardless of soil type or rotation strategy, activity for the P mineralizing enzyme AKP was higher at harvest than before planting, which aligns with patterns in previous agricultural research with corn and soybeans (Hou et al. 2012; Zhu et al. 2018; Hellerstein et al. 2019) suggesting the high P content in dredged sediments require microbially-driven mineralization for it to be available for use by both corn and soybean.

In contrast, the N-mineralizing enzymes were minimally influenced by both crop rotation and soil type. Crop rotation and soil type both failed to significantly alter UR activity and IV activity, although IV activity decreased at harvest. However, LAP activity, which is linked to both N and C mineralization through the breakdown of protein in soils (Greenfield et al. 2021), increased in agricultural soils and fresh dredged sediments but decreased in weathered dredged sediments, eliminating any difference between soil types at harvest, regardless of crop rotation. This may indicate that N mineralization in soil amendments containing high N content is reduced (Clark et al. 2019), and aligns with previous dredged sediment condition research in continuous corn following a single growing season suggesting weathered dredged sediments may provide bioavailable forms of N (Chapter 3). These findings suggest that plants grown with either cropping strategy may not have to rely on microbially-driven N mineralization for N; however, as we did not quantify N content, future research is needed to fully understand the N cycle dynamics within a crop rotation and dredged sediment system.

Finally, independent of the effects on plant biomass, constituents of the C cycle were influenced by soil type over time but were not altered by crop rotation. For the enzyme associate with a more complex C breakdown, PPO activity increased over the growing season in agricultural soils and fresh dredged sediments but decreased over the growing season in weathered dredged sediments, resulting in the loss of differences between soil types at harvest. Furthermore, both PER and BG enzyme activities and OM content were higher in weathered dredged sediments than either fresh or unamended agricultural soils, with both BG activity and OM content higher at harvest than before planting, regardless of soil type or cropping strategy. With the breakdown of OM being an important process in soils (Gougoulias et al. 2014), our findings suggest that crop rotation is not necessary for increasing C cycling independent of their effect on biomass. These results align with previous dredged sediment research showing dredged sediments alone are better for C cycling in agricultural systems than unamended agricultural soils (Averett et al. 1990; Sigua 2005; Vermeulen et al. 2005). Additionally, ARS activity, an S-mineralizing enzyme often linked with OM content (Kumar et al. 2022), was higher at harvest than before planting regardless of soil type or crop rotation. This suggests that the benefits from greater S mineralization to an agricultural system (Schoenau and Malhi 2008; Scherer 2009; Kumar et al. 2022) may not be driven by crop rotation or soil type.

We identified several changes to soil properties, some of which were important for changing total plant biomass, but several relationships we expected to see change (such as pH, % silt and activities for AKP, ARS, IV) did not. These differences in expectation versus reality may reflect experimental condition as this experiment is a greenhouse experiment which means plants were kept in controlled conditions and therefore potentially did not rely on soil properties for success as much as they would have in the field. Consequently, future experiments exploring the effect of dredged sediments and crop rotation on plant growth in the field are needed to confirm the outcomes reported here.

<u>Plant responses altered by soil type and rotation strategy</u>

Several measures of plant production changed due to soil type and/or crop rotation independent of their effects on soil properties. For both corn and soybeans, germination rates were higher in unamended agricultural soils than either dredged sediment condition starting around Day 9 of the experiment. Additionally, plant height for soybeans grown in unamended agricultural soil were shorter than soybeans grown in either dredged sediment condition from Week 9 until harvest, which is consistent with previous research which showed dredged sediments had a positive influence on plant growth of varies crops grown in dredged sediments compared to agricultural soils (Canet et al. 2003; Ebbs et al. 2006). These findings may reflect the higher root to shoot ratio for soybeans in agricultural soils than either dredged sediment condition, indicating that soybeans allocated more biomass to below ground growth than above ground growth early over the course of the experiment. Despite differences in germination rates, plant height and the root to shoot ratio by soil type, we failed to find any biologically meaningful differences in the remaining growth and production metrics (*i.e.*, plant stage, leaf count, RGR, below ground biomass, above ground biomass, total biomass) across soil types for soybean or any growth metric for corn. Such a lack of consistent effects of soil type on plant growth for both plant species may suggest the amendment of agricultural soils with dredged sediments does not necessarily dictate plant growth once germinated unless specific changes in soil properties due to the amendments are considered.

Overall plant survival for both corn and soybean varied by soil type independent of their effects on soil properties. Corn survival was ~100% across soil type until Week 12 of the experiment when there was a spider mite infestation in the greenhouse, resulting in the death of approximately half of all plants grown in fresh dredged sediments and agricultural soils and two-thirds of the plants grown in weathered dredged sediments. By Week 18, all corn plants had died and were harvested. As growing seasons for corn average between 20-23 weeks (IPAD USDA 2023), these plant survival results, coupled with the lack of corn reproduction, do not reflect typical survival of corn growth in dredged sediments (Daniels et al. 2007; Darmody and Diaz 2017; Julian et al. *in review*). However, soybean survival was not impacted by the spider mite infestation and instead varied with soil type. Prior to the senescence following reproduction, over half of the soybeans grown in agricultural soils had died, with even more in both the weathered and fresh dredged sediments. Of the corn and soybeans that lived until harvest, we found both corn and soybeans grown in dredged sediments grew better than those in unamended agricultural soils. These findings align with previous greenhouse research (Canet et al. 2003; Ebbs et al. 2006; Brigham et al. 2021) and field research with a combination of corn / soybean rotation and 100% dredged sediments (Darmody and Diaz 2017) which found that plants grown in dredged sediments, especially in rotation, grew better than those plants grown in agricultural soils. However, plant survival based on soil type did not translate to differences in soybean reproduction for number of flowers, number of beans or bean weight.

Conclusion

Crop rotation resulted in minimal changes in soil property or crop responses compared to the changes found with continuous corn across in both amended and unamended soils. While the results from this study do not directly link the combination of crop rotation and dredged sediments to increases in crop yields, they do identify several soil properties which changed with dredged sediment amendment and consequently led to increases in plant growth over a second growing season, regardless of crop species. Here we investigated the effect of dredged sediments on soil and plant properties following a single corn / soybean rotation; however, this rotation is only one of several rotation strategies commonly used in modern agriculture. For example, rotation strategies that use three or more plant species such as corn / cover crop / soybean are becoming increasingly more common (Magdoff 1993). Consequently, to more fully understand how crop rotation alters the effect of dredged sediments on soil properties and plant growth, future research should investigate additional rotational species. Finally, weathered dredged sediments increased soil property improvements more frequently than changes to soil properties from fresh dredged sediments, suggesting the weathered dredged sediment condition may be more beneficial for agricultural systems employing multiply growing season. Findings from this research support the use of dredged sediments, particularly once weathered, to benefit and improve agricultural soils over repeated growing seasons.

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Chapter 5

Conclusion

Soil degradation is a growing issue in agriculture affecting seventy-five billion tons of productive agricultural soils globally (Wallander 2013; Baumhardt et al. 2015; Richardson and Dooley 2017; Scherr and Yadav 2020). To mitigate soil degradation, farmers often employ several field management practices, including cover crop application, crop rotation, and organic soil amendment applications (Hellerstein et al. 2019). The use of dredged sediments as an organic soil amendment has recently gained traction due to its ability to improve the physical, chemical and biological environment of degraded agricultural soils (Canet et al. 2003; Sigua 2005; Daniels et al. 2007; Darmody and Diaz 2017; Brigham et al. 2021; Rúa et al. 2023). Despite these benefits, research evaluating the use of dredged sediments as a soil amendment in combination with other common field management practices is limited. Additionally, since dredged sediments can be applied to soils in two conditions, fresh from the dredging barge or following a period of terrestrial dewatering (Averett et al. 1990; Darmody and Marlin 2002; Sigua 2005), understanding how dredged sediment condition can alter the benefits of these sediments as a soil amendment when used in conjunction with other field management practices is critical for determining their most effective use in agriculture. In the research described in this dissertation, I investigated how using a cover crop, varying application ratio of dredged sediments, sediment condition and crop rotation alter the effects of dredged sediments on soil properties and crop responses.

In a field experiment investigating changes in soil property and crop responses when a cover crop is applied to dredged sediments, I found that dredged sediments in combination with a cover crop increased corn production due to increased microbial-driven mineralization (Julian et al. *in review* Chapter 2). Specifically, increases in N mineralization drove corn yield increases of ~24% when compared to dredged sediments left fallow. Additionally, I found that the physical and chemical properties of the sediments remained stable with the application of a cover crop and dredged sediments were able to withstand heavy precipitation events without becoming oversaturated, despite their high water holding capacity dredged sediments (Sigua 2005). Furthermore, when corn yield was combined across cover crop treatments and compared to the average of the surrounding agricultural fields, dredged sediments produced 20% more corn yield than non-dredged sediments. These findings align with established agricultural research in which the use of a cover crop, with or without the addition of organic soil amendments, increased soil enzymatic activities and improved soil physical and chemical properties to create a stable soil environment and increase crop yields (Fernandez et al. 2016; Sánchez de Cima et al. 2016; Ashworth et al. 2017; Adeli et al. 2019; Raut et al. 2020), suggesting the combination of dredged sediments and a cover crop can be a beneficial combination of field management practices in agricultural systems.

In a greenhouse experiment investigating changes in soil properties and crop responses to dredged sediment condition, I found that weathered dredged sediments at 100% provided a better soil environment for corn growth when compared to unamended agricultural soils while 100% fresh dredged sediments proved detrimental to corn growth (Chapter 3). These results are consistent with prior dredged sediments research using 100% as a growth medium for plants which found that plants grown in 100% weathered dredged sediments outperformed plants grown in unamended agricultural soils (Daniels et al. 2007; Darmody and Diaz 2017; Roddy et al. *in press*; Julian et al. *in review* Chapter 2). However, in applications following the nutrient recovery ratio and EPA guidelines (Hellerstein et al. 2019; US EPA 2013), dredged sediment condition failed to change soil properties or corn performance in any meaningful way (Chapter 3). The findings do not align with higher application amounts of dredged sediments (50-80%) that found crops grown in dredged sediments had higher yields than their agricultural soil counterparts, which indicates the nutrient recovery ratio and environmental guidelines may not be applicable for dredged sediments (Canet et al. 2003; Ebbs et al. 2006; Darmody and Diaz 2017; Brigham et al. 2021; Kiani et al. 2021).

