

# **The effect of fertilization and land use on soil organic matter**

Ph.D. thesis

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## TABLE OF CONTENTS

<b>Acknowledgments</b> .....	<b>iv</b>
<b>List of Tables and Figures</b> .....	<b>v</b>
<b>List of Abbreviations</b> .....	<b>vii</b>
<b>1. Research Background and Significance</b> .....	<b>1</b>
<b>2. Objectives</b> .....	<b>3</b>
<b>3. Literature Review</b> .....	<b>4</b>
3.1. Overview.....	4
3.2. Microbially mediated processes in the soils.....	4
3.3. The emerging view of SOM stability.....	6
3.3.1. Soil microbiome conditions affected the SOM cycle.....	6
3.3.2. SOM stability in a soil system.....	8
3.4. The importance of studying soil C pools in arable soil.....	10
3.5. Soil organic C pools.....	11
3.5.1. Dissolved organic matter pool.....	13
3.5.1.1. DOM significance in the soil system.....	13
3.5.1.2. DOM composition.....	14
3.5.1.3. The origin of DOM.....	15
3.5.1.4. DOM leaching.....	15
3.5.1.5. DOM patterns under agricultural practices.....	16
3.5.2. Aggregate associated organic matter pool (AAOM) pool.....	17
3.5.3. Mineral-phase-associated organic matter (MPAOM) pool.....	19
<b>4. Materials and Methods</b> .....	<b>21</b>
4.1. Study sites.....	21
4.2. Soil sampling date and preparation. ....	24
4.3. DOM measurements.....	24
4.4. Fractionations procedure for AAOM and MPAOM pools.....	24
4.5. Analytical methods for soil C pools and bulk soil.....	26
4.5.1. DOC concentration.....	26
4.5.2. Fluorescence and UV-Vis analysis of DOM.....	26
4.5.3. Measurements in CHNS elemental analyzer and FTIR.....	28
4.6. Soil water content, air temperature, and precipitation Monitoring.....	29
4.7. Statistical analysis.....	30
<b>5. Results</b> .....	<b>31</b>
5.1. Seasonal concentration and composition of DOM under cropland.....	31
5.2. Seasonal concentration and composition of DOM under grassland.....	35
5.3. DOM property variations between grassland and cropland.....	37
5.4. Correlations between DOM parameters.....	37
5.5. The effect of plant coverage, fertilization, and time on the DOM parameters.....	38
5.6. Aggregate stability across land uses.....	42
5.7. Quantity and composition of solid-phase-related soil organic matter.....	43
5.7.1. SOC concentration and SOM compositions in summer.....	43
5.7.1.1. Correlations between solid-phase related SOM variables in summer.....	48
5.7.1.2. Plants and fertilization effects on solid-phase SOM variables in summer.....	49
5.7.2. SOC concentration and SOM composition in Spring.....	53
5.7.2.1. Correlations between solid-phase related SOM variables in spring.....	54
5.7.2.2. Plants and fertilization effects on solid-phase SOM variables in spring.....	55
5.7.3. The seasonal variations of solid-phase SOM compositions.....	58
5.8. Relationships between DOM parameters and the solid-phase-related SOM pools.....	64

<b>6. Discussion</b> .....	<b>69</b>
6.1. Fertilization does not affect DOC concentration on each sampling date.....	69
6.2. DOM parameters over three years of fertilization.....	69
6.3. Land use affected DOM composition seasonally.....	70
6.4. Soil management affected DOM properties by nitrogen input.....	71
6.5. Changes in soil aggregate stability.....	73
6.6. Relationship between SOC concentration and SOM composition.....	74
6.7. Land use affects SOM stability and C storage.....	75
6.8. SOM compositions changed among land uses.....	76
6.9. SOC concentration and SOM composition differed between soil C pools.....	76
6.10. Fertilization effects on SOC concentration.....	78
6.11. Fertilization effects on SOM compositions.....	79
6.12. Plant effects on SOM variables.....	79
6.13. Correlation of SOM in the slow and fast pools with DOM parameters in August.....	80
6.14. Seasonal changes in SOM composition in the slow pool.....	81
6.15. Seasonal changes in SOM parameters in the fast pool.....	81
6.16. Relationship between DOM and solid-phase-related SOM parameters.....	83
<b>7. Conclusion</b> .....	<b>86</b>
<b>Thesis Points</b> .....	<b>87</b>
<b>Summary</b> .....	<b>88</b>
<b>Literature Cited</b> .....	<b>89</b>
<b>Supplementary</b> .....	<b>101</b>

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## LIST OF TABLES AND FIGURES

<b>TABLE 1.</b> Soil parameters in the study sites. ....	22
<b>TABLE 2.</b> Summary for soil porosity properties .....	22
<b>TABLE 3.</b> The amount (kg/ha), the time of fertilizer inputs in cropland.....	23
<b>TABLE 4.</b> Coble’s peaks range in EEM and their indication for DOM characterization.....	27
<b>TABLE 5.</b> DOC concentration and DOM composition seasonal differences.....	35
<b>TABLE 6.</b> The rotated principal component results highlighted the structure.....	39
<b>TABLE 7.</b> The principal component results highlighted the structure of the loading.....	49
<b>TABLE 8.</b> The principal component results highlighted the structure of the loading matrix...51	51
<b>TABLE 9.</b> The principal component results highlighted the structure of the loading matrix...55	55
<b>TABLE 10.</b> The principal component results highlighted the structure of the loading matrix correlation between the SOM variables within the principal components (PCs).....	57
<b>TABLE 11.</b> The SOC content and total N and C/N ratio in each soil pool.....	59
<b>TABLE 12.</b> SOM compositions in each soil pool and bulk soil over August and April sampling dates in grassland and cropland.....	59
<b>TABLE 13.</b> The principal component results highlighted the structure of the loading matrix correlation between the SOM.....	62
<b>TABLE 14.</b> The principal component results highlighted the structure of the loading matrix correlation between the SOM variables.....	62
<b>TABLE 15.</b> The principal component results highlighted the structure.....	63
<b>TABLE 16.</b> The rotated principal component results for (cropland, August sampling date). It highlighted the loading matrix correlation structure.....	65
<b>TABLE 17.</b> The rotated principal component results for (cropland, April sampling date). It highlighted the loading matrix correlation structure.....	66
<b>Figure 1.</b> Soil organic C pools production and stability in arable soil.....	13
<b>Figure 2.</b> Soil microbiome conditions in cropland versus intact soil in grassland.....	18
<b>Figure 3.</b> The study site’s location and the experimental design in Martonvásár, Hungary....	21
<b>Figure 4.</b> The dissolved organic carbon (DOC) concentration in grassland and cropland.....	31
<b>Figure 5.</b> The C/N ratio values in grassland and cropland sites under different crops.....	32
<b>Figure 6.</b> The specific absorbance (SUVA <sub>254</sub> ) values in grassland and cropland.....	33
<b>Figure 7.</b> The biological index (BIX) value in grassland and cropland.....	34
<b>Figure 8.</b> The correlation plot shows the relationship between the measured DOM.....	38

<b>Figure 9.</b> Principle component results for the measured DOM variables.....	39
<b>Figure 10.</b> Principle component results highlighted seasonality of treatments for both land uses for the measured DOM variables .....	40
<b>Figure 11.</b> The correlation plot showed the relationship between total dissolved nitrogen.....	41
<b>Figure 12.</b> The correlation plot shows the relationship between the HIX index.....	42
<b>Figure 13.</b> Aggregate stability ratio in grassland and cropland.....	42
<b>Figure 14.</b> The SOC concentration in grassland and cropland.....	44
<b>Figure 15.</b> The total nitrogen concentration in cropland and grassland.....	44
<b>Figure 16.</b> The C/N ratio in grassland and cropland sites under different treatments.....	45
<b>Figure 17.</b> The relative amount of aliphatic C components in SOM under grassland.....	46
<b>Figure 18.</b> The ratio of polysaccharides in SOM under grassland and cropland.....	47
<b>Figure 19.</b> The ratio of phenolic lignin in grassland and cropland.....	47
<b>Figure 20.</b> The ratio of C/O functional groups in grassland and cropland .....	48
<b>Figure 21.</b> The correlation plot showing the relationship between the measured.....	49
<b>Figure 22:</b> Biplot of the first two components showing the loading values in PC 1 and 2.....	50
<b>Figure 23.</b> PCA results for the measured SOM variables, including grassland.....	51
<b>Figure 24.</b> Biplot of the first two components shows the loading values in PC 1 and 2.....	52
<b>Figure 25.</b> The correlation plot showing the relationship between the measured SOM.....	54
<b>Figure 26.</b> Biplot of the first two components showing the loading values in PC 1 and 2.....	56
<b>Figure 27.</b> PCA results for the measured SOM variables, including grassland.....	56
<b>Figure 28.</b> Biplot of the first two components showing the loading values.....	58
<b>Figure 29.</b> The relationship between the measured SOM parameters for both sampling.....	61
<b>Figure 30.</b> Biplot of the first two components showing the loading values in PC.....	63
<b>Figure 31.</b> Biplot of the first two components showing the loading values in PC.....	64
<b>Figure 32.</b> The correlation plot showing the relationship between the measured solid-phase.....	65
<b>Figure 33.</b> The correlation plot showing the relationship between the measured solid-phase SOM for the slow pool (cropland, April sampling date) within each PC .....	66
<b>Figure 34.</b> The correlation plots showing the relationship between the fluorescence index....	67
<b>Figure 35.</b> The correlation plot showing the relationship between total dissolved nitrogen of DOM with total nitrogen concentration and A/C index with amide N.....	68
<b>Figure 36.</b> Seasonally, the variation in dissolved organic matter (DOC) was in line with a change in soil organic carbon (SOC) in the fast pool in cropland (secondary axis).....	84

