

THE IMPACT OF LAND MANAGEMENT PRACTICES ON SOIL STRUCTURE AND RESPIRATION IN AN HERBACEOUS ECOSYSTEM

by

PRAMOD HEGDE

(Under the Direction of Nandita Gaur and Gerald Henry)

ABSTRACT

Soils are the largest dynamic stock of carbon (C) on Earth. Land management practices like tillage and fertilization can alter soil structure, impacting soil respiration and carbon fluxes. In this study, the effects of land management practices on soil water retention, pore size distribution, and respiration rates were investigated. Although tillage and fertilization had negligible impacts on bulk density and porosity, they significantly altered water-filled pore volume under different pore size domains, a key factor in the regulation of soil respiration. Fertilization reduced water held in storage pores, potentially increasing gaseous exchange, but when combined with tillage, this increased. These findings highlight the significance of pore structure in mediating respiration dynamics and underscore the need for sustainable management practices that balance productivity and carbon sequestration. Future research should explore the influence of structural changes on microbial processes and interactions with environmental conditions to predict soil carbon dynamics better.

INDEX WORDS: [Soil structure, soil respiration, soil carbon]

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DEDICATION

For my parents and sister.

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CHAPTER I

INTRODUCTION

Grasslands represent one of the most widespread terrestrial biomes, covering nearly one-third of the Earth's terrestrial land surface (Suttie et al., 2005). Grasslands provide vital ecosystem services, including biodiversity conservation, water cycling, and carbon sequestration (Bengtsson et al., 2019). On a global level, grasslands contain approximately 50% more carbon than forest ecosystems, with nearly 90% of this being belowground (Bai and Cotrufo, 2022). Yet, these ecosystems are under increasing pressure from anthropogenic disturbances in the form of land-use change, overgrazing, and nutrient enrichment with profound implications for soil structure and biogeochemical cycling (Dondini et al., 2023). In the US, intensive land management has caused huge soil carbon losses, and an estimate of 50–60% of total Soil Organic Carbon (SOC) loss has occurred since the 1870s (Kucharik et al., 2001). The long history of tillage has also depleted SOC in the Piedmont region of the southeastern United States. One of the dominant pathways through which SOC is lost to the atmosphere is soil respiration. It is the evolution of CO_2 through microbial decomposition of organic matter and root respiration, which is regulated by a variety of biotic and abiotic factors, including soil structure, moisture, temperature, and nutrient input. Land management practices, especially tillage and fertilization, affect soil respiration by their interaction with soil physical properties. Understanding this interaction is key to predicting future SOC dynamics in a changing climate and increasing land-use change.

One of the primary pathways through which tillage and fertilization affect soil carbon dynamics is through its modification of soil structure, the arrangement of soil particles and pores within the soil matrix. Soil structure can be assessed from two perspectives: aggregate organization and pore space architecture. Since most soil processes are dominated by pore shape, connectivity, and tortuosity (Rabot et al., 2018; Young and Ritz, 2000), the pore space perspective is particularly relevant to SOC dynamics. Pores regulate water flow and storage, gaseous diffusion, solute transport, and root growth, all of which are critical for carbon cycling (Rabot et al., 2018; Schjønning et al., 2005; Kravchenko and Guber, 2017). Soil organic carbon and pore characteristics have a bidirectional relationship, with SOC changes affecting pore dynamics (Kravchenko et al., 2015), and pore structure changes also affect SOC dynamics (Rabot et al., 2018). Consequently, land management practices that modify SOC inevitably impact soil structure.

Tillage changes soil structure by aggregate breakdown and exposing previously protected organic matter to decomposition. This results in an increase in macropores ($> 50 \mu\text{m}$) (Dal Ferro et al., 2014; Weninger et al., 2019), which are involved in the transport of water and gases (Greenland, 1977). Thus, an increase in macropores, can potentially increase carbon flux through enhanced gas exchange. However, the impacts of tillage on pore size distribution are not consistent across studies. Araya et al. (2022) reported an increase in pores of $0.2 - 50 \mu\text{m}$ size under tillage, and a decrease in macropores, whereas Pagliai et al. (2004) reported decreases in both storage ($0.5 - 50 \mu\text{m}$) and transmission pores ($50 - 500 \mu\text{m}$) under tilled soils. Tillage increased pores of $> 3 \mu\text{m}$ size, which could be one of the reasons for increased soil respiration, as per Lacroix et al. (2021). These inconsistent results, along with variations in pore-size classification, highlight the site-specific nature of tillage effects on soil pore structure. However, understanding the effects of tillage in the sandy loam soils of our study site could help us apply the results to similar soil types in the Piedmont.

NPK fertilization has a less pronounced effect on soil structure compared to tillage. Dal Ferro et al. (2013) found that NPK fertilization increased porosity in the range of $12.5 - 25 \mu\text{m}$, while Schlüter et al. (2011) reported macroporosity development at the expense of smaller pores ($< 1 \text{ mm}$) due to root growth and crack formation. Similarly, Naveed et al. (2014) observed that pores $> 200 \mu\text{m}$ were enhanced by fertilizer

application. However, organic fertilizers have a greater effect on soil structure compared to inorganic fertilizers since they introduce stable organic material that more readily changes the pore size distribution from small to large pores (Dal Ferro et al., 2013; Naveed et al., 2014). However, the fertilization impact on soil respiration is higher than the impact of structural changes. Terrestrial ecosystems are generally nitrogen limited (Vitousek and Howarth, 1991), which restricts microbial decomposition of SOC. Fertilization alleviates this limitation, enabling microbes to use additional nitrogen for decomposition of organic matter, which may result in increased soil respiration. Fertilization also stimulates plant and root growth, incorporating more organic matter into the soil through aboveground litter and root exudation. Enhanced substrate availability would, in turn, lead to enhanced soil respiration. Quantifying how fertilization impacts soil respiration is crucial to balance against elevated crop yields for any carbon loss. However, the huge number of variables interacting with one another makes it difficult to partition the effect of soil structure-induced changes on respiration.

This study aims to understand the effect of tillage and fertilization on water retention, pore structure of the soil, and soil respiration in a herbaceous community. Chapter one focuses on fitting a dual-porosity model to derive soil moisture release curves (SMRCs), which were then used in calculating pore size distribution (PSD). Pore size distribution allowed us to classify pores into different functional classes according to size. In addition, we analyzed soil water retention properties, i.e., air capacity (AC) and available water content (AWC), to understand tillage and fertilization impacts on soil moisture regimes. To gain a better understanding of the different factors affecting soil structure, we carried out a principal component analysis (PCA), which helped to reveal relationships between soil structural, physical, and chemical properties. This provided insights into the interactions between different pore size groups, water retention parameters, and soil physicochemical properties. The second chapter examines the impact of tillage and fertilization on soil respiration, with a focus on understanding how soil structure mediates carbon fluxes. Using a statistical framework, we evaluated the role of soil pore characteristics in controlling carbon dynamics. Since soil structure is inherently related to gas and water transport, we used water-filled pore volume in different pore size ranges as a proxy for soil structure to analyze its influence on respiration

rates. We aim to clarify the mechanisms through which land management activities alter soil carbon cycling and contribute to long-term soil sustainability in herbaceous ecosystems with these integrated analyses.

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CHAPTER 2

EFFECTS OF TILLAGE AND FERTILIZATION ON SOIL STRUCTURE^I

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2.1 Abstract

Grasslands are among the most widespread ecosystems globally, yet human-induced nutrient enrichment and unsustainable management practices are altering their soils, particularly soil structure. Soil structure regulates hydrological and gaseous fluxes, influencing microbial activity, root growth, and nutrient cycling. Understanding how land management practices affect soil structure is crucial for predicting soil health. This study quantifies the effects of tillage and fertilization on soil pore structure and water retention. Soils were collected from five treatments: 1) nitrogen-phosphorus-potassium (NPK) enriched soils, 2) disturbed soils, 3) their combination (NPKD), 4) NPK Cessation and 5) Control. Pore size distribution was determined by fitting the Durner dual-porosity model to water retention data obtained using evaporation (Hyprop) and dewpoint (WP₄C) methods. While treatments did not affect bulk density or available water content (AWC), NPKD increased the proportion of storage pores (0.5–50 μm), whereas NPK reduced them, potentially due to increased microbial activity. Storage pores were strongly correlated with AWC, highlighting their role in water retention. Transmission pores (50–500 μm) remained unchanged across treatments but were highly correlated with air capacity (AC), a key indicator of soil aeration. These findings provide critical insights into how tillage and fertilization influence soil pore structure, with implications for water availability and soil aeration in managed grasslands.

2.2 Introduction

Grasslands are one of the most widespread ecosystems in the world, covering nearly a third of the earth's terrestrial surface (Suttie et al., 2005). They serve as biodiversity hotspots, support food production, and provide essential ecosystem services like carbon sequestration, erosion control, and water supply/regulation. Notably, grasslands play a crucial role in the global carbon cycle, storing 50% more carbon than the world's forests, with nearly 90% of that carbon sequestered in the soil (Y. Bai and Cotrufo, 2022). However, human-induced nutrient enrichment, land use change, and unsustainable management practices are increasingly altering grassland soils thereby changing their biophysical functionality and capacity to provide ecosystem

services, especially altering soil carbon dynamics (Dondini et al., 2023). There is a general consensus that management practices, such as grazing and fertilization in grasslands, lead to changes in soil structure. However, the specific mechanisms through which these changes occur remain poorly understood.

Soil structure is defined by the spatial arrangement of solid particles and voids in the soil matrix. Traditionally, soil structure has been assessed through aggregate stability and bulk density measurements, but recent advances emphasize the importance of evaluating porespace and pore networks, which directly mediate hydrological and gaseous fluxes (Rabot et al., 2018). Since soil pores serve as conduits for water and air movement, changes in their distribution can significantly impact microbial activity, root growth, and carbon cycling (Rabot et al., 2018; Quigley et al., 2018). Therefore, quantifying how land management practices influence pore-size distribution is essential for predicting long-term soil functionality in grassland systems to ensure the effective stewardship of these vital ecosystems.

Grasslands are frequently subjected to two prevalent practices: tillage and fertilization. Tillage in grasslands is necessary to convert their land-use to cropland and influence grasslands across the world. Between 2008 and 2016, the US Midwest lost 2 Mha of grassland due to land-use conversion (Zhang et al., 2021). Although figures are lacking for other regions, we can speculate that the conversion rates are higher in other parts of the world. Fertilization, on the other hand has been an established practice for increasing productivity in grasslands. Fertilization, particularly nitrogen enrichment, has increased dramatically over the past century (Galloway et al., 2008). Tillage and fertilization alter soil structure (Bronick and Lal, 2005), albeit variably based on the edaphic conditions, such as soil organic matter, microbial diversity, soil texture and soil genesis at the site. Consequently, results from a study on a specific soil type cannot be generalized and applied to other soil types. Notably, soil pore size distribution as a measure of soil structure provides a soil type–agnostic metric that helps relate the impact of these practices to changes in carbon storage and fluxes across diverse soils.

Tillage alters soil structure by disrupting aggregates, increasing aeration, and modifying pore-size distribution (Bronick and Lal, 2005). While some studies in agricultural soils report increased macroporosity following tillage (Lipiec et al., 2006), others have observed reductions (Dal Ferro et al., 2014). In grass-

lands, perennial vegetation with more extensive and deeper rooting compared to typical agronomic crops may facilitate better pore structure of soil through increased organic matter input and the production of microbially derived binding agents (Bronick and Lal, 2005; Six et al., 2004). Thus, while extensive research has examined tillage effects in croplands, the same impacts on grassland soil cannot be assumed and are largely unexplored.

Fertilization on the other hand, has long been used to enhance productivity in grasslands by increasing plant biomass and organic matter inputs into the soil, which can influence soil structure (Six et al., 2004). Since nitrogen input also change root structure (T. Bai et al., 2021) and consequently alter the area of contact between root exudates and soils, fertilized grasslands are poised to have different effects on soil structure as compared to their unfertilized counterparts. Studies have also reported increases in root biomass and microbial activity may alter pore connectivity and water retention properties (Schjønning et al., 2005; Naveed et al., 2014) because of fertilization. Despite these potential effects, there is limited research on how fertilization modifies pore-size distribution in non-agricultural soils, such as those in grasslands.

A key challenge in assessing these structural changes is the quantification of pore-size distribution across relevant spatial scales. Various methods exist to characterize water retention and pore sizes, each with advantages and limitations. Image analysis techniques, such as X-ray computed tomography (X-ray CT), have been widely used to visualize pore networks, but they are often limited to micrometer-scale pores and small sample sizes (Schlüter et al., 2018). Alternatively, soil moisture release curves (SMRCs) capture a wide range of pore sizes from the nanoscale ($< 0.001 \mu m$) that influence water retention and soil organic matter dynamics to the centimeter scale ($< 2 cm$) that control microfauna habitat and movement. However, SMRCs rely on model simplifications and the assumption of disconnected cylindrical-shaped pores, which can introduce uncertainties (Weninger et al., 2019; Rabot et al., 2018). Despite these limitations, SMRCs remain a valuable tool for assessing the impact of land management practices on soil hydraulic properties at scales that are relevant for practical applications.