In a follow-up greenhouse experiment with a second growing season, I compared the soil property and crop responses of a corn / soybean rotation to continuous corn grown in two dredged sediment conditions (Chapter 4). I found that a second growing season can enhance the benefits of dredged sediments, including the stabilization of the physical environment and increases in available nutrients and enzyme activities, and were better in weathered dredged sediments over fresh dredged sediments compared to agricultural soils, regardless of what plant species was grown in the second growing season (Chapter 4). My findings reflect similar findings from other organic soil amendment research which established that consecutive growing seasons, either in rotation or continuous cropping, increase the benefits of soil amendment application (Major et al. 2010; Tian et al. 2015; Yucel et al. 2015; Hoover et al. 2019; Ozlu et al. 2019; Song et al. 2019). These findings suggest that dredged sediment use over multiple growing seasons is a favorable field management practice in agricultural systems, regardless of cropping strategy.

The results from my dissertation work have demonstrated that dredged sediments have potential as a soil amendment when coupled with a cover crop (Chapter 2; Julian et al. *in review*); the benefits these sediments can provide agricultural systems are enhanced when the dredged sediments are allowed to weather as opposed to using them fresh from the aquatic source (Chapter 3). Finally, a second growing season can increase the beneficial changes in soil properties of soils amended with dredged sediments, regardless of plant species grown (Chapter 4). Overall, the findings from this dissertation expand our understanding of the benefits of using dredged sediments as a soil amendment to mitigate soil degradation and increase crop production in agricultural soils.

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APPENDIX A

CHAPTER 2 SUPPORTING INFORMATION

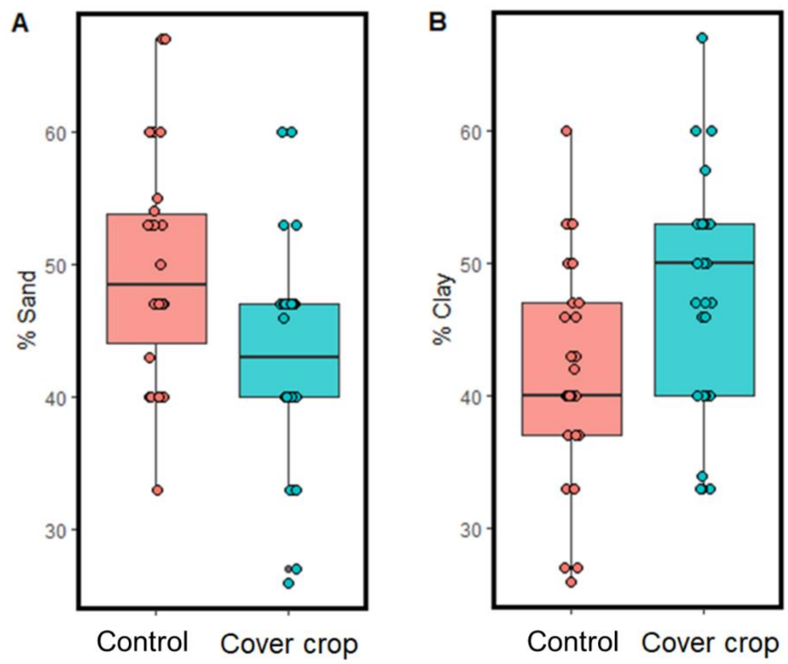

Fig S2.1. The use of a cover crop significantly changed percent sand and percent clay within the experimental plots. The % sand **(A)** was significantly lower in the cover crop plot (blue) compared to the control plot over the winter (pink; $F_{1,18} = 10.35$, $P = 0.0048$, $R^2 = 0.15$) whereas the % clay **(B)** was significantly higher in the cover crop plot compared to the control plot ($F_{1,18} = 2.7380$, $P = 0.0135$, $R^2 = 0.11$). Boxes represent 50% quartiles, with the thick black line indicating the median count and each point representing one plant (n = 30 per treatment).

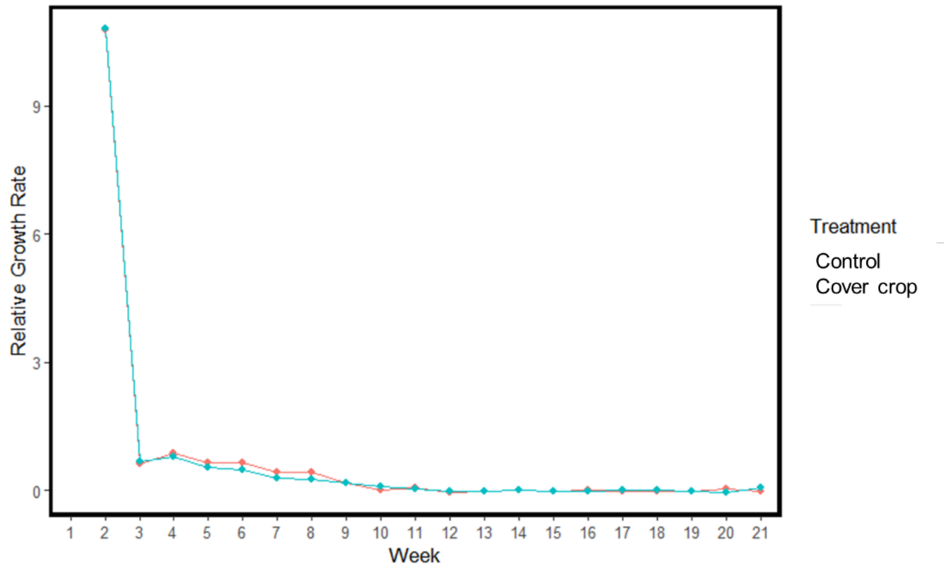

Fig S2.2. Relative growth rate (RGR) in corn did not vary between treatments (P = 1.000). Each point represents the calculated RGR per week.

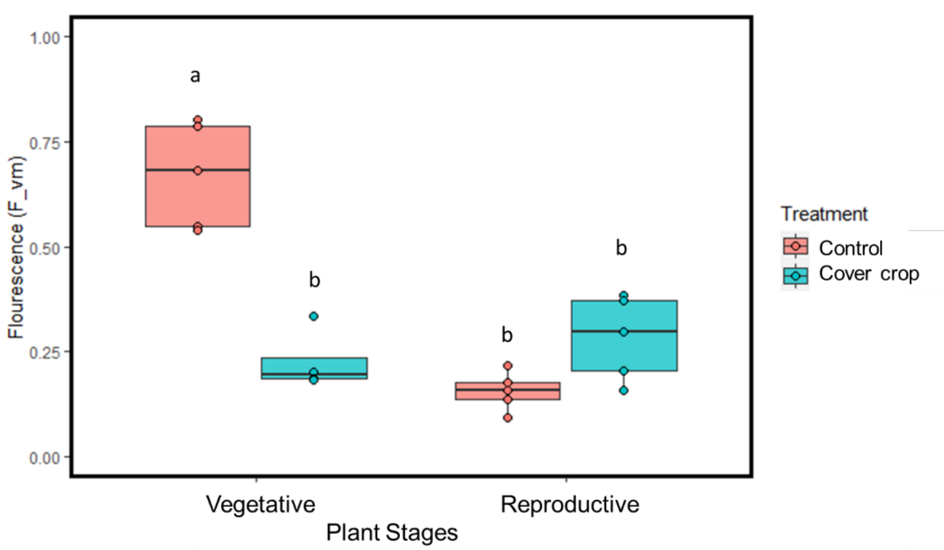

Fig S2.3. Photosynthetic efficiency in corn varied between treatments. Corn grown in the control plot (pink) had higher photosynthetic efficiency in the vegetative stages than corn grown in the cover crop applied plot (blue). However, those differences were lost between treatments during reproduction ($F_{3,35} = 23.34$, $P < 0.0001$, $R^2 = 0.64$). Boxes are 50% quartiles, with the thick black line indicating the median fluorescence (F_{VM}), and each point represents one plant (n = 10 per treatment). Letters indicate significant differences based on Tukey's post hoc analysis ($P < 0.05$).

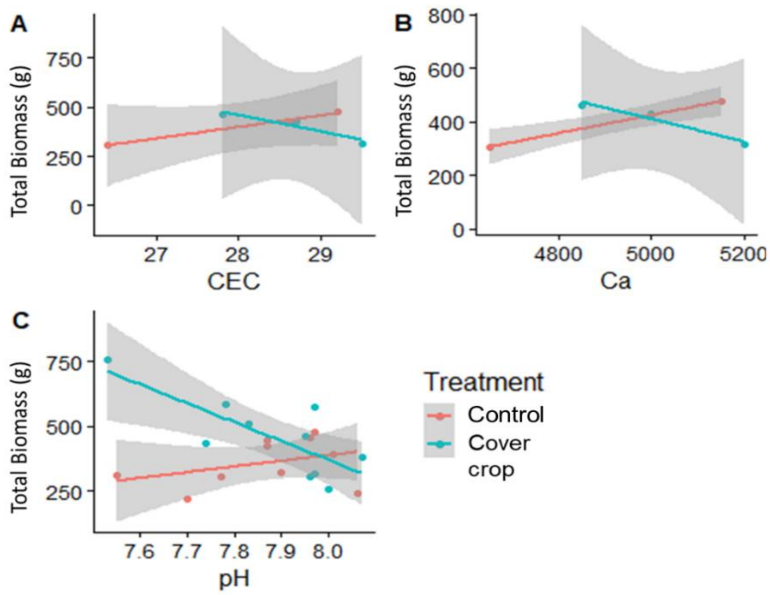

Fig S2.4. A) Cation Exchange Capacity (CEC), **B)** calcium content (Ca^{+}) and **C)** pH significantly influenced plant biomass by treatment (cover crop = blue, control = pink). Shaded regions represent 95% confidence intervals, and each point represents one plant (n = 3 per treatment for CEC and Ca^{+}, n = 10 per treatment for pH).