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## LIST OF ABBREVIATIONS

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<b>Acronym</b>	<b>Notion</b>
MPAOM	Mineral-phase-associate organic matter
AAOM	Aggregate-associate organic matter
OM	Organic matter
OC	Organic carbon
POM	Particulate organic matter
SOM	Soil organic matter
SOC	Soil organic carbon
GHG	Greenhouse gas
CHNS analyzer	Carbon, hydrogen, nitrogen, and sulfur analyzer
FTIR	Fourier transform infrared (FTIR) spectroscopy
DRIFT	Diffuse reflectance infrared Fourier transform spectroscopy
TN	Total nitrogen
DOM	Dissolved organic matter
DOC	Dissolved organic carbon
TDN	Total dissolved nitrogen
EEM	Excitation-emission matrix
BIX	Biological index
HIX	Humification index
FI	Fluorescence index
SUVA	Specific UV absorbance
NPK	Nitrogen, Phosphorus, Potassium
GK	Grassland
CT	Conventional tillage
ANOVA	Analysis of variance
PERMANOVA	Permutational multivariate analysis of variance
PCA	Principal component analysis
PCs	Principal components

## 1. Research Background and Significance

Carbon (C) plays a significant role in the Earth's system. It is the constituent of all organic compounds. Furthermore, C is exchanged between the living and non-living forms in the earth's biosphere and is involved in energy flow. For example, C exchanges on the earth system include the atmosphere (e.g., CO<sub>2</sub>), hydrosphere (e.g., marine life primary production and stored C in ocean sediments), and lithosphere (e.g., C reserved in fossil fuel and plant primary production processes). Soil plays a vital role in the earth's C cycle by having a substantial C reservoir, which moderates climate. Nevertheless, land use changes, including agricultural practices, have been suggested to perturb the natural function of soil to store C (Amundson et al., 2015).

Increasing carbon dioxide (CO<sub>2</sub>) emissions concerns climate change mitigation goals. Initiatives to reduce greenhouse gas (GHG) emissions from agricultural soil, such as 4 per 1000, are aimed at increasing soil organic carbon (SOC) concentration (<https://4p1000.org/?lang=en>). These objectives are a challenge under instant population growth. Hence, it requires proper soil management to increase C storage and preserve soil organic matter (SOM) values. That is an essential approach to satisfying food demand from agricultural lands as FAO shows up to 828 million people are starving (<https://www.fao.org/newsroom/detail/un-report-global-hunger-SOFI-2022-FAO/en>).

Fertilization and crop rotations were suggested to mitigate the soil stress from cultivation practices and restore C storage and ecosystem values. Nevertheless, studies suggest SOM values are lost and concomitant with decreasing soil fertility and increasing CO<sub>2</sub> emission from agricultural soils. For example, a low SOC acquisition was expected in Eastern Europe and Mediterranean arable soils in 2050. It was recommended to imply alternative management practice (AMP), including converting cropland to grassland, reduced tillage practice, and organic matter (OM) addition (Lugato et al., 2014). Furthermore, suggestions have been drawn to imply more improvements in soil management practices to increase C storage in European soils, including Hungarian soil (Merante et al., 2017).

Recently, SOC concentration in soil C pools was reduced due to tillage (Jakab et al., 2023). Hence, conservation tillage was recommended to minimize SOM loss and runoff risks in Hungary (Madarász et al., 2021). The SOM loss in arable soil indicates less C storage, decreased substantial ecosystem services (e.g., reduction in soil fertility for plant growth), and adversely affects the climate change mitigation policy.

There is uncertainty about how C concentration and SOM compositions respond to soil management in agricultural lands. How much C is stored in cropland compared to grassland? Does soil management (e.g., fertilization) mitigate the negative consequences of tillage on C stability? These questions further related to how effective a soil management policy is in long-term experiments in arable soil.

The present work is essential to understand if fertilization can increase SOC concentration and SOM stability in cropland to be almost comparable to intact soils in grassland. Furthermore, SOC concentration and SOM composition can be subjected to local weather parameters. The role of sampling time on SOM composition is not fully understood. Hence, showing the seasonal effects on SOM stability can help to expand our knowledge. These results could also help stakeholders and policymakers decide on the best-balanced soil management practice to secure food demand by preserving SOM values and maintaining C storage in agricultural soils.

## 2. Objectives

The present work aimed to understand the effects of fertilization, crops, and seasonality on the composition and concentration of three soil C pools and bulk soil. These three soil C pools include mobile and stabilized (physically and chemically) organic C. The roles of varying effects from fertilization addition, crops, and sampling time on the SOM composition and SOC concentration in each soil C pool and bulk soil are not fully understood.

Hence, the objectives of the present work would help to understand these effects to know how to preserve SOM quality and decrease GHG emissions from arable soil. Specifically, the current work objectives are listed as follows:

1- To study whether the concentration and composition of the separate soil C pools and the bulk soil are changed seasonally. Specifically, is there a difference in the temporal dynamics of soil C pools (dissolved organic matter, fast and slow pools) and bulk soil between grasslands and arable lands?

2- To understand different long-term nutrient management effects (fertilization) on the concentration and composition of soil C pools and bulk soil in cropland. Does fertilization affect the quantity and quality of OM in soil C pools and bulk soil?

3- To study the effects of tillage on the concentration and composition of bulk soil and recalcitrant SOM pool, mobile SOM pool, and SOM within a soil stable aggregate pool. Specifically, does land use (grassland versus cropland) affect the quantity and quality of OM in soil C pools (dissolved organic matter, fast and slow pools) and bulk soil?

4- To study the effects of different crops on the concentration and composition of SOM within the stable aggregate pool, recalcitrant SOM pool, mobile SOM pool, and bulk soil. Do different plants in cropland (maize, wheat, and diculture) affect the quantity and quality of OM in soil C pools (dissolved organic matter, fast and slow pools) and bulk soil?

5- To determine the role of local weather conditions on mobile SOM pool composition and concentration in arable soil.

6- To study the correlation between parameters characterizing solid-phase-related SOM pools and DOM parameters.

### **3. Literature Review**

#### **3.1. Overview**

This chapter explained the SOM cycle as part of the C cycle in soil. For example, when plant and animal residues are returned to the soil, microorganisms gradually decompose these residues. Hence, a large biomolecule of plant and animal sources will be converted into small-decomposed products and CO<sub>2</sub>. Nevertheless, part of these residues can be protected within a soil matrix, creating a stable SOM. Furthermore, SOM can include diverse constituents due to multiple transformations in its compositions during decomposition processes. Decomposition processes are affected by multiple soil conditions. Soil conditions include soil biotic and abiotic parameters, with OM quality and quantity. A change in soil condition would likely affect the rate of C cycling within a soil system.

#### **3.2. Microbially mediated processes in the soils**

Soil is the most active porous medium comprising the uppermost layer of the earth's continental crust at the interface between the biosphere, hydrosphere, atmosphere, and lithosphere. Soils are recognized from rock by the coexistence of physicochemical and biological interactions and microbial activities. These active interactions lead to SOM accumulation, making the soil a foundation for ecosystem functioning, plant growth, and the most significant C pool on the earth's surface (Weil & Brady, 2016).

SOM comes from various plant and animal residues mixed with soil minerals. SOM includes living and dead OM, regardless of source and decomposition stage. For example, the main SOM components include living parts (e.g., soil microorganism biomass and roots-derived materials). Furthermore, fresh residues, including plant residues, decomposing organic matter, active or labile organic matter, and stable organic matter, were the non-living part of SOM (The Soil Food Web, USDA-NRCS). Hence, organic matter comprises diverse forms and origins, ranging from complex to low molecular weight to a stabilized substance. Specifically, the non-living forms of SOM include dissolved organic matter (DOM), particulate organic matter (POM), mineral-phase associated organic matter (MPAOM), and inert-like material (charcoal) will be the focus during the current chapter.

When OM is introduced into a soil system, various soil organisms break down, decompose, and change OM's physical and chemical characteristics. Decomposition is aided by soil detritivores or macroorganisms (e.g., beetles, ants, and earthworms). These

macroorganisms physically cut plant leaves and fragment soil detritus (e.g., dead organic matter) into tiny fragments. The physical degradation of OM is essential to increase OM surface for microbial activities (i.e., decomposition). Microbial activities (e.g., bacteria and fungi) chemically break down and transform OM composition in the soil. Soil microorganisms start to decompose complex organic compounds (e.g., of plant tissues) by secreting enzymes. For example, plant remains were found to be transformed and gain microbial biomass contents in their compositions, such as amino acid contents, after a series of decomposition by microbial activities (Hopkins et al., 1997).

Microbial activity's role in the SOM cycle is part of the soil food web. Soil food web refers to interactions among soil organisms and their environment and comprises energy transfer among soil organisms (White, 2005). For example, soil microbes colonize the dead OM as a substrate to gain energy during decomposition. Through decomposition, nutrients within organic chemical compounds are converted into a simple inorganic form or mineral elements (e.g., nitrogen (N) in the form of ammonia and phosphorous (P) in the form of orthophosphates). These soil nutrients are essential for plants and microbial growth. That is called a mineralization process.

Furthermore, microorganisms use the remaining C to build their biomass using soil nutrients. That is called an immobilization process. During immobilization processes, nutrients will be unavailable for plant growth until soil microorganisms die or the mineralization process is started again based on the C:N ratio. For example, an organic substrate with a C/N ratio between (1-15) can have rapid mineralization or N releasing compared to a C/N ratio of (>35), resulting in microbial immobilization or N utilization by microorganisms (Brust, 2019). Furthermore, the C:N ratio for plant residues is within a ratio of 50:1 to 100:1, while for bacteria and fungi, it is between 10:1 and 15:1. For example, if a plant residue has a C:N ratio of 134:1, immobilization will be prolonged to support microbial metabolisms (i.e., reproduction and growth) (Beare et al., 1992; Staaf & Berg, 1982).

Hence, based on the C/N ratio, microorganisms can decompose plant residues until the C:N ratio drops into a microbial biomass range. The surplus of N will be used for plant growth, or microbial activities will start again to decompose a high C:N ratio of new OM residues. That highlights the role of the soil C:N ratio in controlling the OM decomposition rate during the SOM cycle within a soil system (Jones & Parsons, 1970).