Soil moisture release curves also provide insights into the functional consequences of structural modifications. Key indices derived from SMRCs include Available Water Content (AWC), Productive Water Content (PWC), and Air Capacity (AC). Available water content and PWC provide direct estimates of the ability of soil to store water and air necessary for optimum plant growth, which is influenced by soil texture, organic matter, and pore connectivity. Meanwhile, AC reflects the proportion of macropores responsible for gas exchange, which is critical for microbial respiration and root aeration (Reynolds et al., 2007). These indices collectively help quantify the extent to which management-induced structural changes translate into functional differences in water retention, microbial habitat, and carbon fluxes.

The objective of this study is to assess the effects of fertilization and tillage on soil structure in grassland soils. Specifically, we aim to evaluate changes in soil water retention and pore structure across a range of pore sizes (0.001 μm to 2 cm) using SMRCs obtained through evaporation and dewpoint techniques (Schindler et al., 2010; Campbell et al., 2007). By linking these changes to land management practices, this study seeks to provide insights into the underlying mechanisms driving soil structural modifications in managed grassland ecosystems.

2.3 Materials and Methods

2.3.1 Site description

The study was conducted in the Whitehall Forest (lat. 33.89 °N, long. 83.35 °W) in Athens, GA. The average annual temperature is 16.9 °C with an average annual precipitation of 1178 mm. The study area consisted of Loblolly pines until five years ago, when they were cleared, and a natural seed bank was allowed to grow. The most common vegetation seen in the study area was *Lespedeza cuneata*, followed by the *Rubus* sp.. The experiment is part of the DRAGNet (Disturbance and Resources Across Global Grasslands) (<https://nutnet.org/dragnet>) project involving five treatments, with five replications each in plots of size 5 x 5m. The treatments are Nitrogen-Phosphorous-Potassium (NPK), disturbance, NPK + Disturbance (NPKD), NPK Cessation, and control. NPK Cessation involves fertilization for the first

five years, followed by cessation. The experiment began in the summer of 2020, and since this study was conducted in the summer of 2023, NPK cessation plots were considered equivalent to NPK plots. The treatments were applied once every summer. NPK treatment involved fertilization using time-release urea, triple super phosphate (TSP) and potassium sulphate at the rate of 10 g m^{-2} by elemental mass. These plots were also treated with a micronutrient mixture (Micromax[®]; BFG Supply Co., Burton, OH, USA) at the beginning of the experiment. Disturbance consisted of removing all standing biomass and rhizomes, and tilling the topsoil using a walk-behind rototiller to a depth of 15 cm.

Table 2.1: Main physical and chemical properties of the top soil measured in the control plots

Property	Mean	se*
Sand (%)	66.6	1.60
Silt (%)	16.8	1.20
Clay (%)	16.6	1.94
Bulk Density (g cm^{-3})	0.90	0.09
OM (%)	5.54	0.73
Total N (%)	0.14	0.03
Total C (%)	2.63	0.60

(a) * - Standard error

2.3.2 Soil Core Collection

In September 2023, 25 soil cores were collected from each plot randomly for water retention measurements. Care was taken to avoid sampling close to the edges to avoid edge effects. Stainless steel cores of height 5 cm and diameter 7.96 cm (250 mL volume) were inserted into the Ap horizon using a rubber mallet and hammering holder for sampling rings. Prior to the insertion of the soil core, care was taken to remove the top of any vegetation. The cores were then capped and transported to the Environmental Soil Physics Laboratory at the University of Georgia, where they were stored in a refrigerator to prevent moisture loss.

2.3.3 Lab measurements

Soil Physical Properties

Bulk density (ρ_b) samples were taken in July 2023. The soil was sieved first for gravel $> 2\text{ mm}$ and the remaining material was oven-dried at 105°C for 24 hours to obtain the dry soil weight. Bulk density was obtained by dividing the oven dry weight by the volume of the container. Porosity (ϕ) was calculated using bulk density and the particle density as 2.65. The equation used to calculate porosity is as follows:

$$\phi = 1 - \rho_b / \rho_s \quad (2.1)$$

where ρ_s was the particle density of 2.65 g/cm^3

The total C and N were measured using combustion analysis, in which the samples are combusted in an oxygen atmosphere at 1350°C . Organic matter content was measured using the Loss on Ignition (LOI) method.

Soil Moisture Release Curve measurement

A complete Soil Moisture Release Curve (SMRC) was developed to evaluate the relationship between Matric Potential (ψ) and Volumetric Water Content (VWC)(θ_v). The HYPROP evaporation method was used for the wet range (0 to -0.1 MPa) and the WP4C dewpoint potentiometer method was used for the dry range (-0.1 to -300 MPa). Both of these systems were developed by the METER group (METER group, Pullman, CA, USA).

The HYPROP setup was as described by Schindler et al. (2010). The setup involves measuring the tension values at two depths using tensiometers of length 2.5 and 5 cm. The soil cores were prepared by saturating them from the bottom for at least 24 hours. Once saturated, the soil core was placed on the HYPROP base, and the top of the core was left open to the atmosphere for evaporation to take place. The difference in the soil mass over time is measured by the automated weighing scale to calculate the VWC. The matric potential was calculated based on the average of the two tensions. We used the HYPROP for

tension values in the wet range of up to -100 KPa/ -0.1 MPa. Tension values in the dry range were measured using a WP₄C dewpoint potentiometer.

The WP₄C measurement was conducted for four subsamples prepared from each HYPROP core. Once HYPROP measurements were done, the soil was air-dried for at least a week. Out of the four subsamples, one was without moisture manipulation, while the other three received increasing amounts of moisture using a dripper to elevate water content slightly above initial conditions. Anywhere between 3-5 g of soil was taken in stainless steel sampling cups diameter of 4 cm and depth 1 cm and placed inside the measurement device. Each measurement last around 20-30 minutes. Following WP₄C measurements, the samples were dried in the oven at 105 °C to estimate the dry weight.

2.3.4 Analytical Models

Hydraulic Retention Models

The dual-porosity model developed by Durner (1994), suitable for structured soils with macropores was used to predict a complete SMRC using the pairs of θ_v versus matric potential (ψ). This equation was used to fit a curve to the points obtained from the HYPROP and WP₄C. LABROS SoilView Analysis software (METER group, Pullman, CA, USA) was used to pre-process the raw data before they were used to fit the curve. Pre-processing included providing the oven dry weight of the soil core for improved accuracy of the measured volumetric water content and inputting tare weights of the soil sampling rings. The dual porosity model is as follows:

$$S_e(h) = \frac{\theta - \theta_r}{\theta_s - \theta_r} = \frac{w_1}{[1 + (-\alpha_1 h)_1^n]^m} + \frac{w_2}{[1 + (-\alpha_2 h)_2^n]^m} \quad (2.2)$$

where θ_s and θ_r are the saturated and residual water content, α [L^{-1}], n and m are fitting parameters, w_1 and w_2 are weights of each region.

Curve fitting was performed using the R programming language Team (2021) through an optimization procedure based on Simulated Annealing (Kirkpatrick et al., 1983). The lower and upper bounds for the

optimization were informed by a literature review. A maximum of 1000 iterations were set to ensure optimal performance, and the iteration process stopped when the Residual Sum of Squares (RSS) reached a minimum. The optim function in R was used to search for parameters that minimized the residual function, which, in this case, was eq.2.2.

The optimized parameters obtained from the fitting process were used to generate curves for the lab data points. Supplementary Table 1 presents the optimized parameters for all samples, along with their RSS, R^2 , and other relevant metrics. Based on this, soil water retention indices were calculated:

- Field Capacity (FC), defined as the volumetric water content at matric potential of -10 kPa ($\psi = -1.02$ m).
- Growth Cease Point, volumetric water content at Matric Potential of -450 kPa ($\psi = 49.95$ m), when plant growth stops.
- Permanent Wilting Point (PWP), volumetric water at -1500 kPa Matric Potential ($\psi = 153.06$ m), when the plant dies.
- Available Water Content (AWC), calculated as the difference between FC and PWP.
- Productive Water Content (PWC), calculated as the difference between Field Capacity and Growth Cease Point.
- Air Capacity (AC), difference between volumetric water content at saturation and Field Capacity.

$$AC = \theta_S - \theta_{FC} \quad (2.3)$$

AWC and PWC are important indices that quantify the portion of water available for plant use, while Air Capacity reflects soil aeration which is essential for root growth and microbial activity.

Pore Size Distribution (PSD)

A Pore Size Distribution (PSD) is a frequency distribution curve of different sized pores in a soil core. The matric potential from SMRC can be converted to pore diameter using the Young-Laplace capillary rise equation which is expressed as follows -

$$d = 2 * \frac{2\sigma \cos \alpha}{\rho_w g h} \quad (2.4)$$

where σ is the surface tension [MT^{-2}], α is the contact angle, ρ_w is the density of water [ML^{-3}], g is the acceleration due to gravity [LT^{-2}], d is the diameter of the pore [L] and h is the matric potential [L].

Hydraulic capacity function $C_w(h)$, which is the slope of the retention curve was obtained by deriving θ with respect to h ;

$$C_w(h) = \frac{d\theta}{dh} \quad (2.5)$$

Substituting θ from eq.2, we get

$$C_w(h) = \frac{\alpha_1^n (\theta_s - \theta_r) m_1 n_1 (-h^{n_1-1})}{[1 + (-\alpha_1 h)_1^n]^{m_1+1}} + \frac{\alpha_2^n (\theta_s - \theta_r) m_2 n_2 (-h^{n_2-1})}{[1 + (-\alpha_2 h)_2^n]^{m_2+1}} \quad (2.6)$$

The hydraulic capacity function along with the diameter obtained from eq.4 can be used to obtain a Pore-Size Distribution curve, where the y-axis containing the hydraulic capacity function is the relative volume of pores for each value of d.

Soil Pore Volume Proportions

The PSD was used to generate a cumulative distribution curve that quantifies the proportion of soil pores within defined size ranges. Specifically, the volumetric water content (measured on the y-axis) was normalized by the total porosity of the soil core to represent pore space. Based on thresholds established in the literature (Greenland, 1977; Głab, 2014), the pore space was partitioned into five domains: bonding space ($< 0.005 \mu m$), residual ($0.005 - 0.5 \mu m$), storage pores ($0.5 - 50 \mu m$) transmission pores

(50 – 500 μm), and fissures ($> 500 \mu m$). In this classification, fissures, the largest pores, primarily serve as microfauna habitat and have a limited role in governing soil hydraulic properties. Transmission pores facilitate gravitational drainage, air movement, and root penetration, while storage pores retain plant-available water. Residual pores, which hold water that is rarely lost, function as reservoirs of nutrient ions (Greenland, 1977; Araya et al., 2022). Thus, the storage and transmission pore domains are the most critical for understanding soil water dynamics in this study.

2.3.5 Statistical analyses

Levene's test was carried out to assess the normality of data. One-way ANOVA was carried out to test the effects of treatment of different parameters. Tukey's HSD test was done as a post-hoc test to differentiate the means ($P < 0.05$). ANOVA assumptions of normality (Shapiro-Wilk test) and homogeneity of variance (Levene test) were tested for all parameters. All statistical analyses were done using R software (Team, 2021). Correlation between parameters were determined using Spearman's Rho value from the corplot package (Wei and Simko, 2024). Principal Component Analysis was done on normalized data using the prcomp function from the stats package and visualized using the factoextra package (Kassambara and Mundt, 2020).

2.4 Results

2.4.1 Bulk Soil Parameters: Bulk Density, Total Porosity and Water Retention

While there were numerical differences, none of the bulk soil parameters showed any statistically significant differences at the 95% confidence level ($P < 0.05$) based on the treatments. The mean bulk density was highest in soils subjected to a combination of fertilization and disturbance (NPKD), measuring $0.94 g cm^{-3}$, whereas the lowest bulk density was recorded in the disturbed plots ($0.87 g cm^{-3}$) (table.2.2). Although bulk density varied across treatments, these differences were not statistically significant ($P <$

0.05). Porosity did not vary much, even numerically, demonstrating that disturbances did not have an effect on the bulk representation of soil structure in these soils.

Soil water retention properties exhibited treatment-dependent variations (Table 2.2). The highest AWC was observed in NPKD ($0.31 \text{ cm}^3 \text{ cm}^{-3}$), while the lowest was in NPK-treated plots ($0.25 \text{ cm}^3 \text{ cm}^{-3}$). This trend suggests that disturbance, when combined with fertilization, may enhance water retention. However, the differences were not statistically significant at the 95% confidence interval due to the high variance within treatments. Similar trends were observed for PWC.

Air capacity, an important parameter for assessing root-zone aeration, was highest in the control plots and lowest in the disturbance-only plots varying by a factor of 1.2 even though the statistical test did not show significance at the 0.05 level. ($P < 0.2$).

Organic Matter was not different between treatments ($P < 0.05$). Soils treated with NPKD had the lowest OM content, while the control had the highest.

Table 2.2: Means with SE in parenthesis of soil physical characteristics and soil moisture retention indices.

Treatment	BD (g/cm^3)	Porosity (%)	AWC (m^3/m^3)	PWC (m^3/m^3)	AC (m^3/m^3)	OM (%)
Control	0.90 (0.09)	66.11 (0.57)	0.26 (0.02)	0.25 (0.02)	0.26 (0.02)	5.54 (0.74)
Disturbance	0.87 (0.04)	67.16 (0.26)	0.28 (0.02)	0.25 (0.02)	0.21 (0.01)	4.90 (0.67)
NPK	0.88 (0.06)	66.79 (0.41)	0.25 (0.03)	0.24 (0.03)	0.25 (0.02)	4.66 (0.35)
NPKD	0.94 (0.05)	64.45 (0.30)	0.31 (0.004)	0.30 (0.01)	0.22 (0.01)	4.28 (0.41)

2.4.2 Pore Size Distribution (PSD) and Pore Volume Proportions

A PSD (Fig.2.1) curve was derived from the dual porosity model, showing the frequency distribution of different pore sizes. A clear distinction was observed between disturbed and undisturbed plots, regardless of fertilization. Disturbed plots exhibited an earlier peak compared to non-disturbed plots, indicating that the most abundant pore sizes in disturbed soils were smaller. However, disturbance resulted in a broader pore-size distribution, whereas undisturbed plots displayed a more pronounced peak at larger pore sizes.