APPENDIX B

CHAPTER 3 SUPPORTING INFORMATION

Agricultural Soil compared to Application Ratios of Dredged Sediments

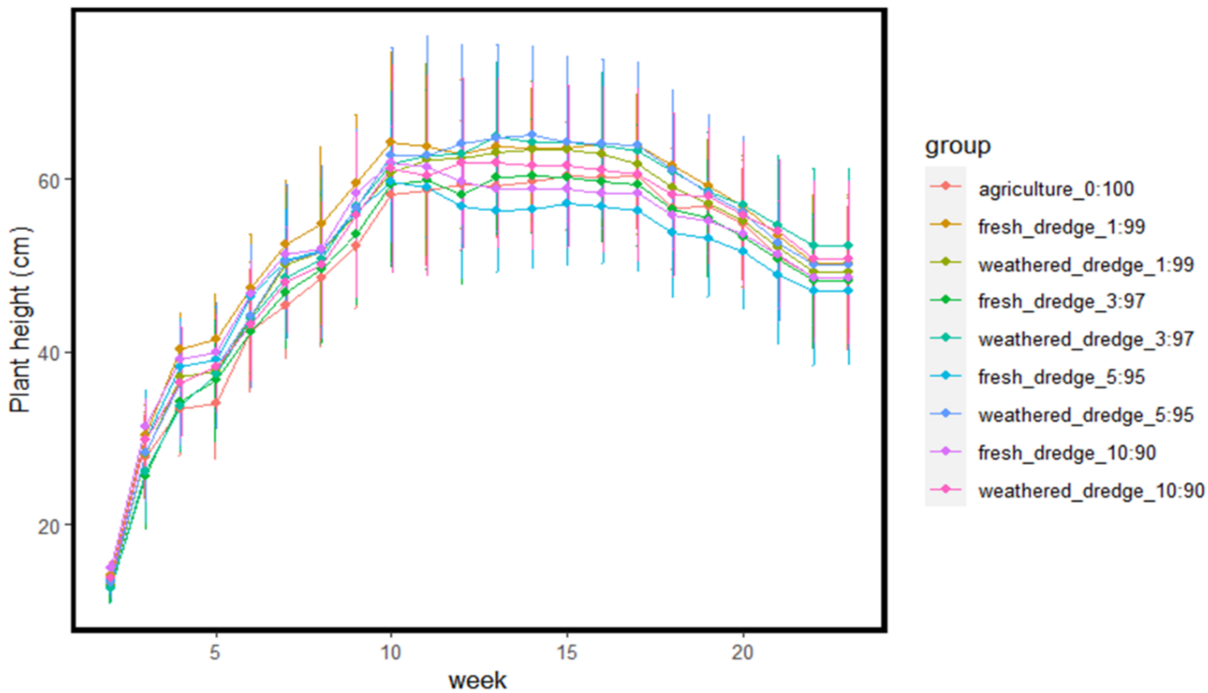

Fig S3.1. Height for corn grown in the soil type and application ratio combinations varied between the 5:95 fresh and 5:95 weathered dredged sediment applications during Weeks 13-15 and 17-18 ($P = 0.0278$, $R^2 = 0.25$, $F_{8, 302} = 2.16$). Each point with associated standard error bars represents the average height per week. Additionally, the colors represent the different soil type and application ratio combinations as follows: 100:0 agricultural soils = light pink, 1:99 fresh dredged sediments = tan, 1:99 weathered dredged sediments = olive, 3:97 fresh dredged sediments = green, 3:97 weathered dredged sediments = teal, 5:95 fresh dredged sediments = light blue, 5:95 weathered dredged sediments = indigo, 10:90 fresh dredged sediments = purple, and 10:90 weathered dredged sediments = hot pink.

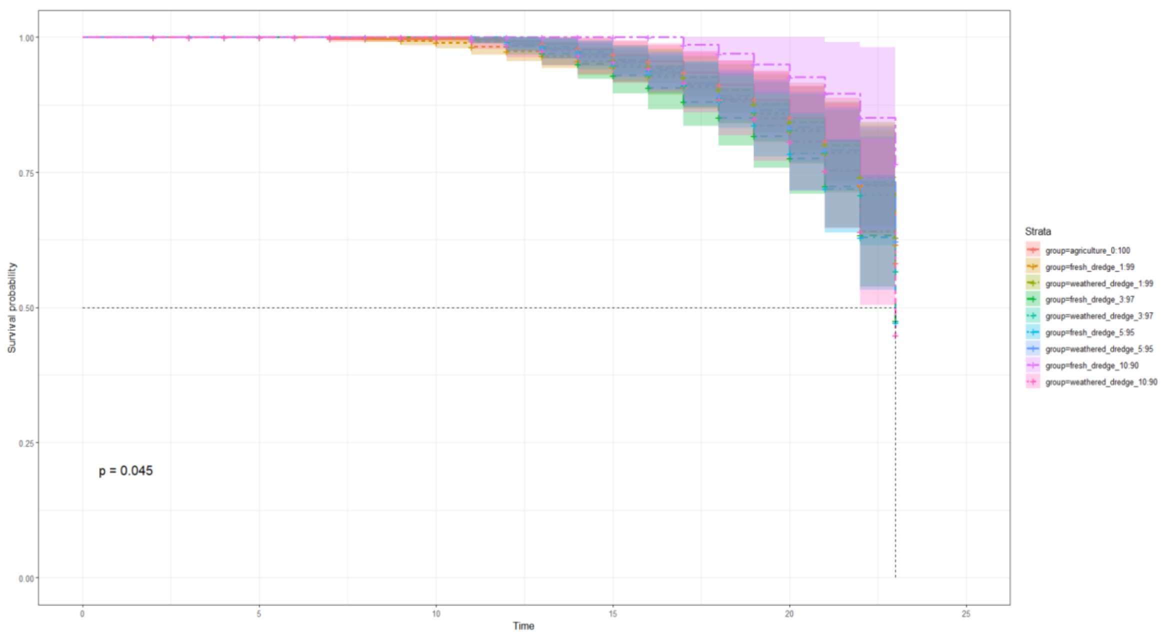

Fig S3.2. Survival rates for corn varied when grown in different soil type and application ratio combinations, with every combination losing between 1-5 plants throughout the growing season ($X^2_{8,\,160} = 15.9$, $P = 0.04$). Colors represent the different soil type and application ratio combinations as follows: 100:0 agricultural soils = light pink, 1:99 fresh dredged sediments = tan, 1:99 weathered dredged sediments = olive, 3:97 fresh dredged sediments = green, 3:97 weathered dredged sediments = teal, 5:95 fresh dredged sediments = light blue, 5:95 weathered dredged sediments = indigo, 10:90 fresh dredged sediments = purple, and 10:90 weathered dredged sediments = hot pink, with shaded areas indicating 95% confidence intervals.

A. Tests for Canonical Dimensions, Application Ratios					
Dimension	Canonical Correlation	Mult. F	df1	df2	P - value
1	0.3577	2.6354	63	403.8148	< 0.0001
2	0.6020	1.9643	40	272.0000	< 0.0001
3	0.8096	1.6958	19	137.0000	< 0.0001

B. Standardized Canonical Coefficients, Application Ratios			
	Dimension 1	Dimension 2	Dimension 3
Soil properties			
Ca^+ content	0.0091	-0.0090	0.0107
pH	-0.0138	0.0094	-0.0024
EC	-0.0118	-0.0195	0.0089
CEC	-0.0271	-0.0169	-0.0287
P content	0.3761	0.0205	-0.3216
OM content	0.2519	0.0141	-0.2512
Mg^+ content	0.4019	-0.0073	-0.3458
K content	0.0438	-0.0059	-0.0063
Moisture content	-0.0096	0.0009	0.0409
BD	0.0772	0.0550	-0.0144
% sand	-0.0415	-0.0141	-0.0173
% silt	-0.0605	-0.0001	-0.0377
% clay	0.0374	-0.0419	0.0660
AKP	-0.0014	-0.0505	-0.0059
ARS	0.0098	0.0335	-0.0017
BG	-0.0015	-0.0004	0.0091
IV	-0.0153	-0.0040	-0.0299
LAP	0.0161	-0.0270	-0.0058
PER	0.0029	-0.0069	-0.0040
PPO	0.0031	-0.0158	0.0155
UR	0.0066	0.0201	0.0369
Plant responses			
Biomass	-0.0318	-0.0167	-0.0816
Leaf count	-0.0613	0.0151	0.0628
Height	0.0114	-0.0774	0.0202

Table S3.1. Associations between soil properties and plant responses for the soil type and application ratio combinations were found through a CCA, where all three canonical dimensions were significantly different from each other (**A**; $P < 0.0001$), with several soil properties and plant responses influencing the three canonical dimensions (**B**).