Furthermore, the soil C/N ratio significantly indicates OM quality and status. For example, a high C:N ratio of plant litter inputs (e.g., such as in waxes and lignin) can restrain microbial activities from metabolizing fresh OM or partly decomposed OM derived from plant

residues compared to a low C:N ratio (Cyle et al., 2016). That highlighted the importance of the C:N ratio as an indicator for OM quality, which can control a continuation of the OM decomposition process rate and, in turn, SOM stability and C storage in a soil system.

In parallel to the C:N ratio, decomposition is also affected by the OM compositions. For example, microorganisms prefer to decompose labile OM while leaving a recalcitrant OM, which is difficult to decompose (Staaf & Berg, 1982). Furthermore, recalcitrant OM, such as lignin-based compounds, will remain in the soil system. As microorganisms die, these remaining OM residues merge with microbial remains, creating an old, decayed, and resistant OM to decomposition. Hence, a series of OM decompositions suggested the creation of a recalcitrant OM, which comprises numerous organic compounds, including polysaccharides and humic acids (Martin & Haider, 1971). Once recalcitrant OM is incorporated into soil minerals, it creates a persistent and stable SOM. Hence, the chemical complexity of OM controls SOM stability. That is the historical view of soil C cycling.

### **3.3. The emerging view of SOM stability**

In a historical view of SOM formation, based on OM quality, microbes can mineralize OM, preferably with a labile quality or not a recalcitrant constituent. Furthermore, the humification model showed that OM inputs could be stabilized as humic complex molecules. Nevertheless, the emerging model provides a consolidated view of OM stabilization. It indicated that OM will be continuously decomposed into tiny fragments. Based on the soil microbiome conditions, these tiny parts will be chemically stabilized by interaction with fine soil minerals.

Furthermore, these fragments can also be physically secured by being a part of soil aggregate through occlusion. It is an emerging view of SOM stabilization in a soil system (Lehmann & Kleber, 2015). The following subsections outline chemical and physical SOM stabilization under soil microbiome conditions.

#### **3.3.1. Soil microbiome conditions affected the SOM cycle**

Recalcitrant organic molecules or their resistant ability to decomposition processes are affected by soil microbiome conditions, which include microbial community, soil minerals, environmental drivers, and enzyme kinetic, more than “intrinsic molecular recalcitrance” (Kleber, 2010).

Soil microbiome refers to microorganisms and their habitats. It has biotic (e.g., a microbial community) and abiotic variables (e.g., soil minerals, oxygen (O<sub>2</sub>) availability, C:N ratio, and soil moisture) that are in an equilibrium state and, in turn, C storage and SOM stability are affected. That confirmed how SOM is complex spatially within the nanometer scale (Lehmann et al., 2008). For example, each gram of soil can contain thousands of microorganisms affected by soil C:N ratio, pH, moisture, quality and quantity of OM, redox potential (indicating oxidation and reduction status in a soil system), P, and N availability (Fierer, 2017). That is the emerging view of soil C cycling.

Hence, SOM stability in the soil C cycle is less related to the chemical complexity than SOM solubility, occlusion within the aggregate, opportunity to form organo-mineral complexes, microbial community role, nutrient availability, and OM quantity and quality (e.g., as an energy substrate for microbes), moisture content, and soil redox reaction. For example, both biotic (e.g., microbial products, rhizosphere, and plant roots) and abiotic factors (e.g., soil moisture and soil aggregate stability) were found to control SOM stability (Doetterl et al., 2015; Paul, 2016; Schmidt et al., 2011).

Hence, SOM transformation was considered endless and can be continued within a soil system. For example, there was no evidence that OM decomposition formed a persistent humic substance in the soil system (Lehmann & Kleber, 2015). Furthermore, OM residues might be partitioned between soil microbial metabolites, stay undecomposed or partly decomposed, stabilize by complexation with soil minerals, or leave the soil system as CO<sub>2</sub> to the atmosphere under a soil microbiome condition.

Furthermore, the cost-benefit of microbial metabolisms is also an essential factor affecting the SOM stability in soil systems. For example, soil functional complexity increased the metabolic demand of microbial communities and their fitness or energy investment to decompose SOM (Lehmann et al., 2020). The interlocking interactions among a co-location of molecular diversity, spatial heterogeneity, and temporal variability of SOM can cause a high OC molecular diversity. It highlighted soil functional complexity's role, which suggested decreasing SOM decompositions. That is another complexity of the soil microbiome, which affects SOM decomposition and its stability in a soil system.

SOM stability can also be affected by a change in the initial state of SOM compositions (e.g., aromatic and carbohydrate contents or initial distribution of C resources). For example, microbial activities increased carbohydrate production when the soil microbiome had low carbohydrate content, using an incubation experiment for a soil-derived dissolved organic matter (DOM) (Kalbitz, Schwesig, et al., 2003). Hence, as a labile form of OM, carbohydrates

can either be favorably decomposed by microorganisms or produced as microbial byproducts. That showed the importance of microbial contribution to soil microbiome and SOM compositions. These contributions affected SOM stability (Veum et al., 2014).

### **3.3.2. SOM stability in a soil system**

SOM comprises a mixture of heterogeneous materials, ranging from fresh plant residues to microbial products, to resist SOM against decomposition. For example, lignin, cellulose, and hemicelluloses were the most abundant plant residues that stabilize soil structure. These plant residues can be stabilized in soils by interacting with soil minerals, occlusion within a soil aggregate, or selectively accumulated due to their chemical complexity (Lützow et al., 2006). The stabilization of SOM in the soil system can be summarized as the following:

First, selective preservation indicated that recalcitrated organic compounds had less degradability due to their complex composition (Lützow et al., 2006). For example, polymers of aromatic rings (e.g., lignin) and polymethylenic molecules (e.g., lipids) were resistant to degradation compared to other OM residues (e.g., cellulose) (Derenne & Largeau, 2001).

Second, SOM can be stabilized by interaction with fine soil minerals. For example, associations with fine soil minerals make a stabilized SOM persist in a soil system for a long term, over 100 to 1000 years (Lehmann et al., 2020; Totsche et al., 2018). Furthermore, there were different ways that OM can be associated with soil minerals:

A- SOM can be stabilized through a ligand exchange mechanism. Ligand exchange can stabilize organic compounds in soil minerals at a low pH (4.3-4.7) in which carboxylic acids of OM replace protonated hydroxyl groups in the mineral surface and form a stabilized complex with a fine soil mineral (Kögel-Knabner & Amelung, 2021).

B- The cation bridge mechanism is also a significant process by which SOM stability increases. For example, clay minerals had a negative charge due to isomorphous substitution, and OM tends to have a negative charge with a much higher cation exchange ability. Hence, polyvalent cations can accelerate an association between clay and OM by attracting positive charge cations within a soil system (Chapman, 1965). Such an attraction also includes organic molecules with a positive charge, which can be attracted to a negative soil clay charge. The importance of these physicochemical soil indicators for SOM stability was highlighted in Hungarian forest soils (Juhos et al., 2021).

C- SOM stability is affected by the coexistence of fine soil minerals (e.g., silt and clay) with microbial byproducts. For example, microbial activities during decomposition can

facilitate SOM associations with fine minerals. For example, surface conditioning of a soil mineral by microbial polysaccharides and protein excretion was suggested to improve the stability of organo-mineral associations between OM and soil minerals (Kögel-Knabner et al., 2008).

Cation bridges, organo-mineral associations, and microbial byproducts are essential for aggregate formation in a soil system. For example, organo-mineral association comprises a basic unit of microaggregate formation, which increases SOM stability in a soil system. Furthermore, microbial byproducts, plant root hair, and fungal debris can be entered and occluded within the microaggregate and physically protected within its structure. Such microbial byproducts (e.g., OM-derived carbohydrates) can stabilize OM fragments by increasing microaggregate formation and stability, and, in turn, OM can be occluded within these microaggregates (Verchot et al., 2011).

Third, OM occlusion in a soil aggregate can increase its stability. Besides the spatial inaccessibility, deficiency in O<sub>2</sub> within soil aggregate decreases microbial activities (Lützow et al., 2006). For example, free particulate organic matter (fPOM) can be outside soil aggregates or can be occluded in soil aggregate (oPOM) (Six et al., 2000). If POM is not occluded, POM has a shorter residence time in soil due to increasing the spatial accessibility of OM to be attached by microorganisms and, in turn, undergo decomposition (Bol et al., 2009; Mueller et al., 2009).

Furthermore, the coexistence or availability of soil cations with fine soil minerals can affect SOM stability by affecting organo-mineral associations. Soil cation exchange capacity (CEC) is a reversible chemical reaction. For example, exchangeable cations refer to the following bases (e.g., Ca<sup>+2</sup>, Mg<sup>+2</sup>, and K<sup>+</sup>), and Ca<sup>+2</sup> is found in the most common and followed by other cations (Thomas, 1982). These soil base cations are replaced or exchanged with other cations per soil mass within the soil microbiome based on base saturation (or potential acidity).

Base saturation refers to the soil system's balance between acidic cations (e.g., H<sup>+</sup> and Al<sup>+3</sup> ions) and former basic cations. By that balance, the adsorption of soil cations in a soil microbiome occurred during CEC. For example, increasing acidic cations concentration over basic cations (e.g., Ca<sup>+2</sup>) can decrease soil pH. Under acidic conditions (or soil pH less than 7), soil fertility is negatively affected by reducing soil nutrient availability and microbial activities with increasing OM decay. Nevertheless, soil with a high OM and lime (CaCO<sub>3</sub>) input and clay content can resist a change in soil pH (i.e., a high soil buffer capacity) (Goulding, 2016; Goulding et al., 1989).

The ability of soil fine minerals (e.g., clay) to secure C storage comes from having a high specific surface area (SSA). SSA refers to the ratio of surface area to volume. Based on that, a positive relationship was found between SOC concentration and a high SSA of a soil mineral (e.g., clay minerals) (Mayer, 1994). That can explain the importance of fine soil minerals in controlling the adsorption process with OM as a chemical protection for SOM to sequester C for a long time in a soil system.