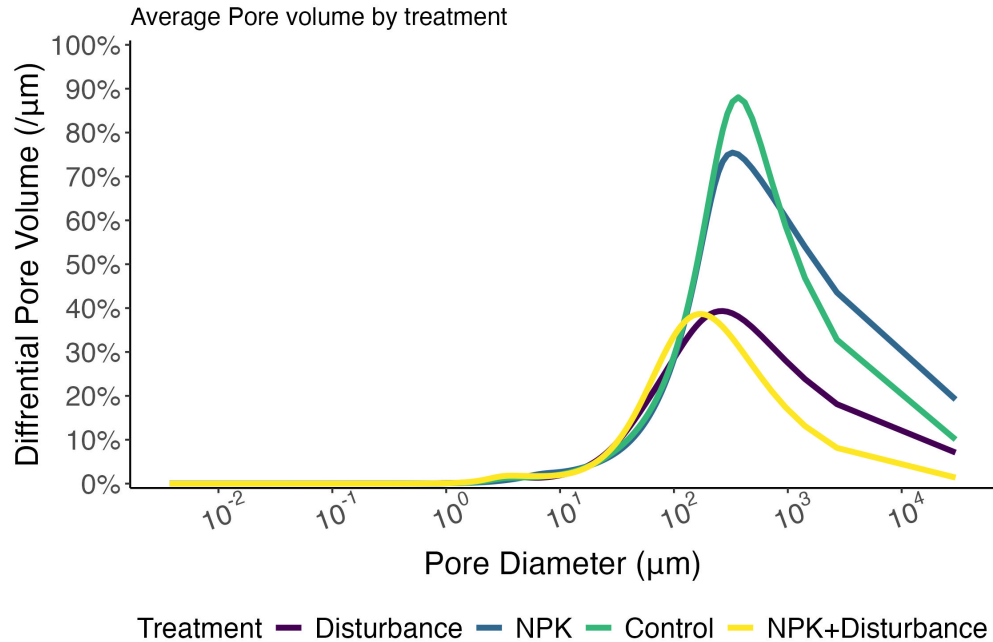


Figure 2.1: Frequency Distribution of treatment averages.

Figure 2.2 represents the proportion of porevolume under different poresize domains. Storage pores ($0.5 - 50 \mu\text{m}$), pores that store water for plants and microorganisms accounted for the largest proportion of total pore volume averaging about 55%. Treatment effects on storage pore volume was evident, with NPK-treated plots accounting for the lowest; however, it was not significantly different from control plots. In contrast, NPKD plots had the highest storage porevolume proportions, significantly differing from NPK plots, but not different from control plots.

Transmission pores ($50 - 500 \mu\text{m}$) which facilitate water movement and root growth contributed approximately 30% of the total pore volume and did not show any significant differences across treatments. However, treatment effects were significant for fissures ($> 500 \mu\text{m}$) which are crucial for root growth. Unlike storage pores, soils treated with NPKD were significantly lower than NPK plots, suggesting that disturbance alters macropore structure.

Residual pores ($0.005 - 0.5 \mu\text{m}$), which retain water tightly and serve as reservoirs for nutrient ions, were significantly affected by treatment ($P < 0.05$). Notably, NPKD plots had lower residual pore volume

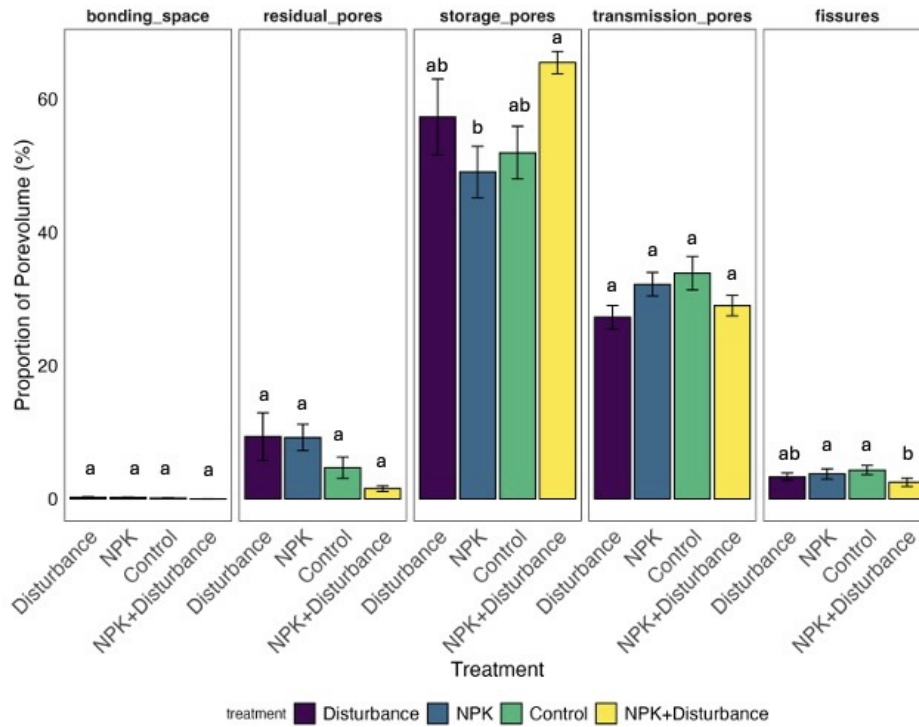


Figure 2.2: Porevolume proportion of different treatments. Bars within the same pore-size shown with the same letters are not statistically significant ($P < 0.05$)

than both disturbed and fertilized plots, suggesting a combined effect of these treatments on fine-scale pore domains. Additionally, control and fertilized plots exhibited similar behavior across all pore-size domains, except for residual pores, where significance was at ($P < 0.1$).

2.4.3 Multivariate Analysis of Soil Properties

We conducted a PCA of different soil structural and physicochemical properties to explore relationships. The biplot (Fig. 2.3) shows the loading of each variable in the first and second principal components, explaining 35.6% and 18.9% of the total variation respectively.

Dim1 which explains the largest proportion of variance mostly has soil structural properties as its variables. Residual pores, transmission pores and bonding space have a positive loading on this principal

component, whereas storage pores, AWC, and PWC have a negative loading. This suggests that soils that have better transmission properties exhibit reduced water retention, whereas those with higher water retention capacities have limited transmission properties.

Dim2 explaining 18.9% of the total variance is mostly comprised of soil physico-chemical properties. Bulk density and % clay have a negative loading, while total nitrogen, total carbon, OM, and root density have a positive loading. This suggests that soils with higher OM showed higher porosity.

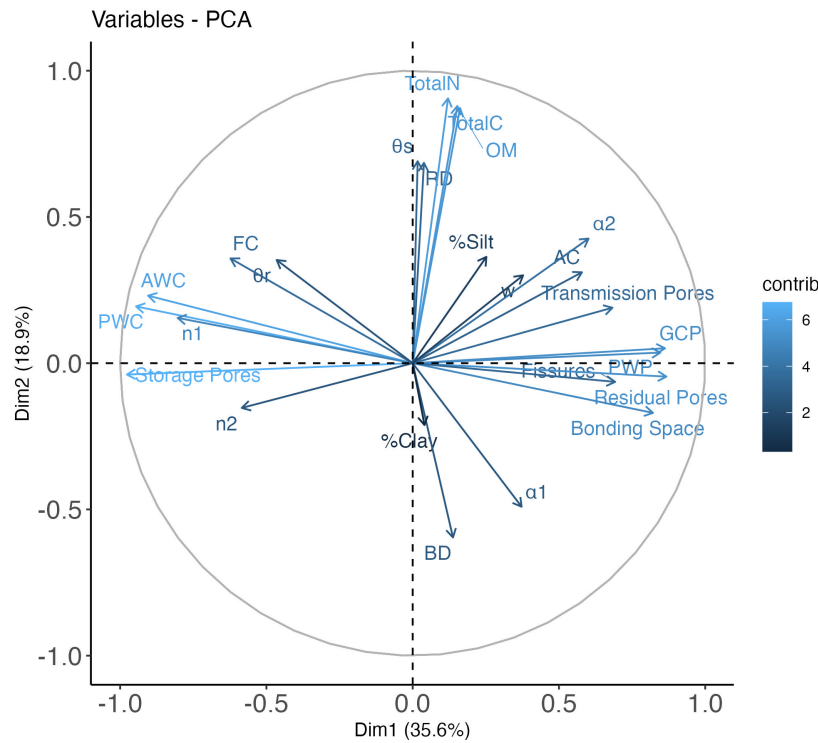


Figure 2.3: Principal Component Analysis Biplot showing various soil structural and physico-chemical properties. The X-axis represents the first principal component explaining 35.6% of the total variance, and the Y-axis represents the second principal component explaining 18.9% of the total variance.

2.5 Discussion

Most studies have investigated the individual effects of fertilization or tillage on soil hydraulic properties, yet few have tried to understand their combined effects. Our findings suggest that the interaction between

these two management practices has significant implications for soil structural properties in herbaceous-dominated ecosystems.

2.5.1 Bulk Density and Compaction

Treatments did not significantly alter bulk density. But bulk density values trend towards increasing with the combination treatment of tillage and fertilization. This could be attributed to enhanced microbial decomposition of OM due to increased substrate availability from fertilization and improved aeration from tillage. Organic matter plays a critical role in reducing bulk density by increasing aggregation (Six et al., 2002; Haynes and Naidu, 1998; Tisdall and Oades, 1980). Thus, the depletion of OM likely contributed to the observed compaction. Increased soil compaction can have long-term consequences, including reduced infiltration, lower root penetration, and diminished soil carbon storage capacity (Shah et al., 2017; Dal Ferro et al., 2014). But, the highest bulk density observed in NPKD soils of $0.9 \text{ (g cm}^{-3}\text{)}$ is 50% lower than the value that might restrict root growth (**kaufmann2010comparison**).

2.5.2 Pore Structure and Distribution

Pore size distribution was strongly influenced by treatment effects, particularly residual and storage pores. We observed an increase in storage pores, potentially enhancing soil water retention (Araya et al., 2022). However, the increase in storage pores occurred at the expense of residual and transmission pores, which are essential for nutrient exchange and gas exchange respectively. Xu et al. (2018) found that transmission pores increased with increasing OM content. We speculate that the marginal decrease ($P < 0.1$) in transmission pore proportion in both disturbed soils was due to the lower OM content in these treatments. Disturbance could be the most important factor affecting soil pore structure without the enrichment of organic matter (Głąb et al., 2013).

Interestingly, NPK fertilization alone produced the lowest proportion of storage pores, contrary to expectations that fertilization enhances aggregation and porosity. Although Naveed et al. (2014) did not directly examine storage pores, they observed an increase in pores larger than $200 \mu\text{m}$ with higher

fertilization levels. We theorize that the apparent decrease in storage pores in our study may reflect a shift toward larger pore sizes that fall outside our measurement range.

The broader PSD indicates a better distribution of pore sizes, capable of releasing water over a range of potential, and indicates a soil with a developed structure (Araya et al., 2022). Our findings that disturbed soils having a better-developed structure is opposed to the observations made by Araya et al. (2022) who found that not disturbing the soils helped develop better structure. Jabro and Stevens (2022) also found similar results, where tilled soils had a higher peak compared to undisturbed soils. Both of these studies have found results that oppose our study.

2.5.3 Water Retention and Available Water Content

Changes in pore structure influenced soil water retention. Soils with higher storage pore volumes exhibited greater water retention ($P < 0.15$), as reflected in the higher AWC and PWC observed in NPKD treatments. The PCA analysis supported this relationship, showing a positive correlation between storage pores and soil water-holding capacity. Spearman correlation (Supplementary Fig. ??) between storage pore proportion and AWC was strong ($R = 0.90$), supporting the PCA analysis. While increased water retention can be beneficial for plant-available moisture, it may also lead to reduced oxygenation, since oxygen diffusion through water is approximately 10,000 times slower than through air (Currie, 1965).

In contrast, undisturbed soils with a higher proportion of transmission pores retained less water, likely due to their greater connectivity and faster drainage capacity associated with their large diameter (50 – 500 μm). The observed trade-off between water retention and aeration suggests that land management practices must balance these opposing effects to main soil function and productivity.

2.5.4 Soil Aeration and Anoxia Potential

The marginal decline in transmission pores in disturbed soils and their strong correlation with AC ($R = 0.90$), raises concerns about soil aeration and the potential for anoxic conditions. Transmission pores facilitate gas exchange, and their reduction may restrict oxygen diffusion, particularly in wet conditions

(Greenland, 1977). This is supported by the marginal increase in AC in undisturbed soils, which suggests better aeration compared to disturbed soils. While tillage is traditionally associated with increased aeration, our results indicate that it may instead lead to a shift in pore size distribution that favors water retention over gas diffusion. These findings align with Wang et al. (2012), who reported that undisturbed soils had a higher proportion of macropores ($> 90 \mu m$), whereas tilled soils exhibited a greater abundance of storage pores. Moreover, Głab (2014) found that increasing soil compaction can reduce transmission pore volume, a trend that was observed in our study as well, albeit not significantly. The reduction in gaseous diffusion capacity could have important implications for microbial respiration and greenhouse gas emissions, particularly in grassland ecosystems where aeration dynamics influence carbon and nitrogen cycling.