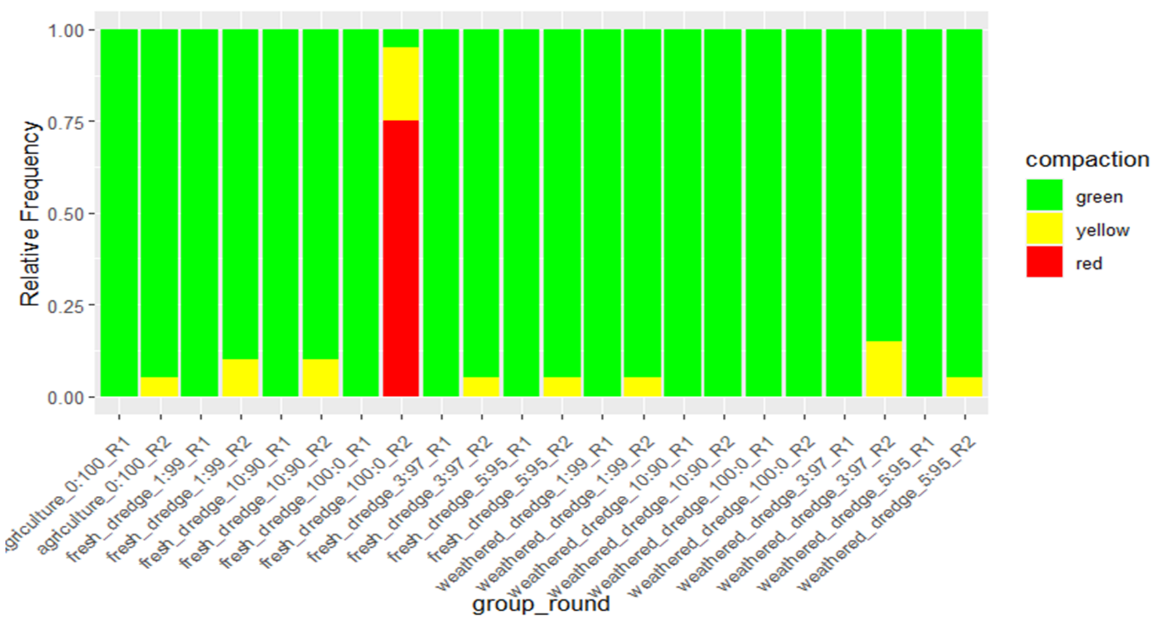

Fig S3.3. Increased soil compaction was measured at harvest for all application rate and soil type combinations except 10:90 and 100:0 weathered dredged sediments, which maintained ‘low’ compaction. Colors represent levels of compaction where ‘low’ = green, ‘moderate’ = yellow, and ‘high’ = red.

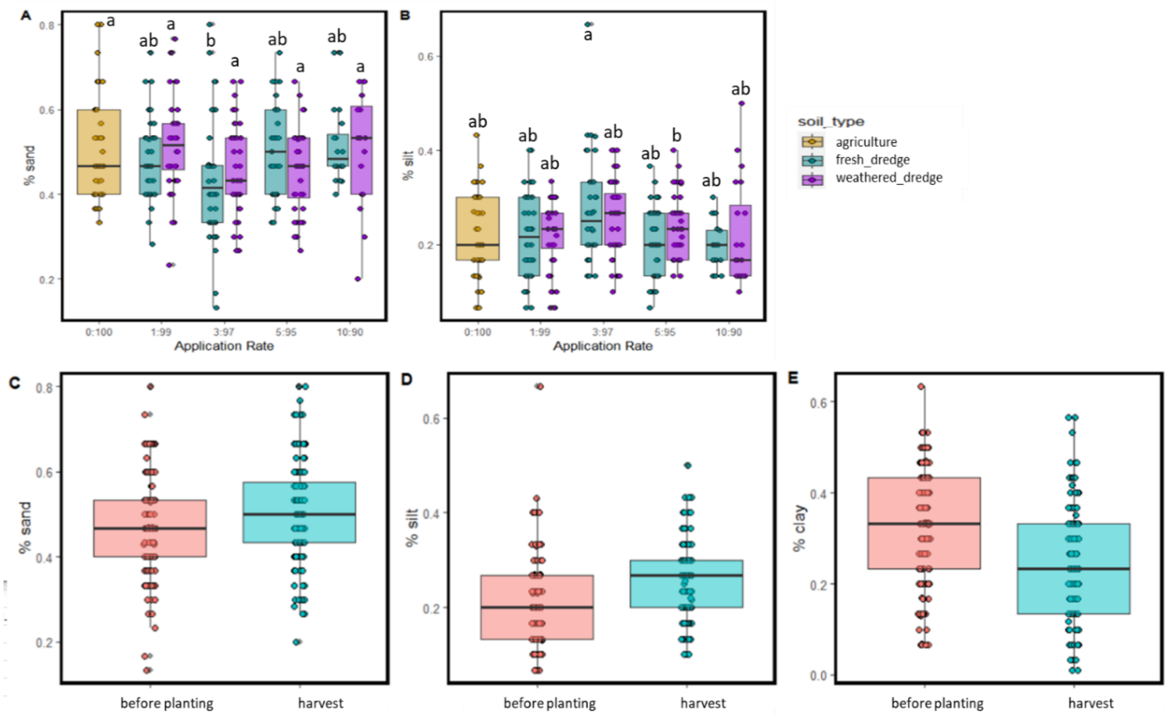

Fig S3.4. Soil texture constituents of sand (**A**; $F_{8,\,289} = 3.206$, $R^2 = 0.13$, $P = 0.0016$) and silt (**B**; $F_{8,\,298} = 3.662$, $R^2 = 0.36$, $P = 0.0004$) varied by soil type and application ratio combination. Additionally, soil texture shifted from clay-loam to a more sandy-clay-loam over the growing season and shifted depending on soil type [sand (**C**; $F_{1,\,289} = 10.65$, $P = 0.0012$, $P = 0.0016$); silt (**D**; $F_{1,\,298} = 43.356$, $R^2 = 0.36$, $P < 0.0001$); clay (**E**; $F_{8,\,298} = 28.86$, $P < 0.0001$)]. Colors represent **(A, B)** each soil type such that agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple and **(C, D, E)** collection period 'before planting' is pink and 'harvest' is blue. Letters indicate significant differences based on Tukey's post hoc analyses ($P < 0.05$).

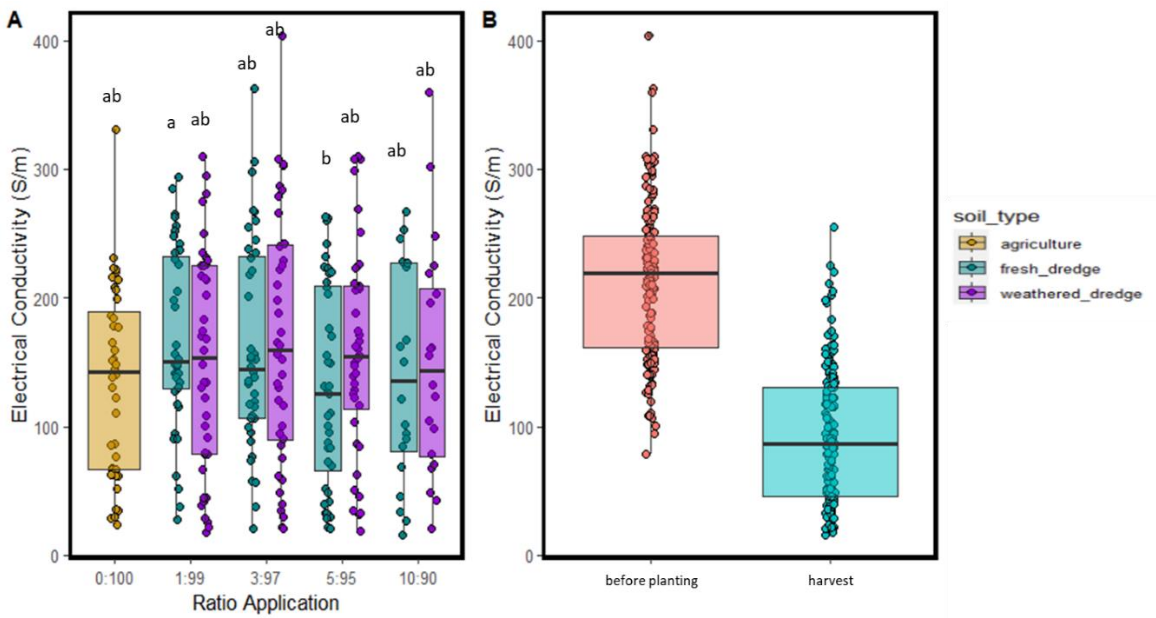

Fig S3.5. (A) Electrical conductivity (EC) was higher in the 1:99 fresh dredged sediment application compared to the 5:95 fresh dredged sediment application, while all other soil type and application rate combinations were no different ($F_{8, 298} = 2.27$, $P = 0.0226$). **(B)** EC decreased over the growing season ($F_{1,289} = 305.6$, $P < 0.0001$). Colors represent **(A)** each soil type such that agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple and **(B)** collection period 'before planting' is pink and 'harvest' is blue. Letters indicate significant differences based on Tukey's post hoc analyses ($P < 0.05$).

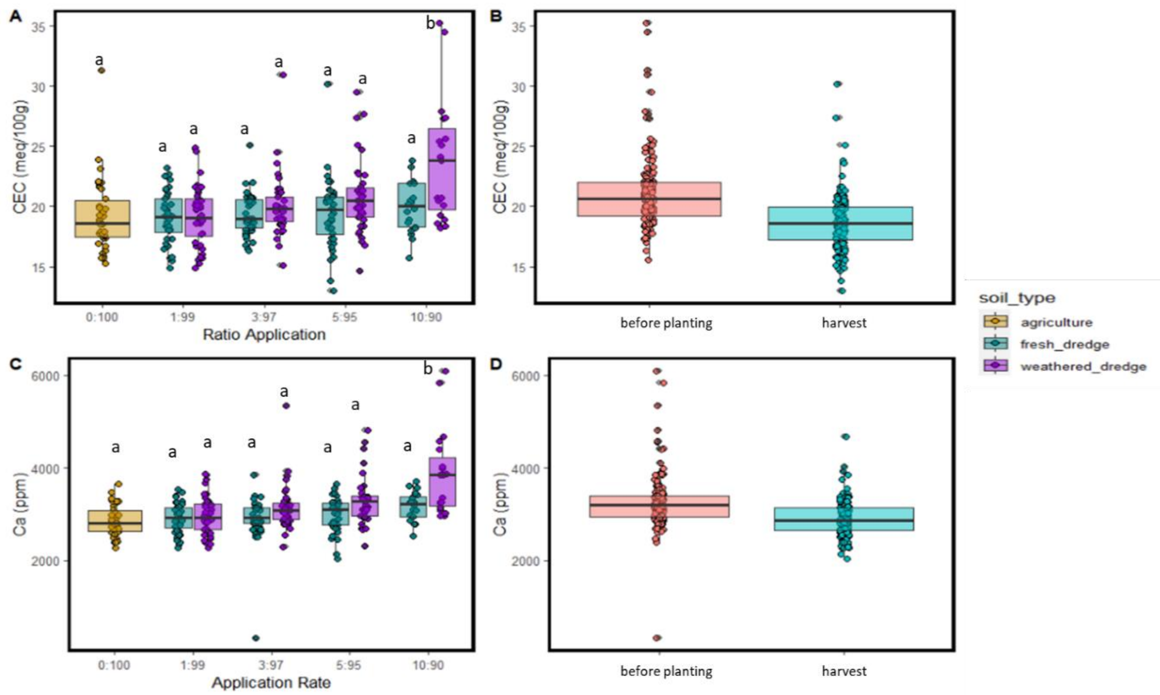

Fig S3.6. Cation exchange capacity (**A**; CEC; $F_{8,\,141} = 7.7543$, $P < 0.0001$) and Ca^{+} content (**C**; $F_{8,\,297} = 7.187$, $P < 0.0001$) were higher in the 10:90 weathered dredged sediment application than any other soil type and application rate combination. Additionally, both CEC (**B**; $F_{1,\,146} = 94.19$, $P < 0.0001$) and Ca^{+} content (**D**; $F_{1,\,297} = 30.47$, $P < 0.0001$) decreased over time. Colors represent **(A, C)** each soil type such that agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple and **(B, D)** collection date 'before planting' is pink and 'harvest' is blue. Letters indicate significant differences based on Tukey's post hoc analyses ($P < 0.05$).