Nevertheless, the amount of stabilized SOM in soil horizons can differ based on soil depth. Subsoil can be a long-term sink for SOM stabilization than organic matter in topsoil (Kaiser & Guggenberger, 2003). For example, a massive amount of OM loading reduced SSA in topsoil (0–10 cm) and upper subsoil (<30 cm) than in subsoil (e.g., > 30 cm). Hence, only a tiny mineral surface area was available for further OM sorption closer to the topsoil than the subsoil. That suggested an OM loading in the form of DOM can increase C sink in a subsoil because of a high SSA of a soil mineral besides the anaerobic condition limiting decomposition processes.

### **3.4. The importance of studying soil C pools in arable soil**

During mineralization and immobilization processes, the decay and synthesis of different SOM constituents control organic C circulation in the biosphere through plants, animals, soil, and air. That is the main component of the C cycle. Part of the C Cycle is SOM stability. SOM stability can be increased by the availability of fine soil minerals and high soil aggregate stability (or low soil disturbances).

Nevertheless, manipulating N or C in a soil microbiome can alter the C cycle by affecting SOM stability. For example, the availability or unavailability of a chemical element (e.g., N) over the other element (C) may drive either process (e.g., mineralization or immobilization) instantaneously, affecting SOM stability (Herrmann & Witter, 2008; Jastrow et al., 2007). It showed the importance of intrinsic soil properties in each field, including land use history and soil nutrients management, in affecting soil microbiome condition and C storage. For example, human activities, especially during cultivation, can dramatically affect soil nutrient regimes based on the existing soil management practice. Such an intensity of agricultural practice (e.g., tillage and removing native plant species) can cause soil perturbations.

Soil perturbation hinders the soil's ability to store C compared to its natural state (i.e., before perturbation). For example, a rate of plant material inputs and decomposition (including

metabolic activities) controls the balance of the stored C and loss (e.g., CO<sub>2</sub>) from a soil system to the atmosphere. That balance takes centuries to a few millennia to arrive at a steady state and sustain a stored organic C (Amundson et al., 2015). For example, agricultural practices might change naturally balanced soil microbiome conditions. Hence, SOM stability and C storage would also be changed within each soil C pool.

Previous works have studied SOM in different soils and ecosystems ranging from forests to moorlands. These studies suggested that converting land use from grassland to cropland decreased SOC storage within a soil system (Del Galdo et al., 2003; Minasny et al., 2011; Poeplau et al., 2011; Post & Kwon, 2000). Furthermore, the added OM can increase SOC concentration. Nevertheless, it is based on the amount and quality of OM additions (Bai et al., 2018; Reijneveld et al., 2009; Zhang et al., 2010). For example, based on detrital input and removal treatment, no effect from doubling aboveground litter input on SOM pools in forests can also highlight the role of the priming effect (Lajtha et al., 2018), which refers to the interaction between old OM and newly inputted or fresh OM. This interaction increases an old OM decomposition (Kuzyakov et al., 2000). Nevertheless, compared with regular litter inputs, SOC concentrations were increased from a doubling of added detrital residues (Juhos et al., 2021).

Contrary results were found in previous studies, often because of different soil textures and other spatial and temporal differences. Hence, various agricultural practices were recommended to be reassessed (Chenu et al., 2018) by studying each site variation's role, which could affect SOM stabilizations (Lajtha et al., 2018). That is a necessary approach, as agricultural soil management practices can vary in their effects on SOC concentration (Bai et al., 2018). Besides, incompatible results require additional research (Bolinder et al., 2020). Hence, expanding the scientific knowledge about soil C pool patterns in arable soil is essential.

### **3.5. Soil organic C pools**

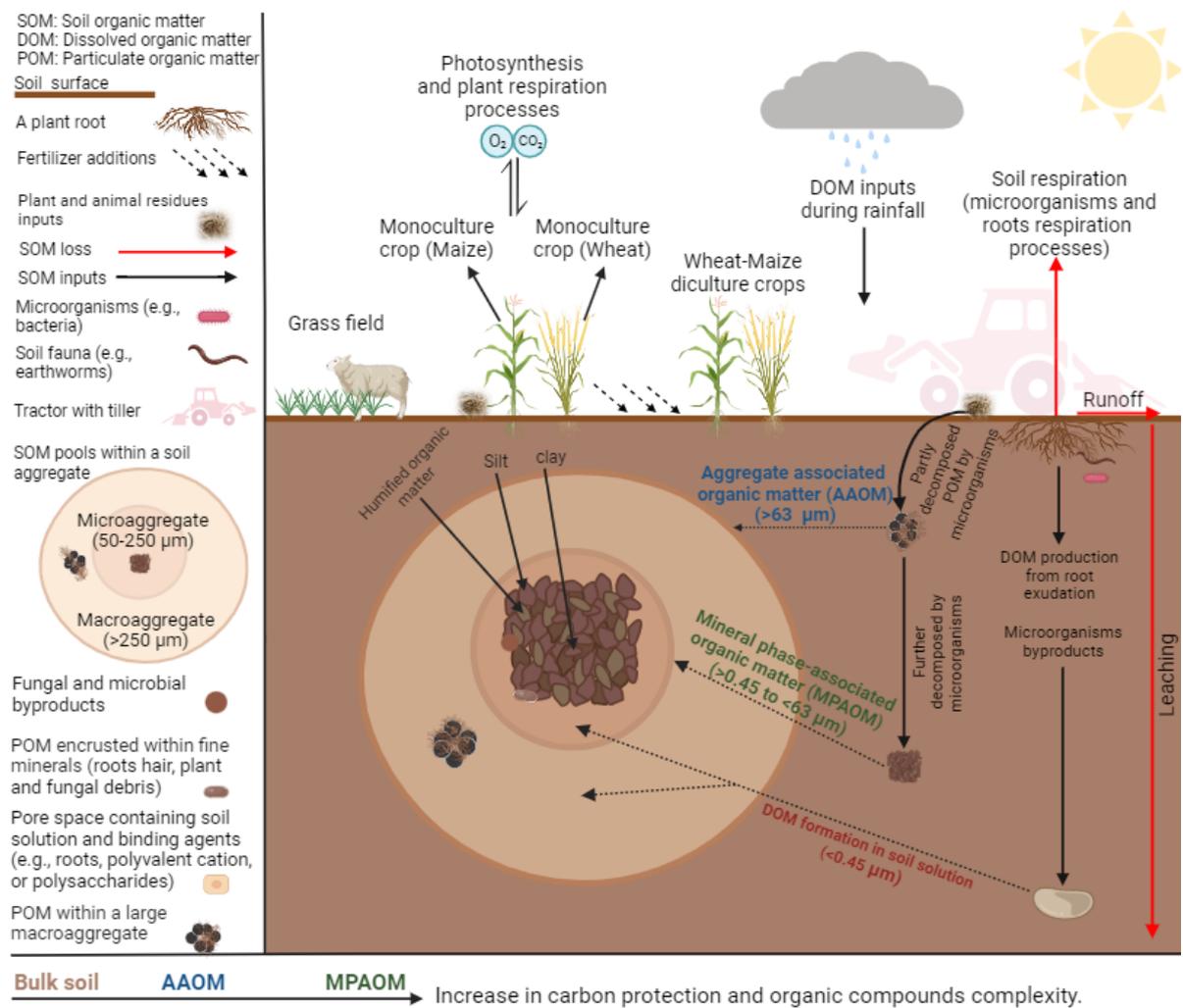
SOM contains substantial soil C pools. A considerable amount of world C was transferred from atmospheric CO<sub>2</sub> into “long-lived” soil C pools. For example, soil C pools have three times (3.3) higher C concentration than the atmosphere and four times (4.5) more stored C than the biological pool. C sequestration in soil C pools affects C sink and C stock in the earth. The C stock refers to the size of SOC for each soil profile, such as soil C pool in 2 meters of soil depth (Batjes, 1996). Globally, 2500 gigatons (Gt) of soil C are primarily

included within soil organic carbon (SOC) (1550 Gt), while the rest is in soil inorganic carbon (SIC) (Lal, 2004).

Soil C pools can be classified into five soil C pools: A- Dissolved organic matter (DOM); B- SOC in mineral-phase-associated organic matter (MPAOM); C- SOC in aggregate-associated organic matter (AAOM); D- Particulate organic matter (POM) as a labile pool; E- Chemically resistant soil organic carbon (rSOC) as a passive pool (Poeplau et al., 2013; Zimmermann et al., 2007). MPAOM and AAOM pools together comprised 75% of the total SOC, while DOM contributed 4% of the total SOC (Zimmermann et al., 2007).

The variability in the C cycling rate among soil C pools can affect soil fertility and C storage in arable Chernozem soils. Chernozem is a common soil reference group in Eurasia and North America. It is characterized by a high SSA of a soil mineral, CEC, base saturation, silty to loamy soil texture, and a medium amount of high-activity soil clay minerals (Kögel-Knabner & Amelung, 2021). Furthermore, it has high biological activities, including microbially derived biomass. Besides, it has a high bioturbation process (i.e., soil mixing by animals). Hence, the Chernozem is called a black soil earth because it is fertile and attractive for cultivation (i.e., high C storage and nutrient availability).

Furthermore, fertilization, land uses, and sampling time (or seasonality) effects on SOM composition in bulk soil, MPAOM (or slow pool), and AAOM (or fast pool) are not fully understood, especially under different land uses and soil nutrient management (fertilization) in arable Chernozem soil (Figure 1).



**Increase in SOM production:**

1. Increase in plant residue inputs; 2. Increase in organic matter (OM) and/or organic fertilizer additions.

**Increase in SOM stability:**

1. Conservation agriculture; 2. Increase in aggregate stability; 3. Increase in soil depth; 4. Increase in fine soil minerals (clay); 5. Increase in surface areas of minerals (e.g. smectite); 6. Increase in polyvalent cations.

**Figure 1.** Soil organic C pools production and stability in arable soil. In a soil holistic system, local weather and soil microclimate conditions, land use, nutrient management, soil biological and physiochemical properties, and their interactions impacted OM formation and stability in each soil C pool. The figure was created with BioRender.com.