2.5.5 Implications and Future Directions

Overall, our findings highlight the complex interactions between soil structure, water retention, and aeration under different land management practices. While tillage and fertilization in combination may enhance soil water retention, they also increase compaction and reduce aeration, potentially leading to anoxic conditions. These changes have significant implications for soil health, plant growth, and carbon sequestration in managed grassland ecosystems. Future research should explore the long-term effects of repeated tillage and fertilization on soil structural stability, particularly in relation to microbial activity and greenhouse gas fluxes. Additionally, strategies such as conservation tillage or organic amendments could be investigated as potential approaches to mitigate compaction and maintain a balance between water retention and aeration.

2.6 Conclusion

Our study highlights the complex interactions between tillage, fertilization, and soil structure in sandy loam soils. While fertilization alone appears to degrade soil physical properties by reducing storage pores,

the addition of disturbance helps counteract some of these negative effects, potentially increasing water retention. These findings suggest that integrated soil management strategies like reduced tillage and organic amendments could optimize soil structure by improving aggregation and water retention. Future research should focus on the long-term effects of tillage and fertilization in grasslands and the role of soil pore architecture in mediating microbial activity and greenhouse gas fluxes.

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Appendix



Figure 2.4: Picture of one of the plots from the summer of 2023 following disturbance treatment.



Figure 2.5: HYPROP setup that measures matric potential and volumetric water content simultaneously to obtain a soil moisture release curve.



Figure 2.6: Augered soil from the HYPROP soil core. An auger is used to remove a hole from the bottom of the HYPROP soil core in order to insert tensiometers.

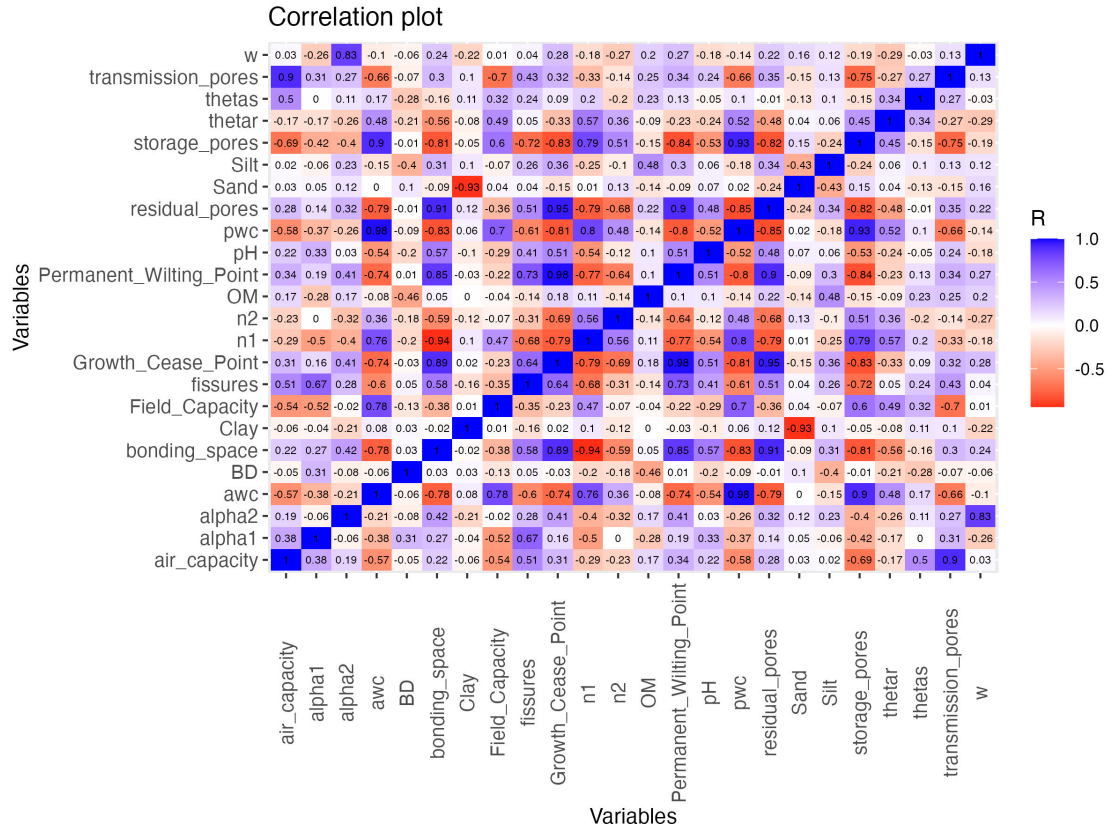
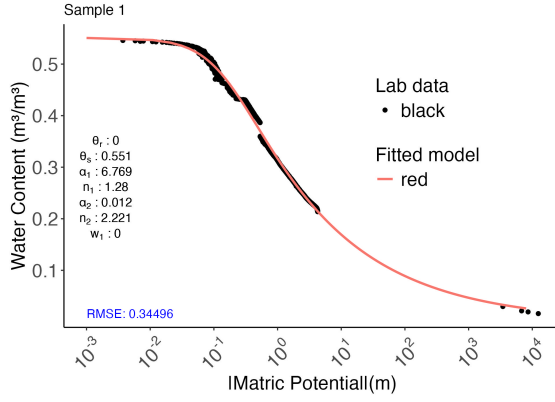


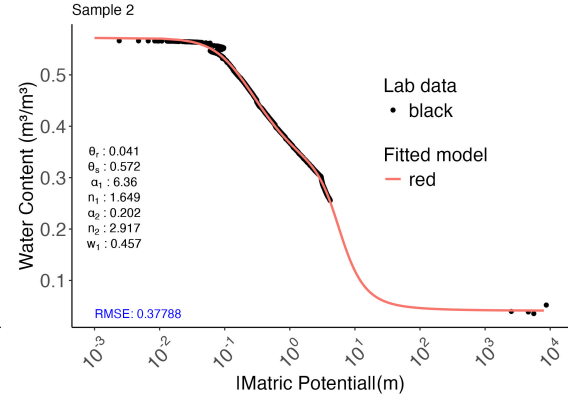
Figure 2.7: Correlation plot. Darker violet values indicate stronger positive correlations, whereas darker red values are more negative correlations.



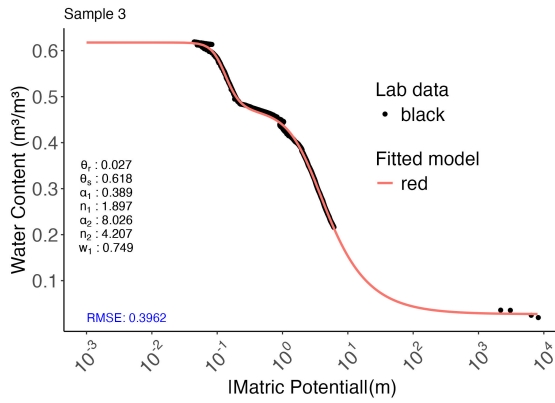
Figure 2.8: WP₄C dew point potentiometer



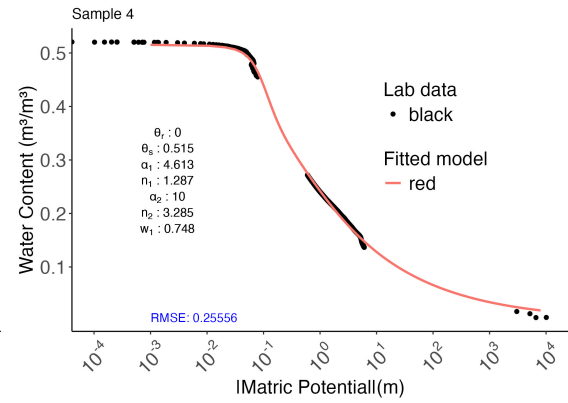
(a) Sample 1



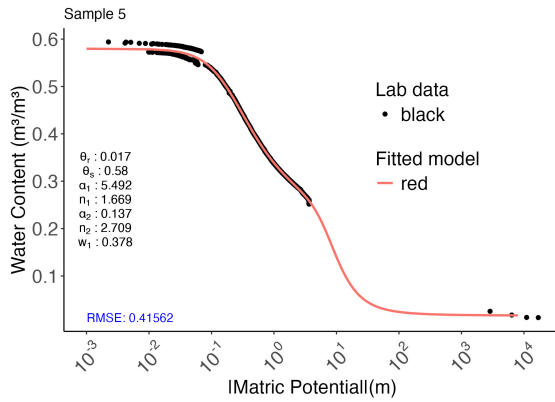
(b) Sample 2



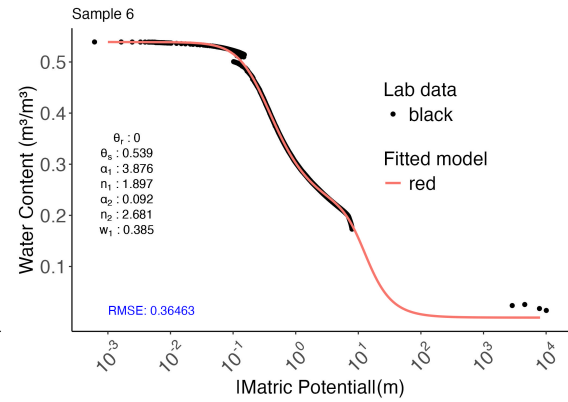
(c) Sample 3



(d) Sample 4

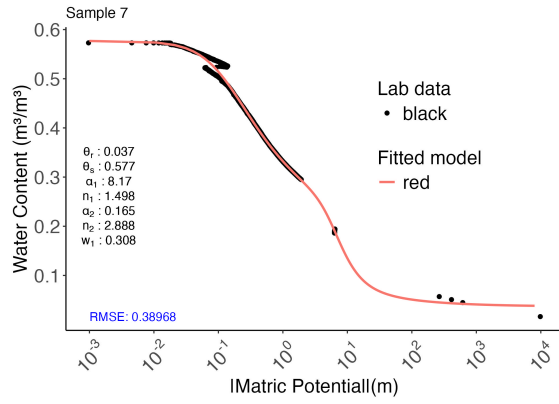


(e) Sample 5

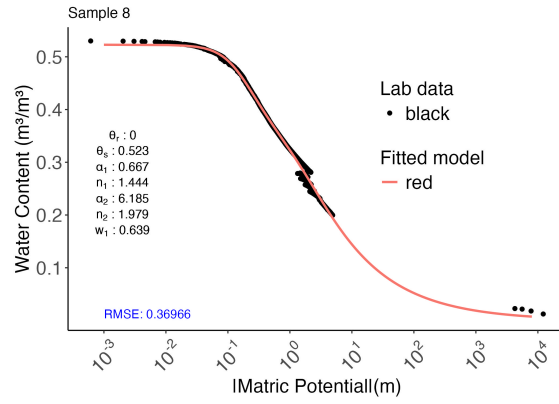


(f) Sample 6

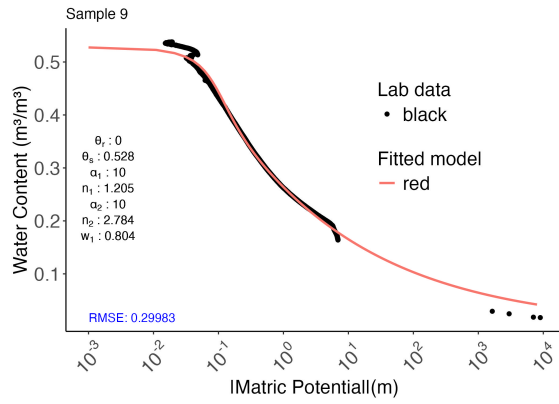
Figure 2.9: Soil moisture retention curves for plots 1 - 6