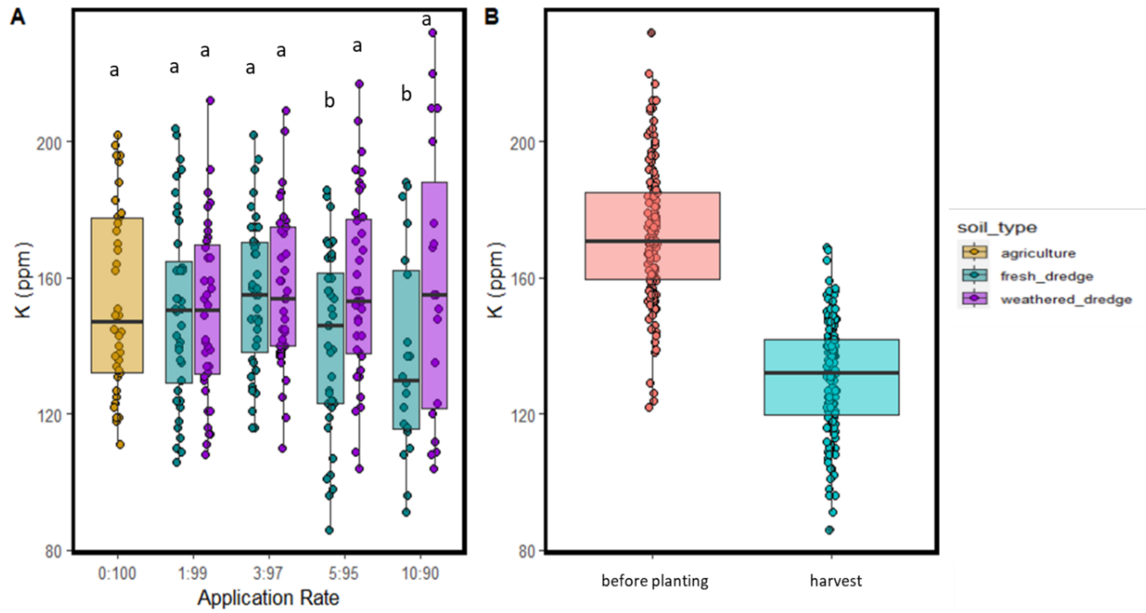

Fig S3.7. Potassium (K) content was consistent in all soil type and application ratio combinations except 5:95 and 10:90 fresh dredged sediments, which had lower K content (**A**; $F_{8, 293} = 6.879$, $P < 0.0001$). K content decreased over the growing season (**B**; $F_{1, 293} = 521.02$, $P < 0.0001$). Colors represent **(A)** each soil type such that agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple and **(B)** collection period 'before planting' is pink and 'harvest' is blue. Letters indicate significant differences based on Tukey's post hoc analyses ($P < 0.05$).

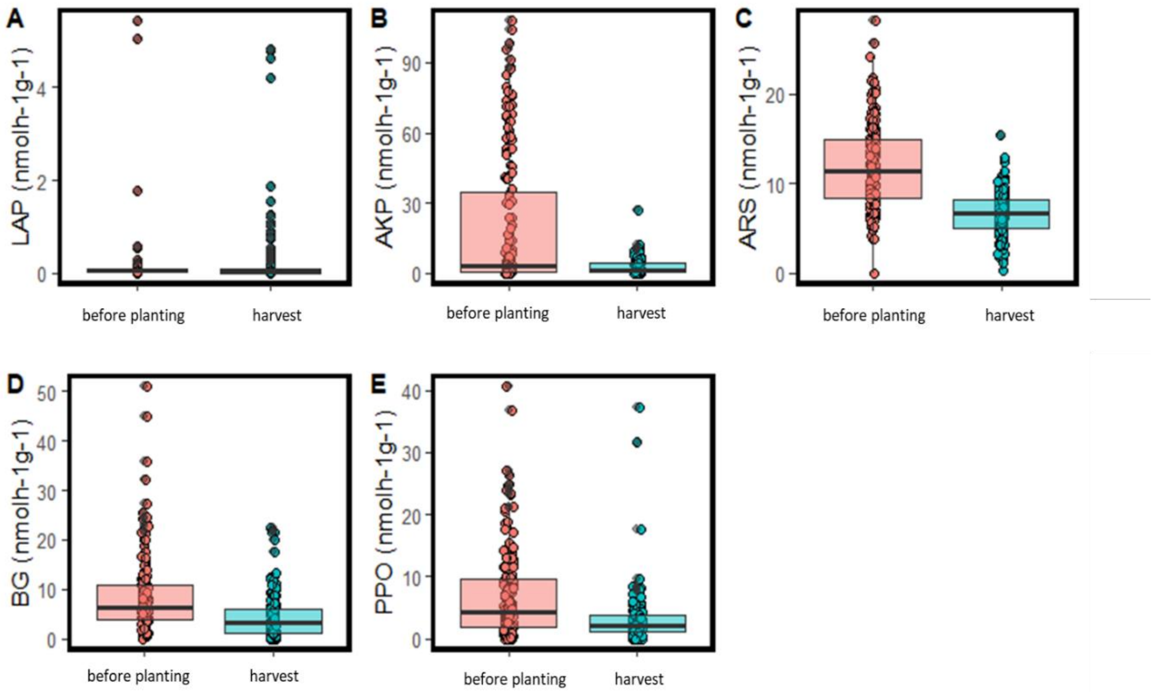

Fig S3.8. (A) Leucine aminopeptidase (LAP) activity increased over the growing season ($F_{1, 298}$ = 5.935, P = 0.0153), while **(B)** Alkaline phosphatase (AKP; $F_{1,298}$ = 11.65, P = 0.0007), **(C)** arylsulfatase (ARS; $F_{1,298}$ = 35.05, P < 0.0001), **(D)** β-glucosidase (BG; $F_{1,298}$ = 36.67, R^2 = 0.32, P < 0.0001), and **(E)** polyphenol oxidase (PPO; $F_{1,298}$ = 10.95, P = 0.0011) activity all decreased over the growing season of corn grown in different soil type and application ratio combinations. Colors indicate collection periods ‘before planting’ in pink and ‘harvest’ in blue.

Unamended Agricultural Soil compared to 100% Dredged Sediments

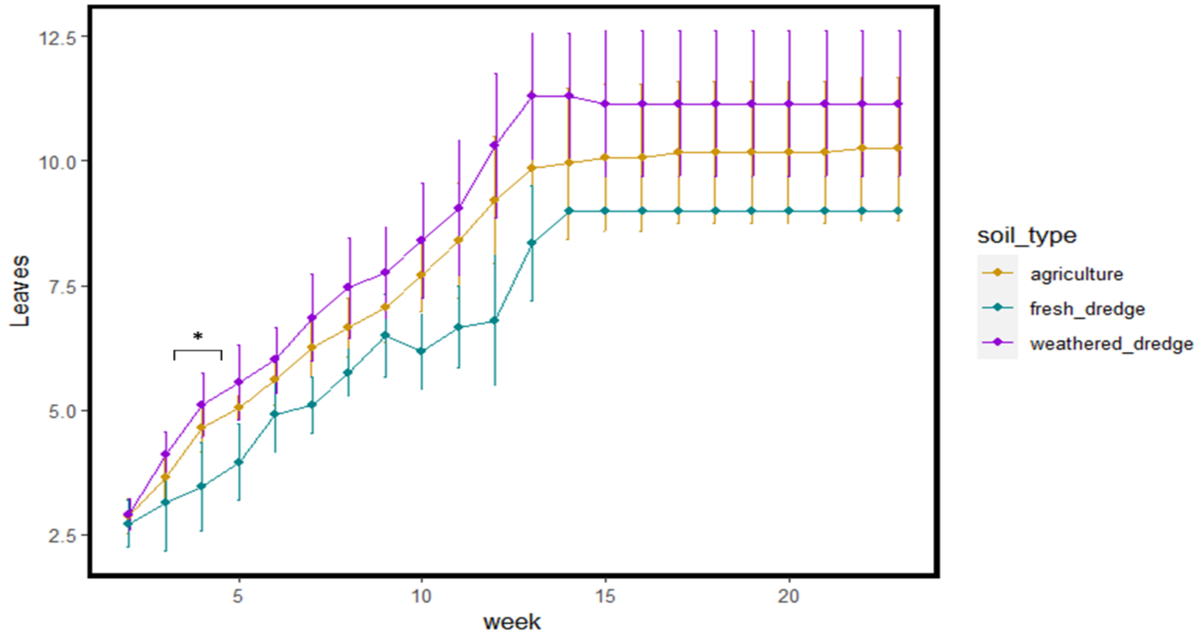

Fig S3.9. There were significant differences in leaf count over time between the 100% soil types such that the corn grown in the fresh dredged sediments had consistently fewer leaves than the agricultural soils, while corn grown in the weathered dredged sediments had more ($F_{2, 974}$ = 326.53, R^2 = 0.49, P = 0.0139). Colors represent each soil type, such that agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple. Asterisk (*) indicates significant differences based on Tukey's post hoc analyses (P < 0.05).