### 3.5.1. Dissolved organic matter pool

#### 3.5.1.1. DOM significance in the soil system

DOM is a heterogeneous mixture of water-soluble compounds. It includes thousands of reactive and mobile organic molecules containing C, H, O, N, S, and P. It plays a vital role in nutrient cycling, becoming a source of nutrients for plant growth. Furthermore, it improves soil aggregate formation and serves as a food source or a substrate for microbial growth (Zsolnay, 1996).

DOM is a significant soil C pool as it is a direct function of SOM. For example, DOM is a valuable soil quality indicator in agricultural soils (Gregorich et al., 1994; Silveira, 2005). It was suggested that a positive relationship existed between an increase in dissolved organic carbon (DOC) and soil C stocks (Fröberg et al., 2013). Furthermore, DOC was suggested to indicate the SOM degradation state (Lajtha et al., 2018).

As DOM is a mobile part of SOM pools, it is present naturally and percolates within the soil system. DOM percolation is affected by its composition, location within the soil profile, and solubility. DOM comes from soluble organic molecules of root exudates and byproducts during decomposition processes (Figure 1). DOM may also refer to soil microbiome conditions. For example, soil moisture and temperature are, in some cases, linearly related to DOM production by microbial activities (Christ & David, 1996). A definite knowledge about the DOM pattern under the field condition was uncertain (Kalbitz et al., 2000). That makes DOM an ever-changing property of SOM.

A change in local weather parameters could affect decomposition activities. These decomposition activities can be changed by seasonal variations, controlling DOC production (Herbert & Bertsch, 1995; Lu et al., 2007). Such a change includes variations in DOM production by soil microbes, controlled by the kinetic of a soil microbiome condition (e.g., temperature) and affected by substrate quality (O'Donnell et al., 2016).

### **3.5.1.2. DOM composition**

Once the soil gets wet, DOM becomes reactive mobile and undergoes convection and diffusion processes in all soil compartments based on DOM composition. DOM composition can have hydrophilic constituents and hydrophobic acids. For example, hydrophobicity contributed to 50% of DOM components with humic material, especially fulvic acids, contributing to a large percentage (Herbert et al., 1995).

Hydrophobic acids were a precursor of humic substances with recalcitrant soil humin with lignin (less degradable and not water-soluble) than hydrophilic acid. Hydrophilic acid contains a higher oxidative biodegradation degree with more microbial polysaccharide origin and carbohydrate composition than hydrophobic. For example, hydrophobic acids were more aromatic than other organic materials (Dai et al., 1996). Nevertheless, hydrophobic was considered an intermediate of OM decomposition, which could undergo decomposition processes to form hydrophilic and CO<sub>2</sub> (Guggenberger et al., 1994). Hence, DOM is a dynamic

and sensitive SOM pool to soil microbiome conditions, and its production and solubility differ among SOM compositions (e.g., aromatic versus aliphatic composition).

### **3.5.1.3. The origin of DOM**

DOM was found to be controlled by microbial activities and continually decomposed and produced. Its biolabile part is considered a food source for microbial growth. Nevertheless, DOM can also be produced during decomposition processes as a byproduct. For example, the newly supplied byproduct of microbial materials from decomposition processes accelerated DOC concentration in summer times from different soils ranging from moorland and organic forest floor to sandy soils (Kaiser et al., 2001; Tipping et al., 1999; Zsolnay & Gorlitz, 1994). Such an increase in DOC concentration after heavy rainfall in summer rainstorms after a dry period on a forest floor was found (Kaiser et al., 2001). An increase in DOC production in organic horizons under favorable soil conditions, especially warming temperatures, was also detected (Dai et al., 1996; Tipping et al., 1999).

Decomposition can change DOM compositions and origin. For example, the mobilized DOM contained a degraded plant material with high synthetic microbial products (Guggenberger & Zech, 1993). Furthermore, plant materials were transformed from cellulose and hemicellulose to water-soluble microbial carbohydrate products on the forest floor. Based on soil cultivation practice, DOM can have a complex DOM composition or not, which can be used as a proxy for land use effects on SOM properties (Jakab et al., 2022). Hence, soil microbiome conditions can change DOM compositions to be more soluble or vice versa. DOM composition in arable soil can also depend on soil management, including fertilization and tillage. It can affect the DOM pattern and its leaching through soil layers.

### **3.5.1.4. DOM leaching**

DOM leaching is the primary way of SOM transportation among soil layers. As DOM is a dissolved and mobile form of SOM, DOM can be leached among soil horizons. The leaching rate can be affected by soil mineral availability through soil horizons. The role of soil minerals in DOM adsorption was stated (Tipping et al., 1999). It makes DOM have a labile SOM component and a stable part. Hence, part of DOM was stable compared to another part, which can respond physically or chemically to changes in the soil system (Leifeld & Kögel-

Knabner, 2005) agreed with (Kalbitz, Schmerwitz, et al., 2003). Hence, the presence of soil minerals in a soil horizon changed DOM adsorption, stability, and leaching.

Furthermore, the DOM leaching rate can differ based on its composition. DOM had various organic compounds (e.g., carbohydrates, aliphatic and aromatic compounds, and amino acids) in hydrophilic and hydrophobic acid fractions (section 3.5.1.2). Hydrophobic acid concentration decreases with increased soil depth because of the interaction with soil minerals. Based on that, recalcitrant compounds or stable DOM components have a higher extent of sorption than labile DOM compositions (Kalbitz et al., 2005). Hence, DOM composition and DOC concentration among soil layers were different (Dai et al., 1996; Guggenberger et al., 1994), with an increase in the amount of hydrophilic acid percolated and transported through the soil solution compared to hydrophobic components (Yano et al., 2004).

Furthermore, DOM leaching through the soil layer may be subject to temporal and local weather variability affecting soil microbiome conditions. It occurred by storm events or was affected by rainfall times (van den Berg et al., 2012). That makes soil moisture play a controlling role in the amount of DOC production and leaching in a soil system (Fröberg et al., 2013; Kalbitz et al., 2000).

Such soil microbiome conditions, including the soil cations and pH, affected the DOM leaching rate—for example, higher pH dissociated organic acids of DOM (Andersson et al., 2000). In contrast, DOM solubility and its concentration were increased with a decline in divalent and polyvalent soil cations (Guggenberger & Zech, 1993) (section 3.3). Hence, a low soil CEC and base saturation can significantly decrease DOM stability.

### **3.5.1.5. DOM patterns under agricultural practices**

Soil management practices were introduced to increase crop production. Nevertheless, DOM loss by leaching and runoff can contribute to a significant C loss from the soil system. A higher DOC concentration was lost from the agroecosystem than restored prairie grass soil (Brye et al., 2001). For example, different cultivation practices, including tillage, are performed in agroecosystems.

Tillage is a significant practice in croplands to prepare soil for planting crops. Nevertheless, tillage showed varying effects on DOC concentration and DOM compositions. For example, DOM compositions showed more decomposed properties under conventional tillage (CT) than in conservation tillage (Toosi et al., 2012). The CT can refer to a mouldboard

plowing in a deep soil depth (e.g., up to 20 cm) with two further secondary tillage operations (Francis & Knight, 1993).

In contrast, conservation tillage is a part of conservation agriculture (CA). Conservation tillage comprises preserving crop residues on a soil surface and reducing soil mechanical disturbance. It includes no-tillage (NT) and reduced till (RT) (Busari et al., 2015). Generally, NT is a more promising practice for soil resiliency of soil C patterns in cropping systems than CT (Mehra et al., 2018).

Furthermore, crops or land use may also affect the DOM pool. Previous studies did not thoroughly study the effects of crop or land use on DOM composition. One study found that DOC concentration varied among land uses using incubation experiments (Neff & Hooper, 2002). Furthermore, Embacher et al., 2007 showed no uniform pattern from crops on DOC concentration.

Nevertheless, varying effects from fertilization on DOC concentration were found. DOC concentration was weakly related to organic fertilizer and not affected by inorganic fertilizer (Zsolnay & Grolitz, 1994). Nevertheless, DOC concentration doubled in the O horizon from long-term fertilization in N-limited soil systems from a Norway spruce forest (Fröberg et al., 2013). Furthermore, biochar addition increased DOC concentration, while DOM composition was unaffected by this addition in rainfed soil (Zhang et al., 2017). Hence, previous studies focused on different soil systems with contrary findings. Furthermore, fertilization effects on DOM composition are not fully understood in arable soils.

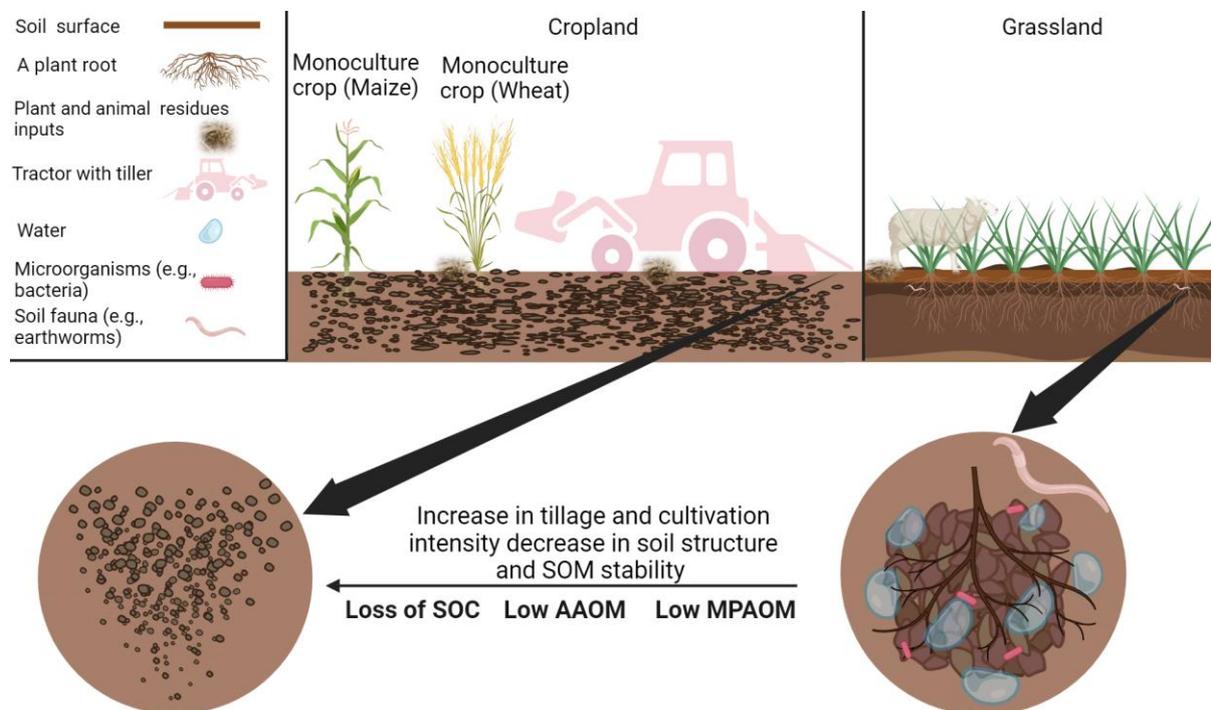
### **3.5.2. Aggregate associated organic matter pool (AAOM) pool**