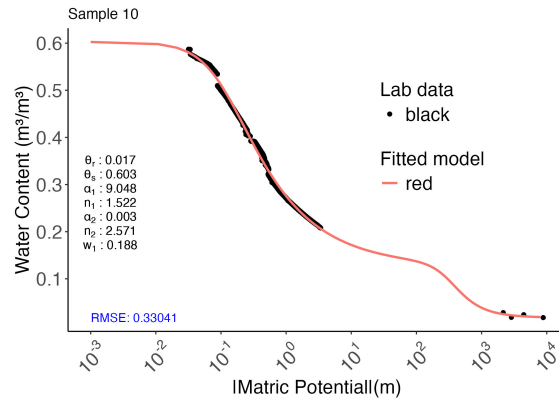
(a) Sample 7



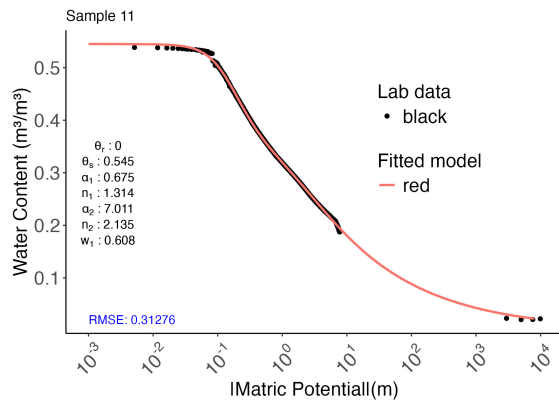
(b) Sample 8



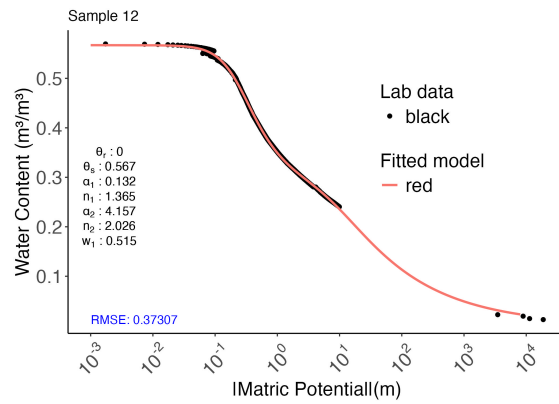
(c) Sample 9



(d) Sample 10

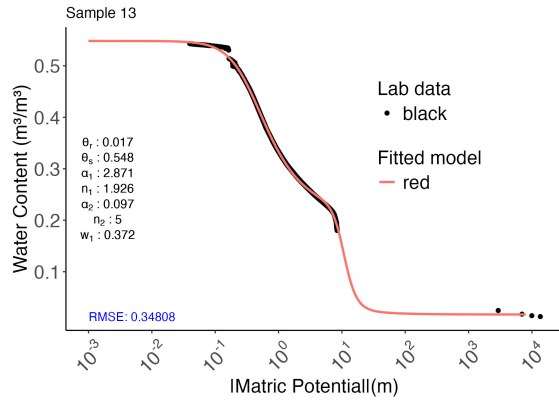


(e) Sample 11

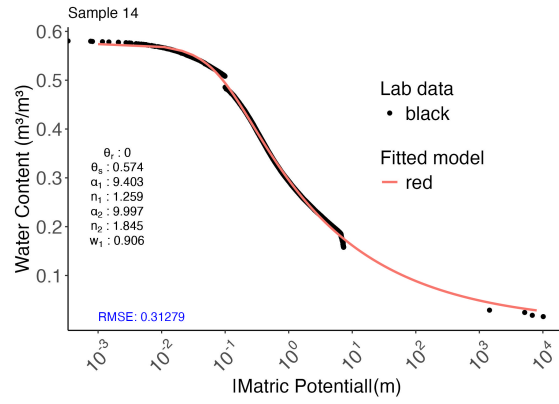


(f) Sample 12

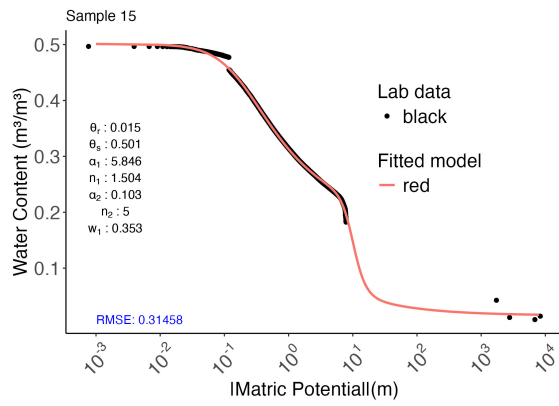
Figure 2.10: Soil moisture retention curves for plots 7 - 12



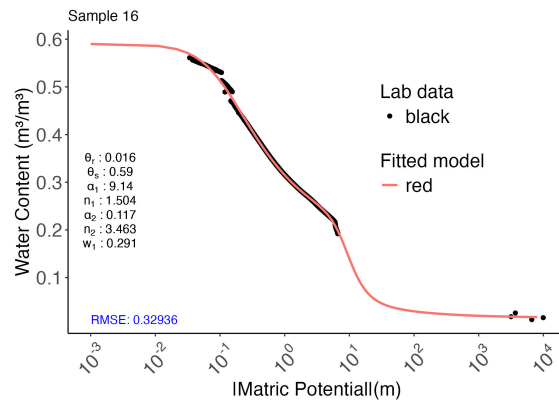
(a) Sample 13



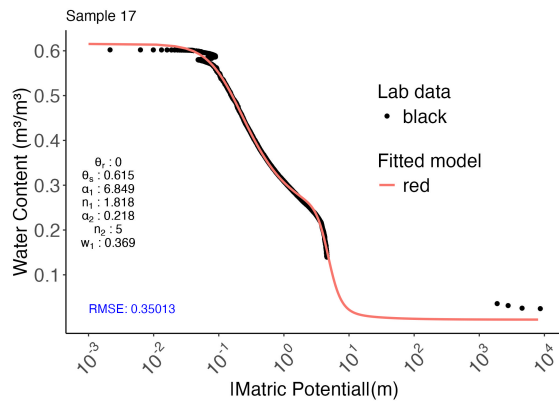
(b) Sample 14



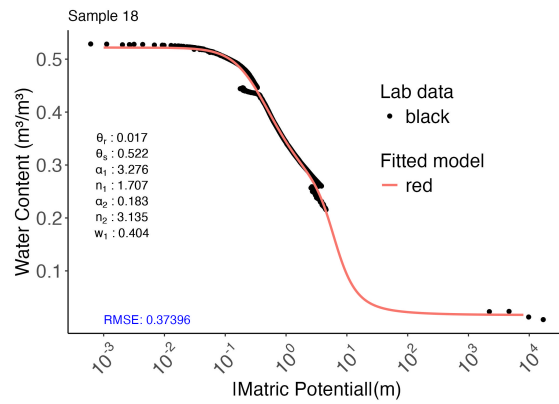
(c) Sample 15



(d) Sample 16

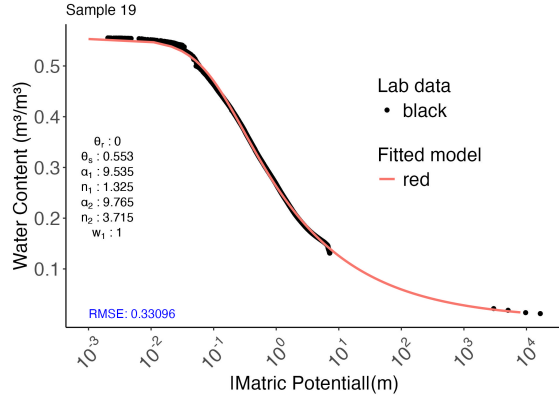


(e) Sample 17

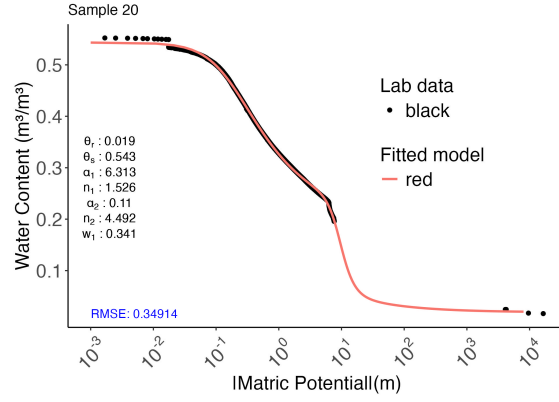


(f) Sample 18

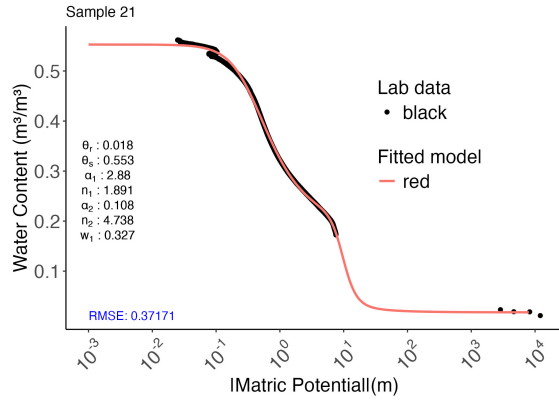
Figure 2.11: Soil moisture retention curves for plots 13 - 18



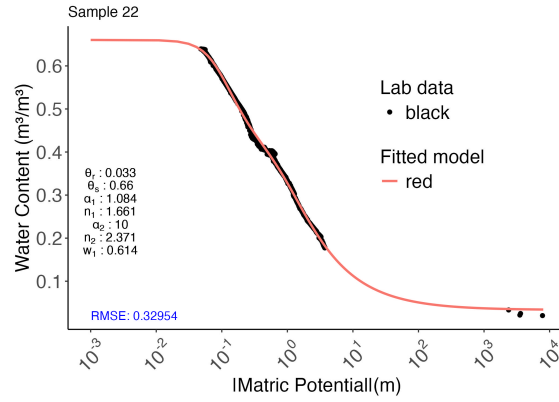
(a) Sample 19



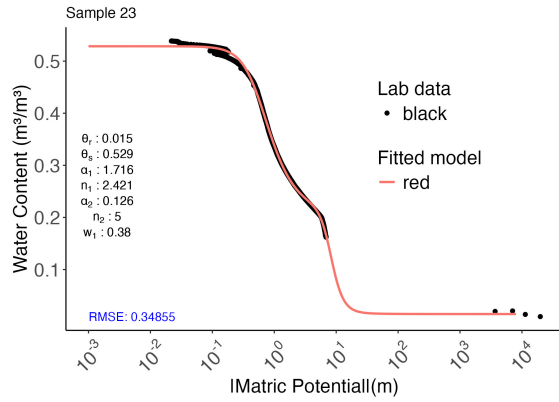
(b) Sample 20



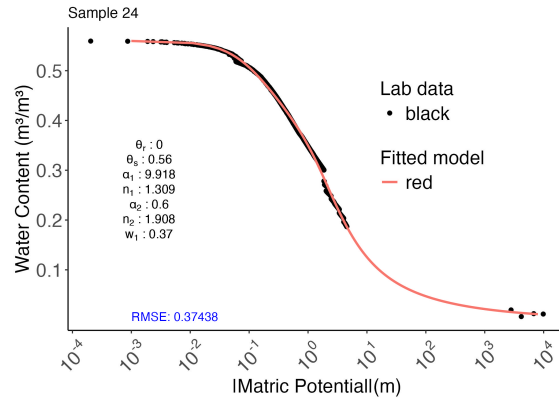
(c) Sample 21



(d) Sample 22

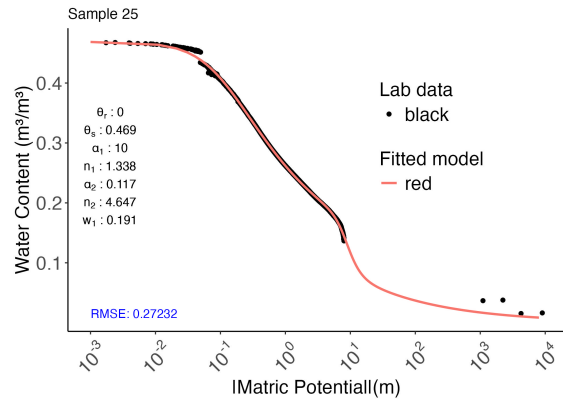


(e) Sample 23



(f) Sample 24

Figure 2.12: Soil moisture retention curves for plots 19 - 24



(a) Sample 25

Figure 2.13: Soil moisture retention curve for plot 25

CHAPTER 3

EFFECTS OF TILLAGE AND
FERTILIZATION ON SOIL RESPIRATION:
VARIATIONS ACROSS SOIL
STRUCTURES^I

^IHegde,P, N.Gaur,F.Morari,R.Abney,G.Henry. To be submitted to *Geoderma*.

3.1 Abstract

Soil respiration is one of the largest fluxes of carbon from terrestrial ecosystems to the atmosphere. It is mechanistically controlled by the movement of air within the soil and the presence of microorganisms. Both factors are directly influenced by the dynamics of moisture and temperature in the soil. Consequently, the relationship between respiration and soil moisture is important in predicting carbon dioxide emissions from soil and their subsequent effects on climate change. Moisture dynamics are governed by soil pore structure and hydraulic properties. This study aims to evaluate and quantify the relationship between soil moisture and soil respiration for variably structured soils. Soils were collected from four treatments: 1) nitrogen-phosphorus-potassium (NPK) enriched soils, 2) tilled soils, 3) their combination, and 4) a control. Soil structure was quantified through the pore size distribution, determined by fitting a Durner dual-porosity model to soil hydraulic properties measured using the evaporation and dewpoint methods. Soil respiration was measured using an automatic gas analyzer. Results indicate that mean respiration was higher in NPKD plots, likely due to the increase in proportion and water-filled pore volume (WFPV) under medium-sized pores ($1.0 - 3.0 \mu m$), that have an optimal demand and supply of oxygen promoting microbial decomposition. Multi-model inference indicates that temperature is the strongest predictor of respiration rates, though WFPV in micropores ($< 0.6 \mu m$) and medium pores also plays a key role. These findings highlight the importance of soil pore structure in regulating respiration and underscore how shifts in moisture dynamics due to climate change could alter carbon fluxes. Understanding these mechanisms is crucial for informing land management strategies aimed at mitigating soil carbon losses.