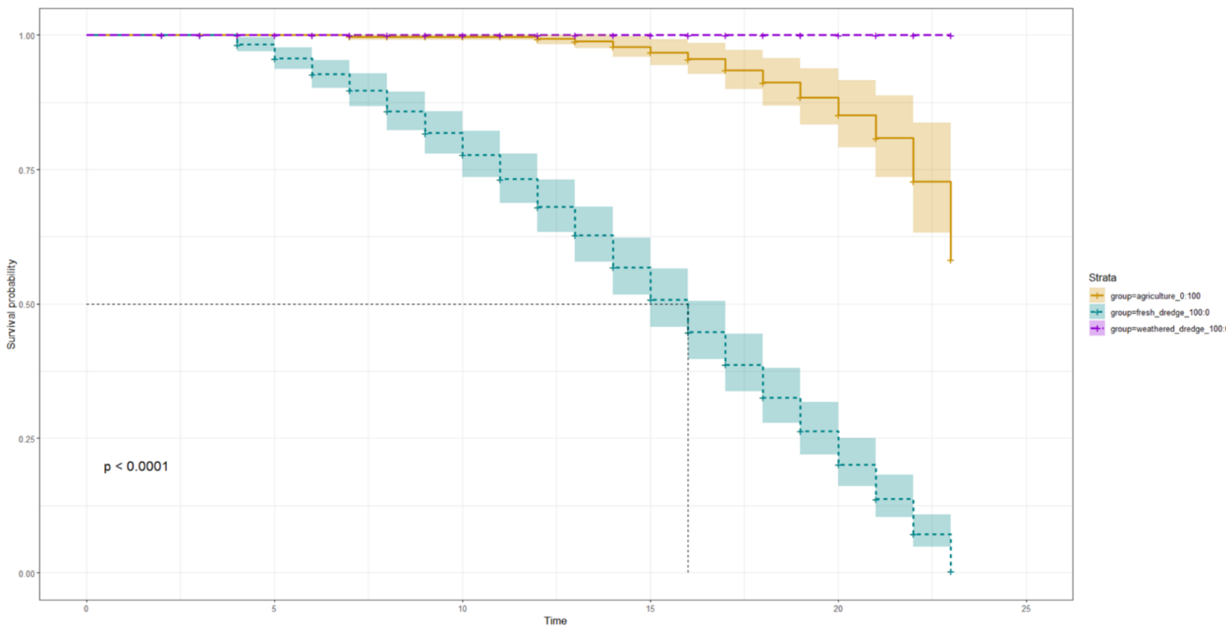

Fig S3.10. Corn survival was minimal in the fresh dredged sediments, with only one plant surviving until harvest, while corn in the agricultural soils only had four plants die off, and no plants died prior to harvest in the weathered dredged sediments ($X^2_{2, 60} = 571$, $P < 0.0001$). Colors represent each soil type, such that agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple, with shaded areas indicating 95% confidence intervals.

A. Tests for Canonical Dimensions, 100% soil types					
Dimension	Canonical Correlation	Mult. F	df1	df2	P -value
1	0.0576	1.7554	63	66.5119	0.0123
2	0.1902	1.4867	40	46.0000	0.0971
3	0.5736	0.9390	19	24.0000	0.5502

B. Standardized Canonical Coefficients, 100% soil types	
	Dimension 1
Soil properties	
Ca^{+} content	0.0318
pH	0.0509
EC	-0.0831
CEC	-0.0039
P content	0.0043
OM content	-0.0040
Mg^{+} content	-0.0079
K content	-0.1795
Moisture content	-0.0224
BD	0.1445
% sand	-0.0795
% silt	1.2490
% clay	-1.1445
AKP	-0.0427
ARS	0.0214
BG	0.0589
IV	-0.1174
LAP	-0.0154
PER	0.0217
PPO	0.0304
UR	-0.0450
Plant responses	
Biomass	-0.0354
Leaf count	-0.0505
Height	-0.0899

Table S3.2. Associations between soil properties and plant responses for the 100% soil types were found through a CCA, where one of the three canonical dimensions were significantly different from each other (**A**; P = 0.0123), with several soil properties and plant responses influencing the canonical dimension (**B**).

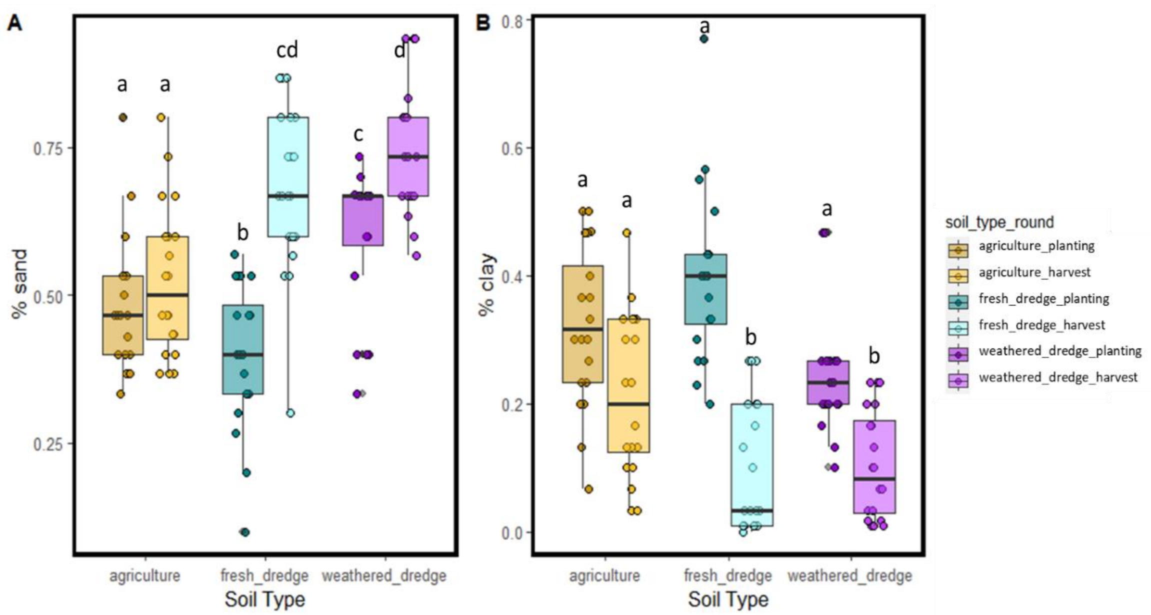

Fig S3.11. Soil texture changed over time in the 100% soil types. Over time, the percentage of sand increased in both fresh and weathered dredged sediments ($F_{1, 114} = 10.42$, $R^2 = 0.43$, $P < 0.0001$), while the percentage of clay decreased ($F_{2, 110} = 5.300$, $R^2 = 0.41$, $P = 0.0018$); however, soil texture constituents remained unaltered in the agricultural soils. Colors represent soil type and shading represents time (darker colors are prior to planting while lighter colors are following harvest): agricultural soils are brown, fresh dredged sediments are turquoise, and weathered dredged sediments are purple and boxes are 50% quartiles, with the thick black line indicating the median value. Letters indicate significant differences based on Tukey's post hoc analyses ($P = 0.05$).

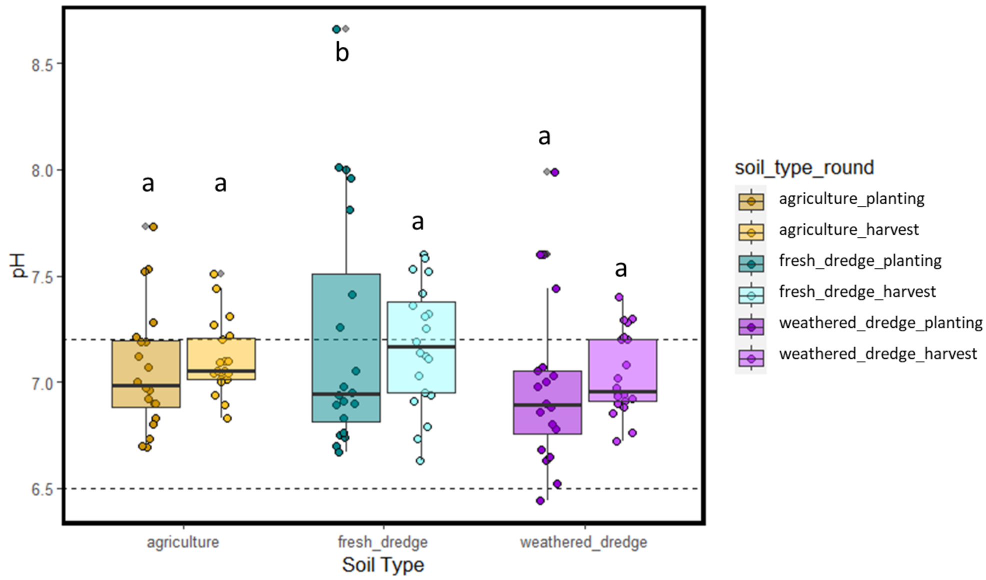

Fig S3.12. Soil pH was altered by the 100% soil treatments over time, such that fresh dredged sediments had higher pH before this was lost so that at harvest there was no difference between soil types ($F_{2,\,57} = 23.64$, $R^2 = 0.59$, $P < 0.0001$). Colors represent soil type and shading represents time (darker colors are prior to planting while lighter colors are following harvest): agricultural soils are brown, fresh dredged sediments are turquoise, and weathered dredged sediments are purple and boxes are 50% quartiles, with the thick black line indicating the median value. Letters indicate significant differences based on Tukey's post hoc analyses ($P < 0.05$).