The aggregate associated organic matter pool (AAOM) or the fast pool contains particulate organic matter (POM) and a labile OM fraction or active SOM. The active soil C fraction (e.g., water-soluble C) can increase soil structure stability but can also be vulnerable to changes in a soil system, such as land use change (Haynes, 2000). Furthermore, POM comes from partly degraded or undecomposed plant residues (von Lützow et al., 2007), and it can be occluded in soil aggregate (oPOM) or free (fPOM) (Christensen, 1992).

AAOM formation and composition can be affected by the fPOM status. When fPOM was outside soil aggregates (Six et al., 2000), fPOM can be entered into a soil aggregate and become stable as oPOM fraction or decay based on the soil microbiome conditions. For example, if it is not occluded inside soil aggregate, POM has a shorter residence time in soil (Bol et al., 2009; Mueller et al., 2009). AAOM can be calculated from the total aggregate C

subtracted from fPOM. Hence, the AAOM pool can be directly related to the state of labile OM (e.g., become within soil aggregate, occluded, or associated with soil minerals). Furthermore, it can help to study SOM status related to soil aggregate under cultivation, including tillage.

Tillage negatively impacted soil structure (Figure 2). Soil physical properties (e.g., bulk density referred to the dry soil weight to its volume) and C storage were negatively affected by tillage (Cookson et al., 2008; Kahlon et al., 2013; Osunbitan et al., 2005). For example, soil aggregation and arbuscular mycorrhizal richness were decreased by CT practice (Kabir, 2005). Recently, the SOC concentration of the AAOM pool was studied and found to be negatively affected by tillage (Jakab et al., 2023). That study raises a further question about the tillage effects on SOM compositions.



**Figure 2.** Soil microbiome conditions in cropland versus intact soil in grassland. The figure was created with BioRender.com. SOM: Soil organic matter; SOC: Soil organic carbon; AAOM: Aggregate associated organic matter; MPAOM: Mineral phase associated organic matter.

Furthermore, soil nutrient management was applied in arable lands to increase crop yield. Such fertilizer addition might increase SOC concentration or can be accompanied by changing SOM composition, which has not yet been fully understood. Theoretical decomposition processes can be changed based on soil microbiome conditions, and such a change in soil microbiome condition (e.g., microbial enzymes and metabolic activities) was suggested to be an effective predictor for soil aggregate stability in agricultural soils (Veum et

al., 2014). Furthermore, the seasonal effects on the AAOM pool concentration and composition are not fully understood.

### **3.5.3. Mineral-phase-associated organic matter (MPAOM) pool**

The mineral-phase-associated organic matter (MPAOM) pool or the slow pool is related to older decayed organic materials (Figure 1). It was characterized by more aromatic and microbial products than in light or labile SOM pools with undecomposed plant and animal residues (Christensen, 1992).

When fine soil minerals can provide building units for aggregate formation, the stability of OM was not the same among various mineral types. For example, soil minerals can have different SSA (Kaiser & Guggenberger, 2003). It can be related to the uniqueness of a mineral's crystalline structure affecting its surface charge, CEC, and SSA. For example, smectite clay mineral had a higher CEC than kaolinite clay mineral.

Other soil clay fractions, including oxyhydroxides (e.g., goethite), can increase SOM stability by ligand exchange (section 3.3.2.). Furthermore, OM can strongly bind into goethite by forming strong surface complexes, especially in micropore mouths. Because of that, OM can be secured against chemical reagents and microbial enzymes (Kaiser & Guggenberger, 2007). The variety and complexity of the chemical structure of goethite, including iron, oxygen, and hydroxyl, give a high SSA and functional groups in goethite. Hence, it increased the goethite capacity to adsorb molecules from a soil solution on its surface.

As OM can be adsorbed to mineral surfaces and chemically protected, OM can also be physically occluded and protected within soil aggregate. For example, OM can be free (e.g., fPOM) or protected (e.g., oPOM) against decomposition (sections 3.5.2) within a soil aggregate. Hence, the MPAOM pool can provide the bases for soil aggregate stability and C storage. That is why MPAOM can also be calculated from aggregate associated C subtracted from intra-aggregate particulate organic matter (IPOM). IPOM is described as OM within aggregate (Six et al., 2000).

As the MPAOM pool is characterized by aromatic and transformed OM, in contrast, light fraction is aliphatic and more vulnerable to a change in a soil system, land use (cropland versus grassland) and fertilization effects on SOM composition within the MPAOM pool are not fully understood. Recently, (Jakab et al., 2023) found that the SOC concentration of mineral phase-associated carbon (MPAOC) pools was negatively affected by tillage. Tillage systems also affected SOM compositions (e.g., aliphatic C and aromatic C-H) (Masoudi et al., 2023).

Nevertheless, SOM composition in the MPAOM pool was studied and found only affected by soil types, not land use, which referred to a change from native plants to cropping systems (Yeasmin et al., 2020). Furthermore, crop rotation practice was found not to affect SOC stock, with a recommendation for further work (Skadell et al., 2023).

Regarding fertilizer effect on SOC within the slow pool, only one study by (Rui et al., 2022) investigated fertilization effects on SOC concentration in Plano silt loam. The finding was that manure addition did not increase the concentration of the MAOM-C pool. Still, it studied a different ecosystem than arable soil. Hence, expanding scientific knowledge by studying MPAOM in fertile arable soil in Central Europe is essential.

Finally, resistant soil organic carbon (rSOC) is extracted from  $<63 \mu\text{m}$  MPAOM soil pool (Zimmermann et al., 2007). The rSOC is related to soil C fraction that cannot be further decomposed or resistant to decomposition and is directly related to inert organic matter (IOM) (Coleman, 1996). An example of IOM was charcoal. Furthermore, rSOC has a turnover time of 100 to 1000 years. Hence, studying the rSOC pool may not reflect SOM conditions in agroecosystems because soil management practices require a long time to affect it.

## 4. Materials and Methods

### 4.1. Study sites

The concentration and composition of soil C pools and bulk soil were studied in cropland and grassland sites in Martonvásár (47.331196 N, 18.789660 E), Hungary (Figure 3). The cropland was part of a long-term experiment established in 1958. The experiment aims to study the role of different fertilizers and manure additions on soil quality and compare them to control plots under different crops. The design consists of a two-factorial split plot, referring to crops (maize, wheat, and diculture, including the former crops) with the main plot (245 m<sup>2</sup>) and the subplot (49 m<sup>2</sup>) for fertilization, which were randomly designed (Berzsenyi et al., 2000). The Grassland site was a quasi-natural site used before as part of a garden in a residential area between 1910 and 1960. After 1960, it was only grass with no residential area, and bush grasses dominated it. There were no elevation differences between the study sites. Based on the world reference base (WRB) (WRB, 2022), the soil reference group is Chernozem.



**Figure 3.** The study site's location and the experimental design in Martonvásár, Hungary (Google Earth Pro, imagery date: 10/15/2017). It showed a randomly designed plot within cropland assigned different colors and numbers classified based on crop type and fertilization.

The study sites' soil parameters and porosity properties were shown (Tables 1 and 2). For the present work, fertilization (treatment) was applied in cropland plots as follows: Unfertilized (control), NPK (110 kg ha<sup>-1</sup> yr<sup>-1</sup>) nitrogen, (45 kg ha<sup>-1</sup> yr<sup>-1</sup>) of phosphorus, and 50 (kg ha<sup>-1</sup> yr<sup>-1</sup>) of potassium, and NPK as in the former with manure. The applied amount of

manure was 30 t/ha in October in each 4th year. Further information about manure's chemical and physical properties was provided (Table S1, supplementary). The applied fertilizers plan in cropland was shown (Al-Graiti et al., 2022) (Table 3). The average rainfall and temperature values were 539 mm and 10.6 °C, recorded between 1958 and 2018 (Mayer et al., 2019).

**Table 1.** Soil parameters in the study sites.

<i>Soil layer (cm)</i>	<i>CaCO<sub>3</sub> (% m/m)</i>	<i>pH<sub>kcl</sub></i>	<i>Clay (% V/V)</i>	<i>Silt (% V/V)</i>	<i>Sand (% V/V)</i>
0-30	1.06	7.50	31.30	51.80	16.90
30-40	3.59	7.50	29.90	53.20	16.90
40-60	29.20	7.72	27.60	46.60	25.80
60-80	29.90	7.69	24.70	43.00	32.30
<80	27.80	7.81	16.60	31.60	51.70

**Table 2.** Summary for soil porosity properties in May 2021 for the topsoil across all treatments and each land use (cropland and grassland), (average  $\pm$  SE, n=3), control referred to no fertilization. Measurement was done based on a previous method (Rowell, 2014).