3.2 Introduction

Soils hold a massive carbon reservoir, containing nearly twice as much carbon as the atmosphere (Scharlemann et al., 2014). This vast storage capacity makes soils a crucial component of global carbon cycling and climate regulation. However, land use changes and agricultural practices have significantly altered

soil carbon stocks. Kucharik et al. (2001) estimated that U.S. soils have lost approximately 30–50% of their original soil organic carbon (SOC) since the onset of agriculture, highlighting both the vulnerability of SOC to disturbance and the potential for soils to act as carbon sinks. One of the primary pathways of SOC loss is soil respiration, a critical component of the global carbon cycle. It is the process by which carbon held in soils is released as CO_2 . It consists of autotrophic respiration, driven by plant roots, and heterotrophic respiration, driven by microbial decomposition of soil organic matter (Ryan and Law, 2005). This process is influenced by biotic factors such as plant and microbial diversity and abiotic factors like soil temperature, moisture, organic matter content, including soil physical properties like structure and texture (Davidson and Janssens, 2006; Ball, 2013). Among these, soil structure plays a fundamental role by regulating air and water movement within the soil matrix, thereby influencing microbial habitat conditions and, subsequently, respiration dynamics.

Soil structure is characterized by the arrangement of soil particles and pore spaces. Pores within the soil matrix control oxygen diffusion, water retention, and substrate availability, all of which directly influence microbial activity and respiration rates (Kravchenko and Guber, 2017). Hence, we utilized water-filled pore volume (WFPV) as a proxy for soil structure in our research. As compared to the more widely used moisture indicator, water-filled pore space (WFPS), which is a bulk parameter, WFPV is derived based on different functional pore sizes. Small pores tend to retain water under greater capillary tension, reducing oxygen availability and potentially suppressing microbial respiration (Moyano et al., 2013; Sexstone et al., 1985). In contrast, large well-connected pores facilitate oxygen diffusion, promoting aerobic microbial activity, which could lead to higher respiration rates (Araya et al., 2022; Keiluweit et al., 2017). However, if microbial oxygen demand outpaces oxygen supply, even large pores can become anoxic, slowing decomposition and potentially stabilizing soil carbon (Lacroix et al., 2021; Keiluweit et al., 2017). Hence, both small and large pores are prone to anoxic conditions and the ensuing reduction in soil respiration. While Kravchenko et al. (2015) reported that the increased presence of atmospheric-connected pores $> 13\mu\text{m}$ increased decomposition, thereby increasing CO_2 flux, Lacroix et al. (2021) set a threshold of $> 3.0\mu\text{m}$ for increased decomposition. It is clear that these size thresholds depend on different factors, and un-

derstanding the interaction between pore size distribution and microbial accessibility to organic matter (OM) is key to determining SOC stability. Resolving these interactions is essential for predicting carbon turnover, particularly in managed ecosystems where structural changes due to land management practices alter pore distribution and function.

Agricultural practices like tillage and fertilization influence soil respiration by modifying soil structure and microbial activity. Tillage disrupts soil aggregates, exposing OM to microbial decomposition, leading to an initial spike in soil respiration (Six et al., 2004; Sainju et al., 2008). Lacroix et al. (2021) found that disturbance increases pores $> 3.0 \mu\text{m}$, potentially leading to increases in soil respiration. However, the disturbance in that study involved sieving and repacking soils, which does not fully replicate field conditions where biotic and abiotic interactions influence soil structure. Understanding the effects of disturbance in grassland soils while environmental factors are involved is important for future research in respiration dynamics.

Fertilization, a common agricultural practice, is increasingly used in grasslands to enhance their productivity (Bardgett et al., 2021). It is projected that global annual N depositions will increase by a factor of 2.5 by the end of the 21st century (Lamarque et al., 2005). Hence, understanding the effects of N deposition on soil carbon dynamics is crucial for climate mitigation. Fertilization alters soil respiration through multiple pathways. While fertilization often increases plant productivity and root exudation, providing additional substrates for microbial respiration, it can also enhance microbial decomposition by alleviating N limitations (Sainju et al., 2008; Kuzyakov and Xu, 2013). However, fertilization can also promote soil aggregation and alter pore structure, physically protecting SOC from microbial decomposition and thereby reducing respiration rates (Six et al., 2004). Additionally, fertilized soils may become anaerobic more quickly than unfertilized soils, limiting microbial activity and lowering CO_2 fluxes (Chirinda et al., 2010). These mechanisms operate simultaneously, and their relative influence depends on factors such as soil type, fertilization duration, and microbial community composition, which may explain the inconsistent findings in previous studies. While our study does not directly assess these factors, we investigate how fertilization affects soil pore structure and, consequently, soil respiration.

Despite the well-established importance of soil structure in soil respiration dynamics (Bronick and Lal, 2005; Ball, 2013), the effects of tillage and fertilization-induced changes in pore structure on soil respiration remain poorly understood. Investigating these mechanisms is essential for predicting how land management influences SOC cycling. This study specifically examines how tillage and fertilization affect soil respiration dynamics and, through a statistical framework, identifies the potential role of soil structure in controlling these dynamics.

3.3 Materials and Methodology

3.3.1 Site description

The study was conducted in the Whitehall Forest (lat. 33.89 °N, long. 83.35 °W) in Athens, Georgia . The average annual temperature is 16.9 °C with an average annual precipitation of 1178 mm. The study area consisted of Loblolly pines until five years ago, which was cleared. Now the major vegetation of this area is native Piedmont grasses. The experiment is part of the DRAGNet (Disturbance and Resources Across Global Grasslands) (<https://nutnet.org/dragnet>) project involving five treatments, with five replications each in plots of size 5 x 5m. The experimental design is a Randomized Complete Block Design (RCBD). The treatments are Nitrogen-Phosphorous-Potassium (NPK), disturbance, NPK + Disturbance (NPKD), NPK Cessation, and control. NPK Cessation involves fertilization for the first five years, followed by cessation. However, since this experiment was conducted in year 4, NPK Cessation plots are considered equivalent to NPK plots for this study. The treatments were applied once every summer. NPK treatment involved fertilization using time-release urea, triple super phosphate (TSP), and potassium sulfate at the rate of 10 gm⁻³ by elemental mass. Disturbance consisted of removing all standing biomass and rhizomes followed by tilling the topsoil using a walk-behind rototiller to a depth of 15 cm. A prescribed burn was also carried out at the study site once a year.

3.3.2 In-situ field measurements

Soil respiration measurement

Soil respiration was measured using an automated soil CO₂ flux system (LI-800, LI-COR®, USA) equipped with a portable smart chamber. The system also recorded soil moisture, temperature, and electrical conductivity (EC) using an attached Stevens HydraProbe. Polyvinyl chloride (PVC) collars with an inner diameter of 20 cm were inserted into the sampling points of each plot to a depth of approximately 10 cm. To minimize disturbance effects on soil structure, the collars were installed at least 24 hours before measurements began. The area within the collar was manually cleared of any vegetation to avoid the interference of vegetation.

Measurements were conducted from the day following a rainfall event until the sixth day. A total of 26 soil respiration measurements were taken per plot, with the majority occurring within the optimal measurement window of 8 AM to 11 AM. However, to capture the drying cycle dynamics, some measurements were also conducted in the afternoon. All measurements included simultaneous soil temperature recordings, which explains the observed spikes in respiration during the afternoon.

Flux values were obtained using the Soil Flux Pro software (LI-COR, USA). The software fits an exponential curve to obtain carbon dioxide flux values. The flux values were then normalized for SOC content to take into account the variation in substrate C values that might affect flux dynamics.

Soil Abiotic Factors

Soil Water Content (SWC) and Soil Temperature were measured using a HydraProbe attached to the automated gas analyzer. Bulk density was measured on soil samples collected in the summer of 2023 using a corer of volume 202.68 cm³ (2.54 cm radius and 10 cm height). The samples were weighed and then dried in an oven at 105 °C for 48 hours. Bulk density and particle density of 2.65 g cm⁻³ were used to obtain porosity. Water-Filled Pore Space (WFPS) was calculated by dividing the SWC obtained from the probe by porosity obtained from eq.2.1.

3.3.3 Lab Measurements

Pore volume proportions

Pore volume proportions were calculated by using a cumulative pore size distribution curve, which was derived from a Soil Moisture Release Curve (SMRC) (Eq. 2.2). The thresholds for various pore-volume fractions were obtained by a previous literature review (Lacroix et al., 2021). Pore size domains included $< 0.6 \mu m$ (micro), $0.6 - 1.0 \mu m$ (small), $1.0 - 3.0 \mu m$ (medium), and $> 3.0 \mu m$ (large). The naming of the pore size domains is just for ease of reference in the study. The limits were set, and the trapezoidal integration rule was used to find the area under the curve to arrive at the proportions of pore volume within that certain pore-size interval.

Water-Filled Pore Volume proportions

Water-filled pore volume proportions of different-sized pores were obtained by subtracting WFPS from the pore-volume proportions starting from the smallest pore-size fraction to the largest since the smallest pores fill up first.

Soil Characteristics

Bulk density, total carbon, total nitrogen and OM were calculated based on the method used in section 2.3.3.

3.3.4 Statistical methods

The effects of treatments on various soil properties were tested using a one-way ANOVA. Before the ANOVA test was conducted, assumptions of residual normality, homoscedasticity, independence, and outlier were visually inspected using plots for verification.

The effect of various factors on soil respiration were tested based on multi-model inferencing. We generated a global model using all predictors of interest and arrived at different submodels using the

dredge() function from library MuMIn (Bartoń, 2024). The best model was selected based on the lowest Akaike Information Criterion corrected (AICc), which is the more suitable identification criterion for small samples (Hurvich and Tsai, 1989). All predictor variables were scaled before consideration as model factors due to their highly varied range.

3.4 Results

3.4.1 Soil properties

The principal physicochemical soil properties for the study site are listed in Table 3.1. Although this was the fourth year of treatment application, we did not find any significant differences in treatment plots.

While we did not see any significant difference in OM, there was a slight dip in OM values with increasing disturbance and fertilization.

Table 3.1: Treatment means and SE of selected physico-chemical characteristics.

Property	Control	Disturbance	NPK	NPKD
Bulk Density (g cm^{-3})	0.90 (0.09)	0.87 (0.04)	0.88 (0.06)	0.94 (0.05)
Porosity (%)	66.11 (0.57)	67.16 (0.26)	66.79 (0.41)	64.45 (0.30)
OM (%)	5.54 (0.74)	4.90 (0.67)	4.66 (0.35)	4.28 (0.41)
Total N (%)	0.136 (0.006)	0.116 (0.004)	0.103 (0.002)	0.097 (0.002)
Root density (%)	0.00138	0.00036	0.00044	0.00036
C:N	19.5 (0.21)	18.5 (0.15)	20.1 (0.15)	19.2 (0.29)

3.4.2 Measurement of CO₂ emissions

Soil respiration measurements varied from 0.00523 to 0.565 mg CO₂ / Kg-C (Figs. 3.1 and 3.2). The highest mean flux was for plots treated with NPK+Disturbance, which was significantly ($P < 0.05$) different from the other treatments (Fig. 3.2). The flux values are higher in 2023 than in 2024, most likely due to the seasonality of soil respiration.

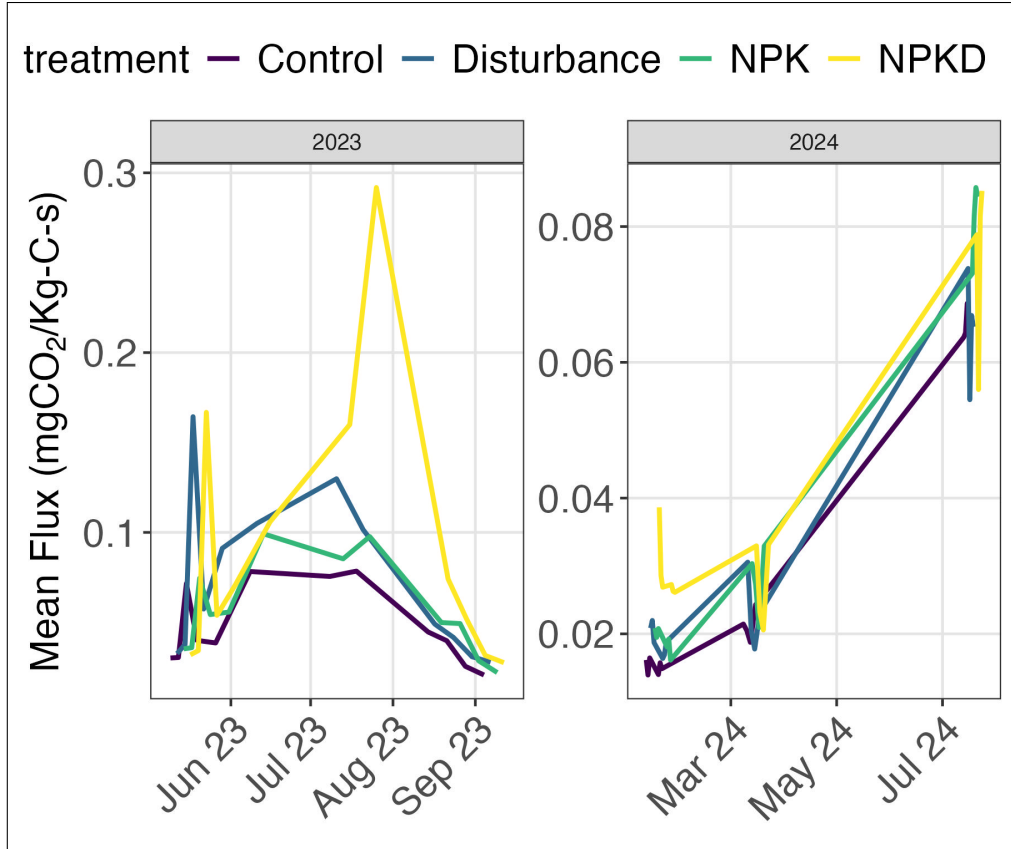


Figure 3.1: Measured soil respiration across the measurement period.