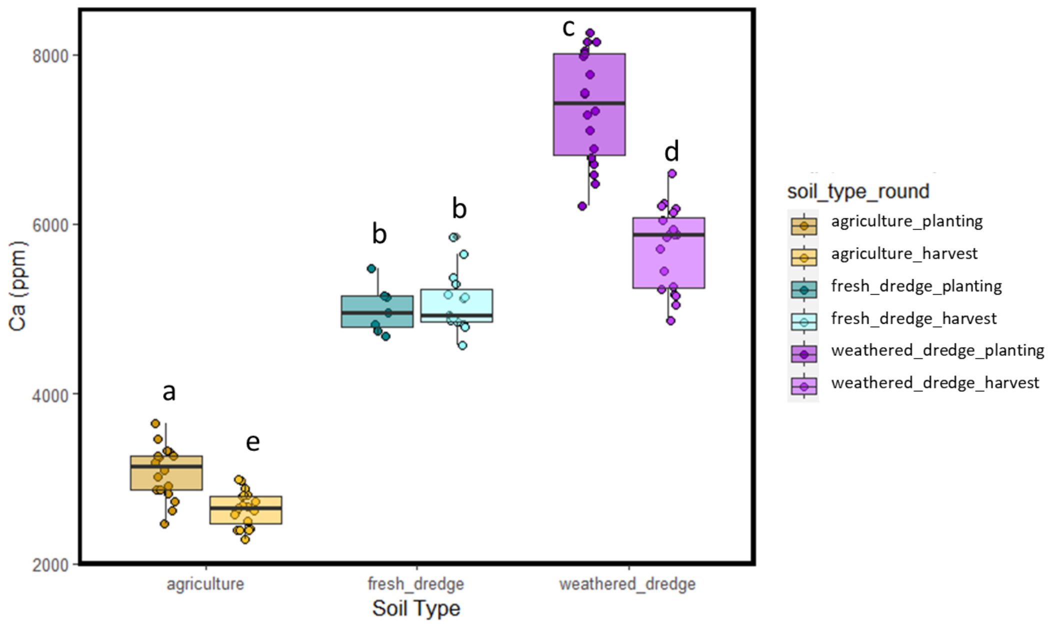

Fig S3.13. Calcium (Ca^+) content follows patterns established with CEC levels, where weathered dredged sediments had a decrease over the growing season but maintained the highest Ca^+ compared to the fresh dredged sediments which maintained higher Ca^+ than that of agricultural soils, and agricultural soils saw a decrease over the growing season ($F_{2, 88} = 15.08$, $R^2 = 0.92$, $P < 0.0001$). Colors represent soil type and shading represents time (darker colors are prior to planting while lighter colors are following harvest): agricultural soils are brown, fresh dredged sediments are turquoise, and weathered dredged sediments are purple and boxes are 50% quartiles, with the thick black line indicating the median value. Letters indicate significant differences based on Tukey's post hoc analyses ($P < 0.05$).

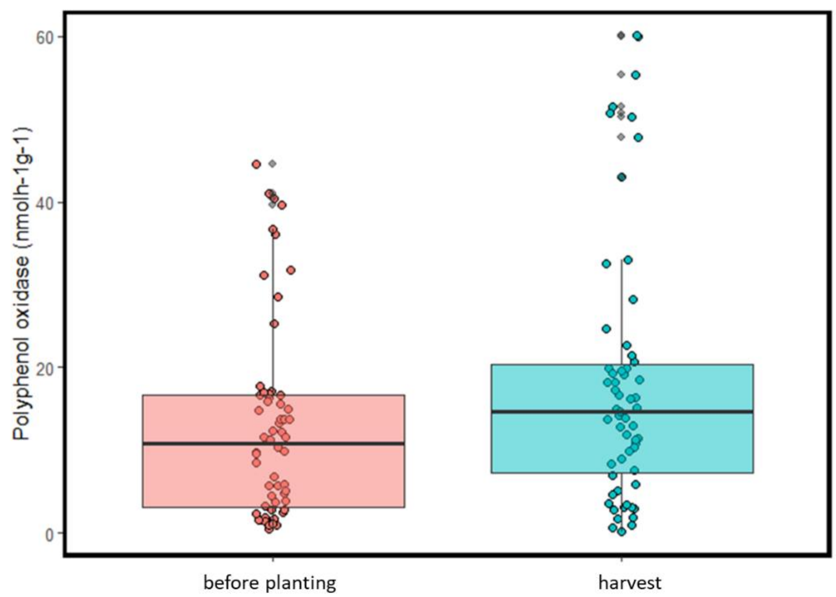

Fig S3.14. Polyphenol oxidase (PPO) activity decreased over the growing season, regardless of soil type ($F_{1,109} = 7.83$, $P = 0.0061$). Colors represent the collection periods 'before planting' as pink and 'harvest' as blue.

Appendix C

Chapter 4 Supporting Information

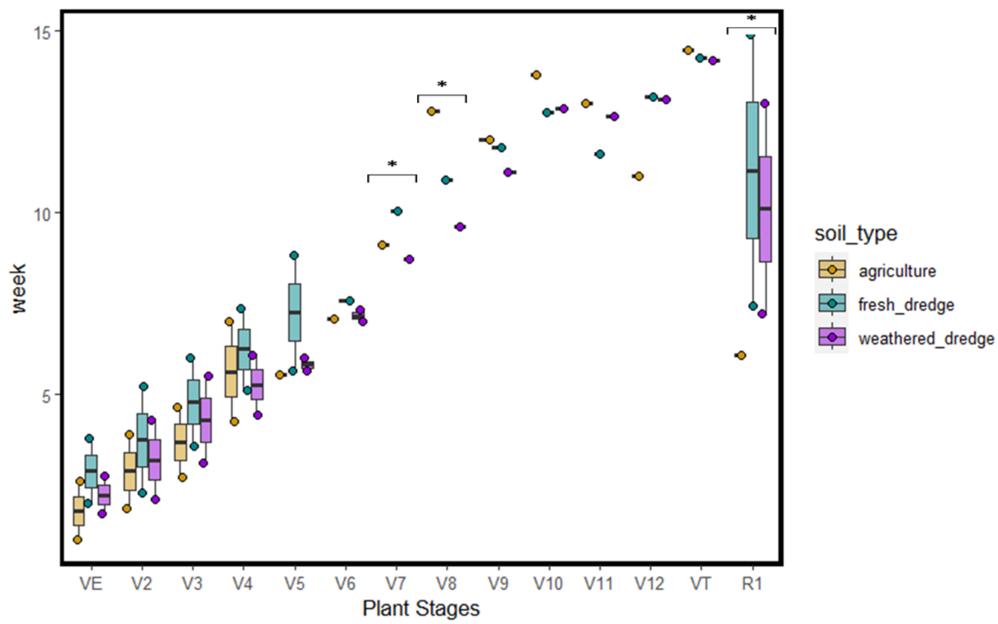

Fig S4.1. Plant stage development varied for corn only when grown in different soil types ($P < 0.0001$, $R^2 = 0.84$, $F_{24, 1038} = 2.7114$). Corn grown in the weathered dredged sediments reached V7 vegetative stage faster than corn grown in the fresh dredged sediments, and V8 was reached faster than in both fresh dredged sediments and agricultural soils. Additionally, only one plant grown in the agricultural soils reached R1. Colors represent soil type such that agricultural soils are brown, fresh dredged sediments are turquoise and weathered dredged sediments are purple. Each point represents one plant (n = 20 for agricultural soils, n = 60 for fresh dredged sediments, and n = 80 for weathered dredged sediments). Asterisk (*) indicates significant differences based on Tukey's HSD post hoc analyses ($P < 0.05$).

A. Tests for Canonical Dimensions, Crop Rotation					
Dimension	Canonical Correlation	Mult. F	df1	df2	P -value
1	0.3507	1.1983	60	170.8913	0.1850
2	0.5627	1.0169	38	116.0000	0.4570
3	0.8175	0.7319	18	59.0000	0.7651

B. Tests for Canonical Dimensions, Continuous Corn					
Dimension	Canonical Correlation	Mult. F	df1	df2	P -value
1	0.2590	1.7469	57	173.7633	0.0032
2	0.5574	1.1124	36	118.0000	0.3279
3	0.8704	0.5254	17	60.0000	0.9293

C. Standardized Canonical Coefficients, corn	
	Dimension 1
Soil properties	
Moisture content	-0.0041
BD	-0.0280
% sand	-0.0012
% silt	-0.0134
pH	0.0141
EC	0.0155
P content	0.0787
K content	-0.0153
Mg^+ content	0.0754
CEC	0.0034
BG	-0.1771
LAP	-0.0121
PER	0.0141
AKP	0.0239
IV	-0.0026

ARS	-0.0013
PPO	0.0021
UR	0.0035
Plant responses	
Biomass	0.0840
Leaf count	0.0888
Height	-0.0764

Table S4.1. (**A**) Tests of canonical dimensions for crop rotation, which resulted in zero of three canonical dimensions as significant from the others (P > 0.05). (**B**) Tests of canonical dimensions for continuous corn, resulted in one of three canonical dimensions as significant from the others (P = 0.0032). (**C**) The standardized canonical coefficients for the first dimension across both sets of variables for continuous corn.