<i>Topsoil Layer</i>	<i>Control</i>	<i>NPK+Manure</i>	<i>NPK</i>	<i>Grassland</i>
<i>Total porosity (v/v %)</i>	48.70 $\pm$ 2.08	47.05 $\pm$ 2.57	49.04 $\pm$ 1.63	55.57 $\pm$ 0.70
<i>Gravitational pores (v/v %)</i>	15.62 $\pm$ 3.42	9.77 $\pm$ 2.11	12.52 $\pm$ 0.70	15.80 $\pm$ 0.87
<i>Bulk density (g/cm<sup>3</sup>)</i>	1.45 $\pm$ 0.06	1.43 $\pm$ 0.03	1.43 $\pm$ 0.02	1.15 $\pm$ 0.02
<i>Adsorptional and capillary pores (v/v %)</i>	33.07 $\pm$ 1.44	37.29 $\pm$ 1.07	36.52 $\pm$ 1.89	39.77 $\pm$ 1.53
<i>Soil specific weight (g/cm<sup>3</sup>)</i>	3.00 $\pm$ 0.23	3.06 $\pm$ 0.22	2.93 $\pm$ 0.14	2.07 $\pm$ 0.04

**Table 3.** The amount (kg/ha), the time of fertilizer inputs in the cropland site, and the sampling dates on the right side when soil samples were collected. A: no fertilization, B: NPK+manure, C: NPK. NPK: Nitrogen, Phosphorus, Potassium. The given amount within the table referred to the B treatment was only for mineral fertilizer, and the amount of manure was applied in October in each 4th year ( 2016 and 2020). The applied manure amount was 30 t/ha on the "B" treatment.

<i>Application date</i>	<i>Treatment</i>	<i>Maize (monoculture)</i>			<i>Wheat (monoculture)</i>			<i>Maize-wheat (diculture)</i>			<i>Soil sampling date</i>
		<i>N</i>	<i>P<sub>2</sub>O<sub>5</sub></i>	<i>K<sub>2</sub>O</i>	<i>N</i>	<i>P<sub>2</sub>O<sub>5</sub></i>	<i>K<sub>2</sub>O</i>	<i>N</i>	<i>P<sub>2</sub>O<sub>5</sub></i>	<i>K<sub>2</sub>O</i>	
<i>10/11/2017</i>	A	-	-	-	-	-	-	-	-	-	
	B	100	45	50	50	45	50	50	45	50	
	C	112	45	70	56	45	70	56	45	70	
<i>4/3/2018</i>	A	-	-	-	-	-	-	-	-	-	<i>3/29/2018</i>
	B	-	-	-	50	-	-	50	-	-	<i>5/23/2018</i>
	C	-	-	-	56	-	-	56	-	-	<i>6/26/2018</i>
<i>9/25/2018</i>	A	-	-	-	-	-	-	-	-	-	<i>8/22/2018</i>
	B	-	-	-	50	45	45	-	-	-	
	C	-	-	-	55	45	45	-	-	-	<i>10/11/2018</i>
<i>11/30/2018</i>	A	-	-	-	-	-	-	-	-	-	
	B	100	45	50	-	-	-	100	45	50	
	C	112	45	70	-	-	-	112	45	70	
<i>3/28/2019</i>	A	-	-	-	-	-	-	-	-	-	
	B	25	-	-	25	-	-	25	-	-	<i>4/23/2019</i>
	C	25	-	-	25	-	-	25	-	-	<i>6/4/2019</i>
<i>10/11/2019</i>	A	-	-	-	-	-	-	-	-	-	<i>7/8/2019</i>
	B	100	45	50	50	45	50	100	45	50	<i>9/9/2019</i>
	C	112	45	50	56	45	50	112	45	50	<i>10/14/2019</i>
<i>3/10/2020</i>	A	-	-	-	-	-	-	-	-	-	
	B	-	-	-	50	-	-	-	-	-	<i>4/27/2020</i>
	C	-	-	-	56	-	-	-	-	-	<i>7/7/2020</i>

There was no conservation tillage practice in the study area. In the experimental area, since 1960, there has been continuous plowing rotational tillage (with a tillage depth of ~20 cm). For winter wheat, the plowing was in September, sowing was in October, and the wheat harvesting was in the summer of the following year. Plowing for maize was in November, the sowing practice was in April, and harvesting was in the fall. After harvest, wheat straw and maize stalks remained on the soil plots.

#### **4.2. Soil sampling date and preparation**

Soil samples were collected from four blocks with different fertilizers and crops (Section 4.1). The present work was aimed at plots containing maize, wheat, and diculture crops (Figure 3). Soil samples were collected from a 5–11 cm depth at the mentioned soil sampling dates (Table 3) over three years. After collecting soil samples, they were placed in plastic bags, air-dried directly after collection in the lab, and sieved (<2 mm). Apparent plant materials and debris were removed from the soil.

#### **4.3. DOM measurements**

Fertilizer additions probably limit the opportunity to use specific tools (e.g., a suction cup, section 3.5.1.6) to collect soil solution directly in the field for DOM measurements. Hence, extraction methods could be a more feasible alternative. An extraction method was used based on previous works (Jones & Willett, 2006; Shepherd et al., 2001). The method summary includes 4 g of soil suspended in 40 mL of distilled water. The solution was shaken for 2 hrs. After that, the suspensions were centrifuged at 4800 rpm for 15 min, followed by filtration with 0.45  $\mu\text{m}$  glass fiber. After that, filtrates were stored at 4 °C.

Four soil samples were collected from each block or plot. Twelve samples were used per crop and treatment. Three more replicates per soil sample were done for DOM measurements. The total sample size for all dates was 1404 (n=1404).

#### **4.4. Fractionation procedure for AAOM and MPAOM pools**

Bulk soil samples were fractionated to investigate the separate SOM pools. Only soil samples from August 2018 (or summer, 22/08/2018) and April 2019 (or spring, 23/04/2019) were fractionated into the MPAOM and AAOM pools. The soil fractionation method was based on (Zimmermann et al., 2007) and updated by (Poeplau et al., 2013). 100 ml of distilled water

was mixed with 30 grams of the bulk soil. After that, ultrasonic agitation was used to stir the mixture for 10 seconds ( $22 \text{ J ml}^{-1}$ ). Then, the mixture was shaken with a glass stick and poured into the sieve ( $63 \mu\text{m}$ ). After that, 2 liters of distilled water was poured into the sieve. This wet sieving method was aimed to separate the soil portion ( $<63 \mu\text{m}$ ) to gain the MPAOM pool from the AAOM part ( $>63\mu\text{m}$ ).

After that, the separated MPAOM pool was put in the fridge for the next day, while the AAOM pool with POM was put in the oven ( $65 \text{ }^\circ\text{C}$ ) overnight. MPAOM pool liquid was filtered using a vacuum pump, with  $0.45 \mu\text{m}$  glass fiber to separate the DOM pool from the MPAOM pool. The glass fiber filter was smoothly cleaned with distilled water in a glass watch to collect the MAPOM pool from the filter surface. After that, the collected soil was dried in the oven overnight ( $65 \text{ }^\circ\text{C}$ ). Once dried, the soil was grounded finely (powdered to  $<50 \mu\text{m}$ ) and saved in plastic bags until the instrumental analysis. This procedure was done for both sampling dates.

Furthermore, the aggregate fraction ( $>63\mu\text{m}$ ) was separated from POM to obtain the AAOM pool using density separation with distilled water ( $1.0 \text{ g cm}^{-3}$ ) (Jakab et al., 2023). For example, after drying in the oven, the AAOM pool with POM crashed. The crashed soil was weighed into three plastic flasks, and distilled water was poured into each flask, using three times the soil weight (e.g., 8 grams per flask mixed with 24 ml of distilled water). After that, they were shaken with hand and put in a centrifuge for 15 minutes (2475 rpm).

After that, the centrifuged flask was poured into  $0.45 \mu\text{m}$  glass fiber to have POM on the filter while what was inside the flask was the AAOM pool. Each of the AAOM and POM pools was dried in the oven overnight. After they were dried, POM was saved in a plastic bag directly. After that, the AAOM pool crashed, grounded finely (powdered to  $<50 \mu\text{m}$ ), and was saved in a plastic bag until the instrumental analysis. These steps were done for August 2018 data.

Similar former fractionation steps were also taken for April 2019 data, except to separate the AAOM pool from POM, sodium iodide (NaI) at density ( $1.6 \text{ g cm}^{-3}$ ) was used (Sohi et al., 2001). After that, the soil was washed with distilled water 2-3 times to remove iodide ions, and then  $\text{AgNO}_3$  was used as an indicator to ensure that the soil had no more iodide.

The present work used a different threshold density ( $1.6 \text{ g cm}^{-3}$  to  $1.0 \text{ g cm}^{-3}$ ) to gather more POM from the AAOM pool. Nevertheless, both distilled water and the NaI yielded a negligible amount of separated POM. Furthermore, according to the FTIR analysis, significant differences between these two methods were not detectable.

The total sample size for both sampling dates was 240 (n=240, or n=120 per sampling date). The cropland site had a total sample size of n=108 (n=36 for each crop (maize, wheat, diculture), within each crop, n=12 for each treatment (control, NPK+manure, and NPK), and within each treatment, n=4 for each soil pool (AAOM and MPAOM) beside bulk soil. The grassland site had a sample size of n=12 (n=4 for each soil pool (AAOM and MPAOM) and bulk soil for each soil sampling date.

Aggregate stability was also measured using the wet sieving method (Kemper & Koch, 1966). For example, during soil fractionation, the AAOM pool with POM was collected in a glass container, and the weight of the container was labeled. After drying the container with soil in the oven, the container's weight with soil was measured and subtracted from the original container weight. The results were the soil weight of AAOM with POM. After calculating the POM weight, the aggregate stability ratio was found by dividing the weight of AAOM by the weight of the soil used for the fractionation procedure (30 grams) and multiplying the value by 100. Furthermore, the sample size for aggregate stability was (n=40) for both cropland (n=36) and grassland (n=4) per each sampling date.

#### **4.5. Analytical methods for soil C pools and bulk soil**

##### **4.5.1. DOC concentration**

TOC/TN analyzer (Shimadzu, Kyoto, Japan) was used to determine WEOC or DOC and total TDN concentrations.