3.4.3 Soil Respiration and abiotic factors

Spearman correlation analysis indicated a moderately strong positive relationship between CO₂ emissions and soil temperature (Fig. 3.3). The Spearman correlation coefficient (R) represents the overall relationship across all treatments, whereas the R^2 values are treatment-specific. The lack of differences between treatment R^2 indicate that treatment does not have an effect on the relationship between soil temperature and respiration.

In contrast, the correlation between mean flux values and WFPS was weak, though statistically significant ($P < 0.05$). This may be attributed to WFPS being a bulk soil parameter, which does not capture the complexity of how soil moisture is distributed and retained within different pore domains.

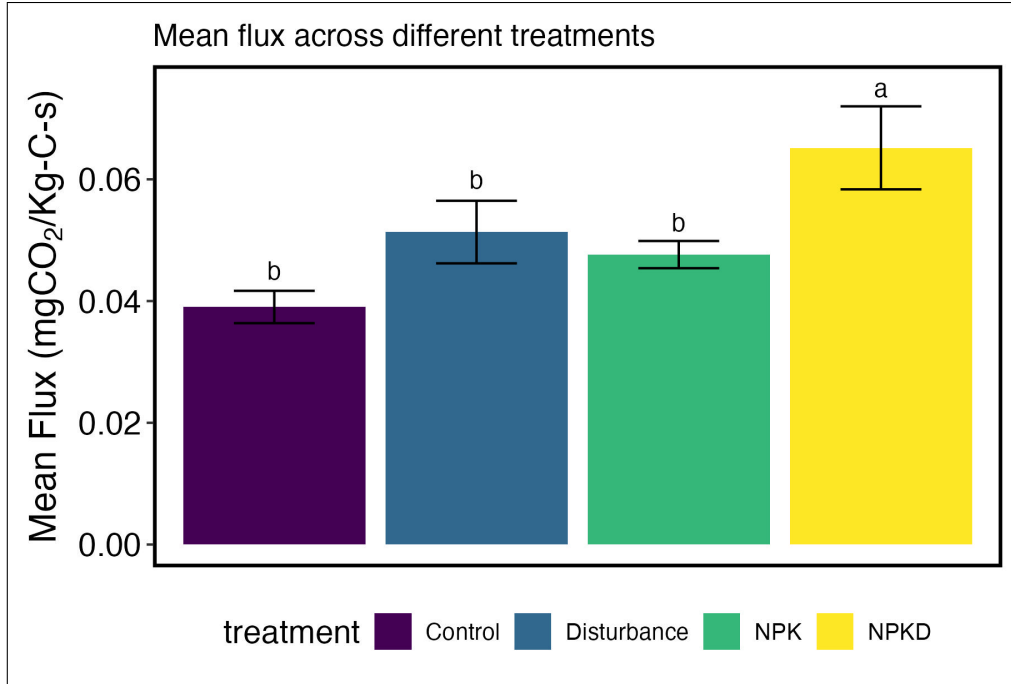


Figure 3.2: Treatment-wise mean of soil respiration.

3.4.4 Water-Filled Pore Volume (WFPV)

Pore volume under different pore-size domains was characterized using a PSD (Fig. 3.5). It was found that on average 75 % of the pore volume was occupied by pores sized $> 3.0 \mu m$. We also found that treatment affected pore volume proportions across all size domains ($P < 0.05$). NPKD increased the proportion of medium-sized pores ($1.0 - 3.0 \mu m$) and decreased the proportion of the smallest size pores measured ($< 0.6 \mu m$), while plots treated with just NPK saw a decrease in the pore volume under the medium pore size domain and an increase in the $< 0.6 \mu m$ range. In the largest pore size domain ($> 3.0 \mu m$), disturbance, irrespective of the addition of fertilization, decreased the pore volume proportion.

Water filled pore volume within the smallest pore size measured ($< 0.6 \mu m$) and the medium-sized pore domain ($1.0 - 3.0 \mu m$) was affected by the treatments ($P < 0.05$) (Fig.3.6). While NPKD increased the proportion of the water-filled pore volume under the medium-sized pore domain, it decreased in the smallest pore size domain, albeit not significantly different from the control. Interestingly, plots treated

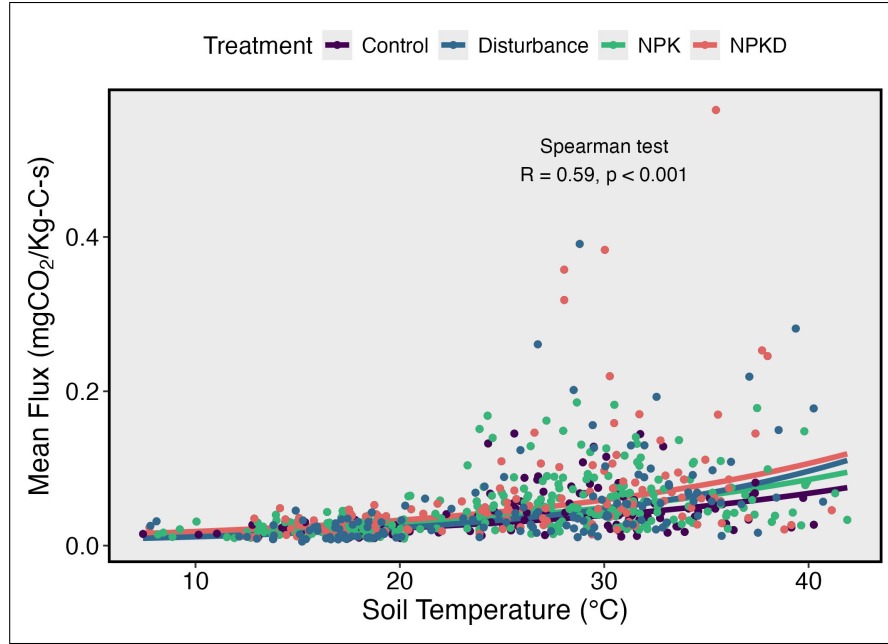


Figure 3.3: Soil respiration shown in relation to soil temperature. The different colors indicate different treatments. The Spearman test coefficients are from an overall correlation analysis.

with NPK increased the WFPV under the smallest domain, while it had a decreasing effect on the medium pore size domain. The lack of differences in the largest pore size domain was mostly due to the high variation within the treatments.

3.4.5 Multi-Model Inferencing

Based on pre-hoc hypothesis, we came up with a number of models to explain the variance in flux. The models and their respective AICc scores are provided in the supplementary materials. The best-performing model based on the lowest AICc was the model containing WFPV under the pore size domains $< 0.6 \mu m$ (Micro WFPV) and $1.0 - 3.0 \mu m$ (Medium WFPV), along with soil temperature and time and day as a random factor. The different models fitted are listed in the supplementary materials. The standardized coefficients of the best-performing model are represented in Fig. 3.7. It is evident that all factors significantly control respiration rates since their interval range does not contain zero. Since these coefficients are

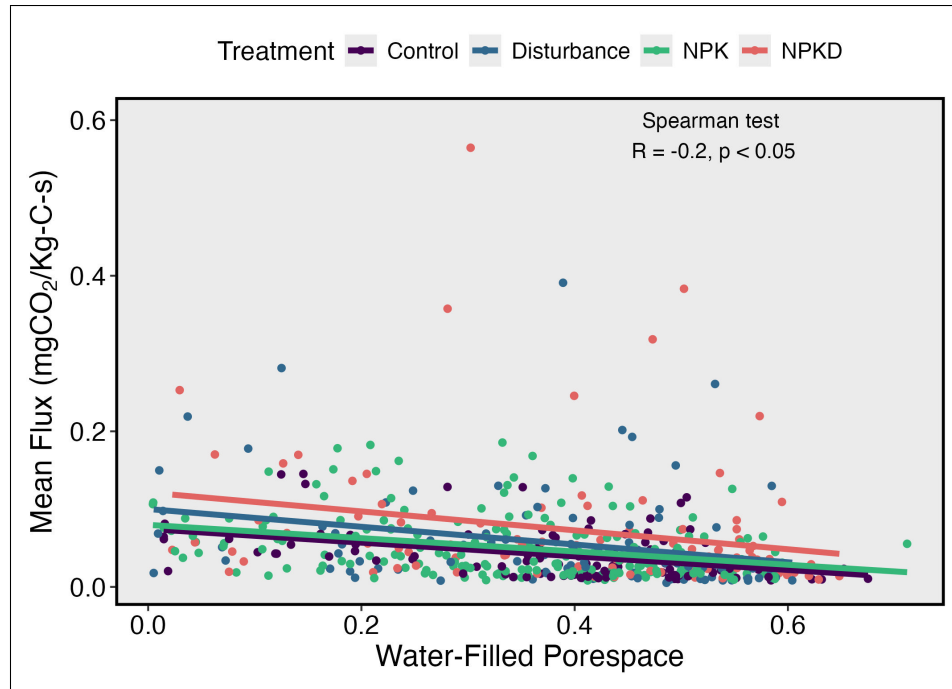


Figure 3.4: Soil respiration shown in relation to water-filled pore space.

standardized, their relative importance can be compared (Grueber et al., 2011). From the graph, it is clear that the soil temperature is the most important factor contributing to respiration rates. Both structural factors seem to be equally important.

3.5 Discussion

Our study aimed to explore the combined effects of tillage and fertilization on soil respiration, a topic that has received limited attention compared to studies examining these factors independently. The results from this study are based on a few assumptions, and thus, any inferences based on these results must consider these limitations. Water filled pore volume is determined using a PSD curve, which assumes cylindrical disconnected pores; however, real-life conditions could vary significantly. Furthermore, soil respiration measurements were made after the removal of vegetation inside the collar, but it should be

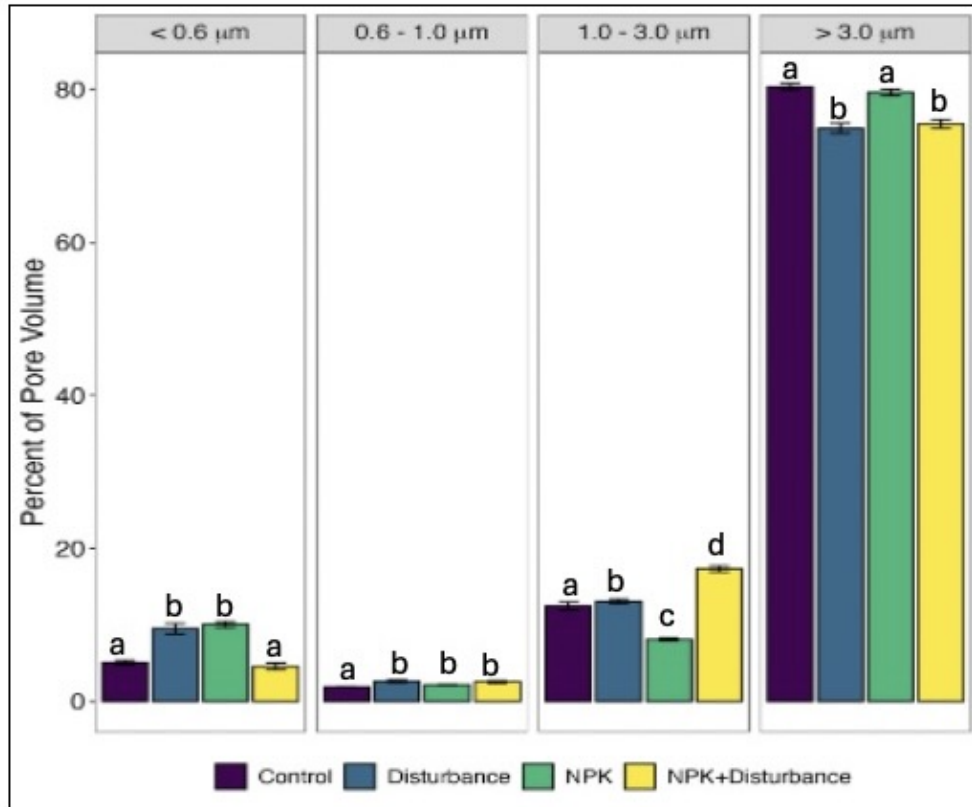


Figure 3.5: Pore Volume in response to treatments

noted that this does not completely remove the effects of root respiration, and the authors acknowledge this drawback of the sampling procedure.

Our findings suggest a synergistic effect of tillage and fertilization, particularly in the NPKD treatment, which exhibited significantly higher soil respiration. The mechanistic origins of this trend appear to be linked to changes in soil pore structure and moisture dynamics rather than conventional physicochemical changes such as bulk density or OM content.