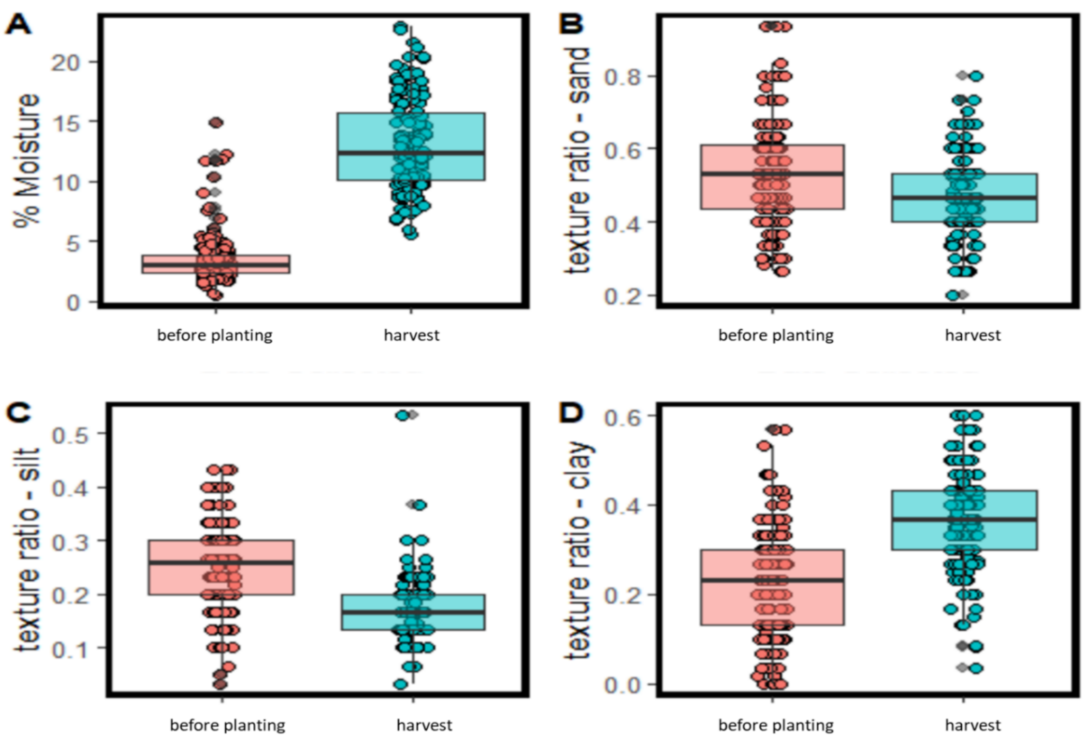

Fig S4.2. Four physical properties were influenced by time alone. Both moisture content (**A**; $P < 0.0001$, $F_{1, 154} = 986.9$) and the percentage of clay (**D**; $P < 0.0001$, $F_{1, 154} = 76.6333$) increased from before planting to harvest, while the percentage of sand (**B**; $P = 0.0015$, $F_{1, 154} = 10.42$) and silt (**C**; $P < 0.0001$, $R2 = 0.25$, $F_{1, 154} = 97.25$) both decreased from before planting to harvest. Color represents before planting as pink, and harvest as blue. Points represent a single soil sample (n = 160 per date collected).

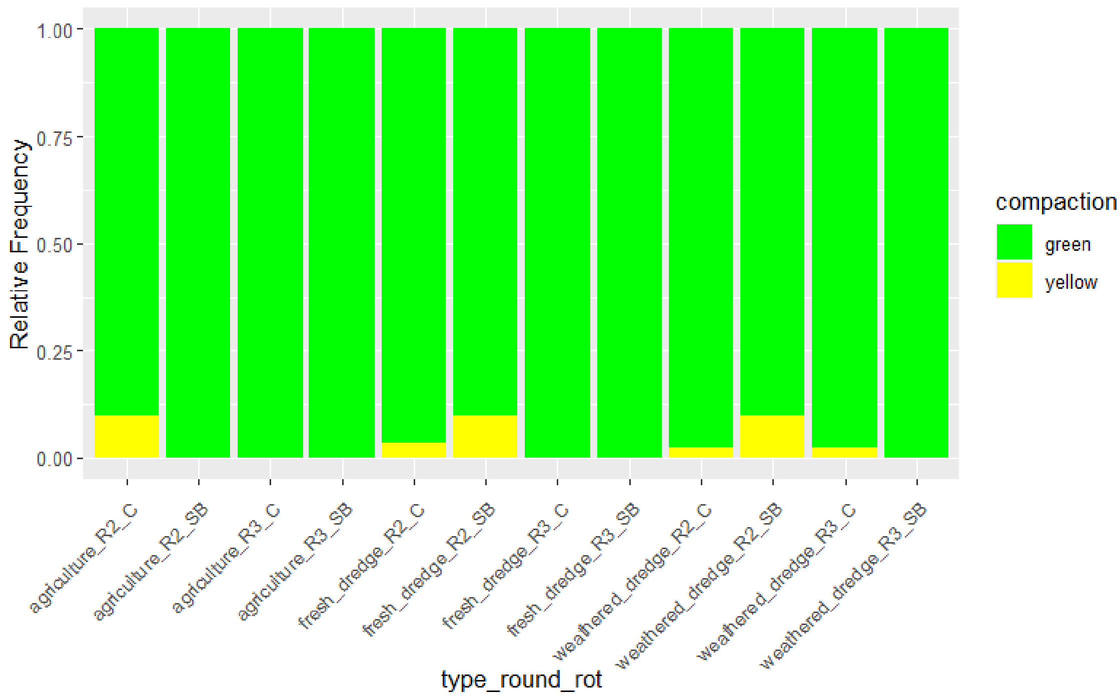

Fig S4.3. Soil compaction remained at 'low' or 'moderate' from before planting ('R2') to harvest ('R3') for all soil types regardless of rotation strategy ('C' = continuous corn, 'SB' = rotation; P = 0.1726, X^2 = 15.22).

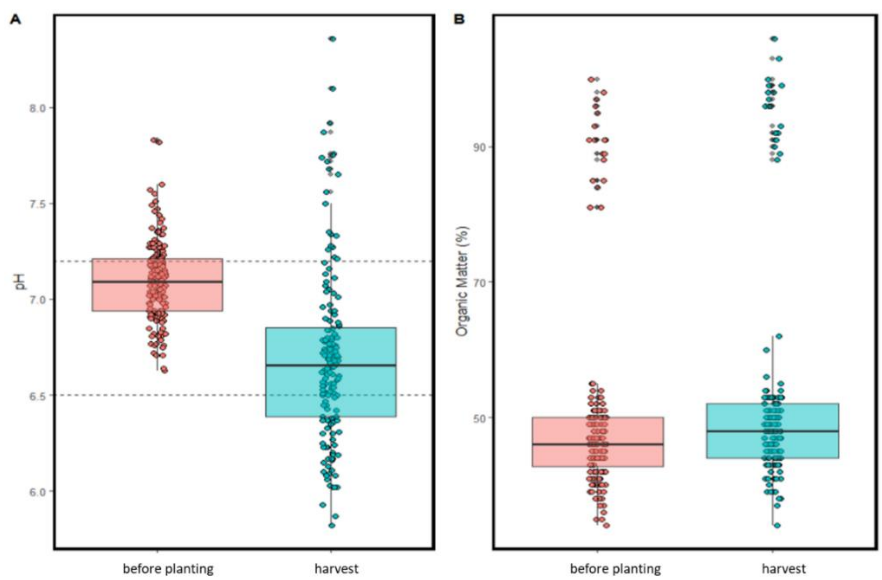

Fig S4.4. Soil pH decreased from before planting to harvest (**A**; P < 0.0001, R^2 = 0.25, $F_{1, 154}$ = 89.38), while organic matter (OM) content increased at harvest compared to before planting (**B**; P = 0.0001, R^2 = 0.11, $F_{1, 151}$ = 15.39). Colors represent dates collected such that before planting is pink, and harvest is blue. Points represent a single soil sample (n = 160 per date collected).

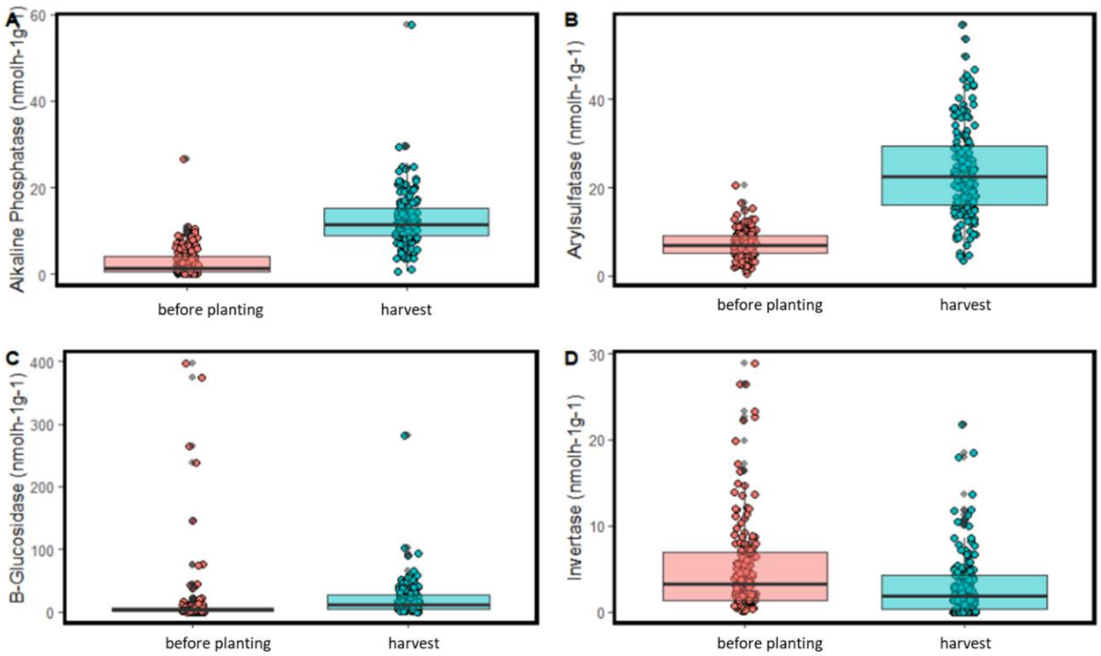

Fig S4.5. (**A**) Alkaline phosphatase (AKP; P < 0.0001, R^2 = 0.33, $F_{1, 154}$ = 108.24), (**B**) arylsulfatase (ARS; P < 0.0001, R^2 = 0.59, $F_{1, 154}$ = 350.0), (**C**) β-glucosidase (BG; P < 0.0001, R^2 = 0.12, $F_{1, 154}$ = 29.69) increased from before planting to harvest, while (**D**) invertase (IV; (P < 0.0001, R^2 = 0.16 , $F_{1, 154}$ = 46.57) decreased at harvest. Color represents before planting as pink, and harvest as blue. Points represent a single soil sample (n = 160 per date collected).

END.