3.5.1 Environmental Controls on Soil Respiration

Soil respiration was strongly correlated with soil temperature, a trend widely observed in terrestrial ecosystems (Lloyd and Taylor, 1994; Davidson and Janssens, 2006). The positive exponential relationship be-

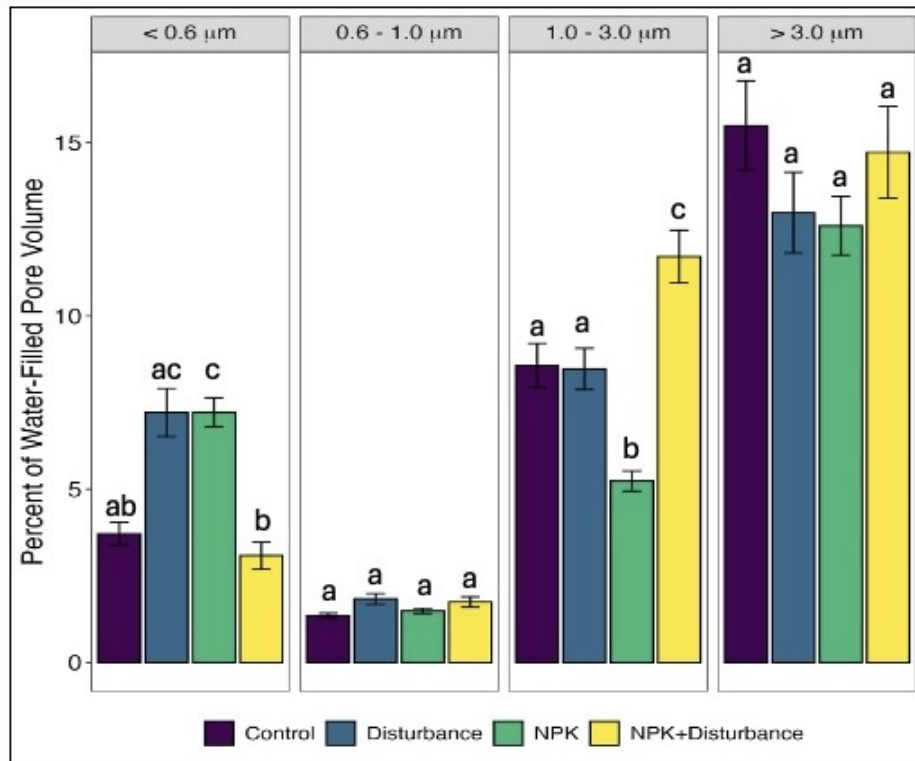


Figure 3.6: Water-Filled Pore Volume in response to treatments

tween soil temperature and respiration agree with previous results in grasslands (Luo et al., 2016). Temperature influences microbial activity by accelerating enzymatic processes, thereby enhancing OM decomposition. The strength of this relationship in our study aligns with findings from temperate ecosystems, where microbial communities exhibit temperature sensitivity in soil CO_2 flux regulation (Ray et al., 2020). Although we did not observe any difference in the relationship between soil temperature and respiration based on the treatment, there were a few extreme values of respiration at higher temperatures, which all belonged to disturbed plots, irrespective of fertilization.

Contrary to studies conducted in grasslands across regions, where soil moisture is often the primary limiting factor for respiration (Luo et al., 2016), our results did not show a significant correlation between respiration and soil moisture. This discrepancy may be due to the relatively mesic conditions of our

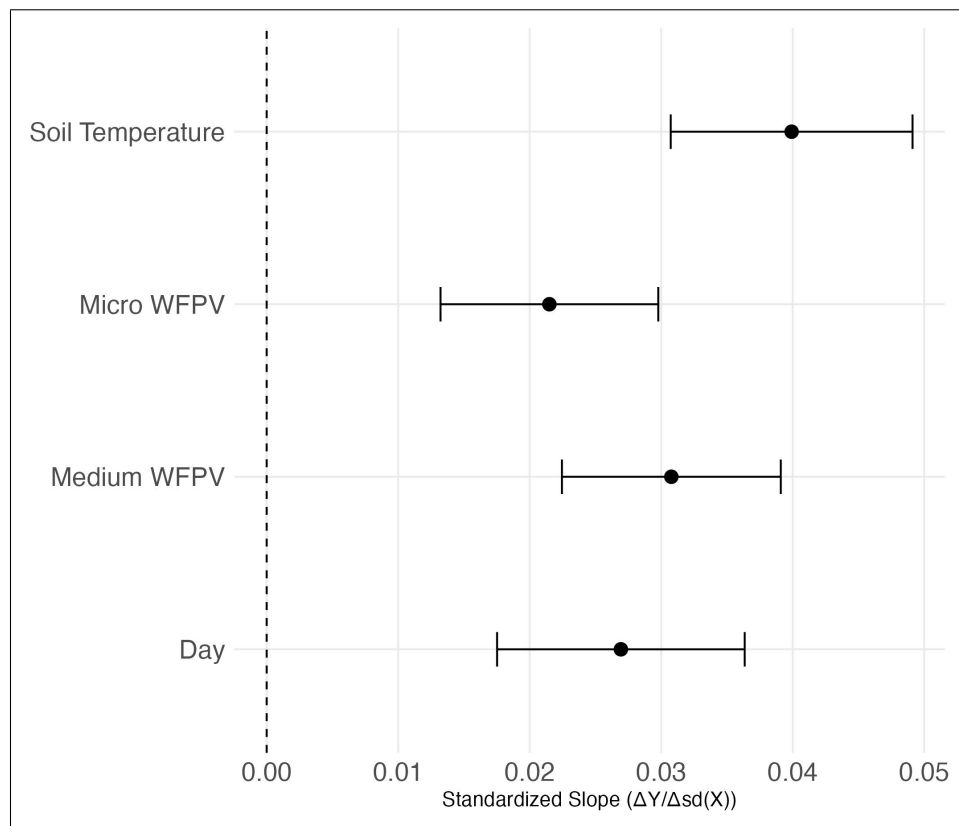


Figure 3.7: Coefficients of the best-performing model.

study site, where water availability was not a major constraint on microbial activity. Previous research suggests that in such ecosystems, respiration responses to moisture fluctuations are often secondary to temperature-driven effects, unless extreme drought conditions occur (Reichstein et al., 2003)

3.5.2 Management Effects on Soil Structure and Soil Respiration

Our study explored the combined effects of tillage and fertilization on soil respiration, an interaction that remains underrepresented in the literature. While previous studies have examined their individual effects, our results suggest a synergistic interaction where the combination of disturbance and fertilization led to the highest respiration rates. This aligns with findings that suggest fertilization enhances substrate availability while disturbance modifies soil structure, collectively stimulating microbial activity (Six et al., 2004; Sainju et al., 2008). Additionally, Luo et al., 2016 reported that soil respiration rates are more closely linked to nitrate ($\text{NO}_3\text{-N}$) concentrations in soil than to ammonium ($\text{NH}_4^+\text{-N}$). In our study, we suspect that the urea application increased ammonium concentrations in fertilized soils, but the aeration caused by additional disturbance in NPKD plots may have shifted the balance towards greater nitrification (Yuan et al., 2022), leading to increased soil respiration.

A key observation was the shift in pore structure associated with the NPKD treatment. Fertilization has been shown to increase soil aggregation (Six et al., 2004), which often enhances inter-aggregate macropores while reducing the prevalence of medium-sized pores that are crucial for microbial respiration. However, in our study, NPKD treatment increased the proportion of water-filled pores in the $1.0 - 3.0 \mu\text{m}$ range, pores considered optimal for microbial activity due to balanced oxygen diffusion and water availability (Lacroix et al., 2021).

3.5.3 Pore Structure as a Key Determinant of Respiration Dynamics

Multi-model inference identified the proportion of water-filled medium-sized pores as a significant predictor of soil respiration, supporting the hypothesis that microbial access to water and oxygen within these pore domains regulates CO_2 flux. Lacroix et al. (2021) found that while micro pores ($< 0.6 - 1.0 \mu\text{m}$)

and large pores ($> 3.0 \mu\text{m}$) tend to induce anoxic conditions and protect carbon from decomposition, medium-sized pores facilitate microbial respiration by maintaining an optimal balance of moisture and gas exchange. Our findings corroborate this by showing that increased WFPV in medium-sized pores under NPKD treatment coincided with higher respiration rates. Moreover, we found that approximately 75% of medium-sized pores ($1.0 \sim 3.0 \mu\text{m}$) remained water-filled, a condition that has been identified as optimal for microbial activity since it maintains adequate moisture and oxygen diffusion (Moyano et al., 2013).

3.5.4 Implications for Carbon Cycling

The observed increase in respiration under the NPKD treatment has significant implications for SOC dynamics. Enhanced CO_2 fluxes indicate accelerated decomposition, which may lead to reduced SOC storage over time unless offset by increased plant biomass inputs. Long-term studies have suggested that fertilization can either increase or decrease SOC stocks depending on the balance between microbial respiration and carbon inputs from plant residues (Khan et al., 2007). The results suggest that a combination of soil structural and chemical factors could be driving respiration responses.

3.6 Conclusion

Our study highlights the importance of considering pore size-specific water-filled pore volume as opposed to considering a bulk water-filled pore space for soils when evaluating soil respiration responses to management practices. While temperature remained the dominant environmental control, the role of medium-sized pores in regulating microbial activity was a key factor. The combined effects of tillage and fertilization increased respiration rates, likely due to shifts in pore structure, water availability and nitrification rates rather than changes in soil OM or bulk density. These findings emphasize the need for further research on the mechanistic links between soil structure, microbial dynamics, and carbon fluxes to inform sustainable land management strategies.

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Appendix



Figure 3.8: A picture of a disturbed plot a few days after the treatment. The picture captures soil respiration measurement using an automatic gas analyzer.



Figure 3.9: Automatic Gas Analyzer (LI-800, LI-COR®, USA), with an attached HydraProbe that measures soil water content and soil temperature.

Table 3.2: Parameters of the top 10 models obtained from Multi Model Inference.

intercept	time	treatment	z_day	z_macro_wfpv	z_medium_wfpv	z_micro_wfpv	z_small_wfpv	z_rs	df	log_lik	ai_cc	delta	weight
0.050	-	-	0.027	-	0.031	0.021	-	0.040	7	1127.467	-2240.757	0.000	0.959
0.050	-	-	0.028	0.009	0.027	0.021	-	0.043	8	1124.825	-2233.423	7.335	0.025
0.050	-	-	0.025	-	0.024	-	0.018	0.039	7	1122.691	-2231.205	9.552	0.008
0.050	-	-	0.027	-	0.031	0.022	0.000	0.040	8	1123.496	-2230.764	9.993	0.006
0.050	-	-	0.023	-	0.034	-	-	0.040	6	1119.584	-2227.036	13.721	0.001
0.050	-	-	0.027	0.010	0.020	-	0.018	0.042	8	1120.239	-2224.252	16.505	0.000
0.103	0	-	-	-	0.028	0.020	-	0.056	7	1118.830	-2223.484	17.273	0.000
0.050	-	-	0.028	0.009	0.027	0.021	0.000	0.043	9	1120.850	-2223.416	17.341	0.000
0.050	-	-	-	-	0.026	0.017	-	0.057	6	1116.580	-2221.027	19.730	0.000
0.050	-	-	0.024	0.011	0.030	-	-	0.043	7	1117.483	-2220.789	19.968	0.000

CHAPTER 4

CONCLUSION

These studies highlight the intricate interactions between soil structure and soil respiration in an herbaceous ecosystem under tillage and fertilization treatments. Soil structure is a significant factor that regulates water and gas transport, plant growth, and carbon sequestration and influences the rate of soil respiration. Understanding these dynamics can aid in creating land management plans that can mitigate the effects of rising anthropogenic CO_2 emissions while promoting soil health.

Our findings show that while disturbance and fertilization did not significantly impact bulk density, porosity, or available water content, they altered the proportion of storage pores ($0.5 - 50 \mu\text{m}$) and fissures ($> 500 \mu\text{m}$). The combination treatment of disturbance and fertilization increased storage pores, which had a strong negative correlation with transmission pores, the dominant pore size domain for water and gas diffusion. This suggests that enhanced water retention under NPKD may come at the expense of water movement. Conversely, NPK reduced storage pore proportion, likely due to changes in rooting structure induced by fertilization. Principal Component Analysis further indicated that transmission pores had a strong positive correlation with air capacity, reflecting their role in soil aeration. Additionally, we found no significant correlations between soil structural parameters and soil texture, suggesting that management-induced pore architectural changes are not dependent on inherent soil textural properties.

Soil respiration was primarily driven by soil temperature, while moisture effects were negligible, when the bulk moisture parameter water-filled pore space (WFPS) was considered. In multi-model inference,

water-filled pore volume (WFPV) in micro ($< 0.6 \mu\text{m}$) and medium ($1.0 - 3.0 \mu\text{m}$) pores were found to be an important factor in controlling respiration rates. These results suggest that land management practices affecting moisture distribution and pore structure could have cascading effects on CO_2 fluxes that could enhance climate change.

While these findings provide valuable insights, they are based on certain assumptions, and scientists using these results to make inferences must keep these limitations in mind. The hydraulic parameters fitted to the dual porosity model describe water movement at equilibrium conditions in discontinuous and cylindrical capillary pores. Additionally, variations in model fitting due to optimization parameters could introduce a degree of uncertainty.

Overall, this research highlights the need for integrated soil management approaches that balance disturbance and nutrient inputs to optimize soil structure and function. It also highlights the importance of considering moisture distribution within different pore sizes, rather than relying on bulk soil moisture measurements, when assessing respiration dynamics. Future research should investigate the long-term consequences of such management. Furthermore, a comparison between the soil respiration rates from laboratory incubations and field measurements is necessary to link controlled experimentation and natural variability. This will allow manipulations under controlled environments in a targeted manner while validating findings against field conditions, providing strength to our understanding of the mechanistic relationship between soil pores and soil respiration.