##### **4.5.2. Fluorescence and UV-Vis analysis of DOM**

Fluorescence spectra were measured using an RF-6000 spectrofluorophotometer (Shimadzu, Kyoto, Japan). The fluorescence phenomenon can be explained by when a molecule is excited (due to energy absorption) to a high energy level; it returns to its original ground state. During that, energy will be lost as light or fluorescence. Hence, an occurrence of fluorescence corresponds to wavelengths of excitation and emission, which can be used to describe a molecular structure (Fellman et al., 2010).

When a DOM pool consists of thousands of molecules, which indicates its chemical complexity, a comprehensive information range can be used from the excitation-emission matrix (EEM) to estimate DOM composition.

In the present work, the eemR package in R (Massicotte, 2019) was used to analyze the fluorescence EEM spectroscopy data to estimate DOM composition using the following measurement parameters: Range Ex (200-450 nm), range Em (200-550 nm), and resolution (5nm). The eemR statistical approach decomposed a complex DOM fluorescence signal in EEM into a quantitative range of wavelengths. These distinct wavelength ranges can be used further to define indices or proxies to characterize or estimate DOM composition. For example, vast amounts of information contained within EEM can be utilized in indices using ratios of fluorescence intensity in different EEM regions.

Metrics of specific wavelengths in EEM can represent a meaningful fluorescent index. For example, five information extracted from EEM were identified (Coble, 1996). These fluorescent components or coble's peaks and their indication are listed (Table 4). The fluorescence properties of OM can also be highlighted using the coble peaks ratio of A/C and T/A. Peak A indicated terrestrial fulvic-like substances; peak C referred to terrestrial humic-like substances; and peak T indicated microbially protein-like substances (Coble, 1996).

**Table 4.** Coble's peaks range in EEM and their indication for DOM characterization. Coble's peak heights were corrected with the extracted DOC concentration. \* Referred to the presence of Tyrosine and Tryptophan's (amino acid or protein derivatives) contribution to SOM. These calculated ratio indices were recently used in a similar study area (Jakab et al., 2022) based on (Baker et al., 2008).

<i>Coble's peak height</i>	<i>Ex (nm)</i>	<i>Em (nm)</i>	<i>Indices or Indicators for</i>
<i>High A</i>	260	380-460	Increasing of humus-derived organic matter
<i>High B</i>	275	310	Indicating a recent microorganism's contributions*
<i>High C</i>	350	420-480	Increasing of humus-derived organic matter
<i>High M</i>	312	380-420	Indicating marine humic-like indices
<i>High T</i>	275	340	Indicating a recent microorganism's contributions*
<i>High T/A</i>	Calculated ratio		A low complex molecular structure
<i>High A/C</i>	Calculated ratio		An increase in complex molecular structure
<i>High (A+C)/(B+T)</i>	Calculated ratio		Indicating a total highly complex molecular structure

Furthermore, the intensity of fluorescence ratios/or indices was used for proportion estimation of humified and microbial sources of DOM (Birdwell & Engel, 2010). For example, These ratios were used to estimate humification and biological indices from EEM using the fluorescence ratio at a fixed excitation (Massicotte, 2019).

Three types of ratios were used. First, the biological index (BIX) refers to the presence of fluorophore peptides and amino acids (Huguet et al., 2009). Second, the fluorescence index

(FI) can help determine the degree of DOM transformation. It ranged between 1.4-1.5, referring to terrestrial and soil sources, while ~1.9 is for microbially derived fulvic acids (McKnight, E. W. Boyer, et al., 2001). Third, the humification index (HIX) indicated high-molecular-weight HA and high aromatic content (Zsolnay et al., 1999).

BIX index was calculated by dividing fluorescence intensity emitted at 380 nm and 430 nm at a fixed excitation of 310 nm (Eq. 1). The FI index was calculated from ratio fluorescence at emission 450 and 500 nm at a fixed excitation (370 nm) (Eq. 2). HIX was calculated at a fixed excitation of 254 nm from the ratio of sum the fluorescence in between 300 and 345 nm and 435 and 480 nm (Eq. 3).

$$BIX = \frac{X_{310,380}}{X_{310,430}} \quad Eq. (1)$$

$$FI = \frac{X_{370,450}}{X_{370,500}} \quad Eq. (2)$$

$$HIX = \frac{\sum_{em=435}^{480} X_{254,em}}{\sum_{em=300}^{345} X_{254,em}} \quad Eq. (3)$$

Furthermore, specific UV absorbance (SUVA) was also used to estimate DOM chemical properties (e.g., aromatic carbon content). SUVA<sub>254</sub> is the UV absorbance at 254 nm, normalized for DOC concentration. Elevated SUVA<sub>254</sub> values indicate increased DOM aromaticity (Weishaar et al., 2003). It was estimated using a UV-Vis spectrophotometer (Shimadzu UV2600i) and a TOC/TN analyzer (Shimadzu TOC-L/TN).

#### 4.5.3. Measurements in CHNS elemental analyzer and FTIR

After bulk soil and soil fractions (related to AAOM and MPAOM pools) were homogenized using a pestle and an agate mortar grounded finely (section 4.4), soils were dried using an oven under (105 °C) for three days to determine SOC concentration. Another set of the bulk and fractionated soils was dried overnight at (40 °C) prior to the SOM composition for the FTIR measurements.

Fourier transform infrared (FTIR) spectroscopy (DRIFT mode) was used to estimate the following SOM compositions: Phenolic lignin, polysaccharides, amide nitrogen (amide N), aliphatic C, aromatic C, C/O ratio for functional groups, and aromaticity (aromatic:aliphatic ratio). Furthermore, the Elementar vario MACRO cube CHNS elemental analyzer (Elementar Ltd., Germany) was used to measure total C and N concentrations.

The FTIR measurement was taken by Bruker Vertex 70 infra-red spectrometer with the drift attachment. After the soil samples were dried and powdered, from each sample, three subsamples for better representativity were made, and then the soil was measured with these

conditions: Sample scan time 64, Resolution: 4 cm<sup>-1</sup>; Range: 4000-400 cm<sup>-1</sup>. The infra-red spectra were baseline-corrected and averaged.

The baseline correction was done using rubberband correction in Bruker Opus 8.1 software (Szabó et al., 2020). Each baseline location refers to the distance between endpoints of a chosen range (Yeasmin et al., 2020). Below each peak, the area was used to determine a relevant absorbance. The relative band area (RBA) was determined as (Eq.1) based on (Demyan et al., 2012).

Organic bands were identified using the previous work ranges with minor changes. The formal organic band includes the following: Aliphatic C (2960–2840 cm<sup>-1</sup>), amide N (1547–1510 cm<sup>-1</sup>), (1465–1360 cm<sup>-1</sup>) for aliphatic C-H bending, phenolic lignin (Lehmann & Solomon, 2010), aromatic C (1680-1580 cm<sup>-1</sup>) (Demyan et al., 2012), and polysaccharides (1175–1148 cm<sup>-1</sup>) (Egli et al., 2010). The OPUS 8.1 software (Bruker, USA) was used to estimate the relevance absorbance for OM compositions.

$$RBA = \frac{\text{Area of a particular band}}{\text{Sum area of all bands}} \times 100 \quad \text{Eq. (4)}$$

Functional groups associated with SOM compositions were estimated using the ratio of C/O function groups (a lower ratio referred to an increase in biological reactivity) and aromaticity (high aromaticity referred to a high decomposition degree) (Veum et al., 2014; Yeasmin et al., 2020) (Eq. 2 and 3).

$$C/O = \frac{RBA_{2970-2830} + RBA_{1680-1560} + RBA_{1465-1360} + RBA_{1550-1510}}{RBA_{1175-1140}} \quad \text{Eq. (5)}$$

$$\text{Aromaticity} = \frac{RBA_{1680-1560}}{RBA_{2970-2830} + RBA_{1465-1360}} \quad \text{Eq. (6)}$$

#### 4.6. Soil water content, air temperature, and precipitation Monitoring

Soil water content (SWC) was observed at 0–7 cm soil depths diurnal temporal resolution using remote sensing data. Air temperature and precipitation data were acquired from a nearby meteorological station in Martonvásár. The following weather parameters were used: Precipitation, average air temperature, and SWC. Numerical variables for weather data were made. For example, the sum of the precipitation of the 7, 14, 21, and 28 days before each sampling date was calculated, and similar calculations were done for the average of SWC and air temperature data.

#### 4.7. Statistical analysis

All data were presented as the mean  $\pm$  standard deviation (SD). If data did follow a normal distribution, one-way ANOVA, followed by a post hoc Tukey test, was used; otherwise, Mann-Whitney U and Kruskal-Wallis tests were used if the data did not follow a normal distribution. Both parametric and nonparametric analyses were done using SPSS statistical software (version 28, IBM Corp., Armonk, NY, USA).

Most of the variables in SOM pools besides bulk soils did not follow the normality standard, which was tested using the Shapiro-Wilk test. Hence, Spearman's correlation coefficient was used to investigate correlations between variables. Furthermore, principal component analysis (PCA) was conducted to create uncorrelated new variables reduced in their dimension and summarize SOM variations within each pool and bulk soil. Outliers were detected as out of a range of 3 to -3 and removed using the Z score (Equation 7).

$$Z_i = \frac{X_i - \mu}{\sigma} \quad \text{Eq. (7)}$$

$X_i$  refers to the value of a measured variable  $i$ ;  $\mu$  was the mean and  $\sigma$  the standard deviation. That statistical approach was done in similar study areas (Jakab et al., 2022). Furthermore, the Z score was used to perform the PCA. The PCA aimed to reduce the parameter dimensions using scree plots (Cattell, 1966; Hatvani et al., 2018). The Bartlett and Kaiser-Meyer-Olkin (KMO) tests examined the PCA validity. PCA was performed using correlation analysis and varimax rotation for the DOM pool. Some DOM parameters were not included in the PCA (e.g., the HIX negatively correlated with coble peak B and T) because they lowered the KMO value ( $<0.5$ ) and reduced the PCA validity (Kaiser, 1974).

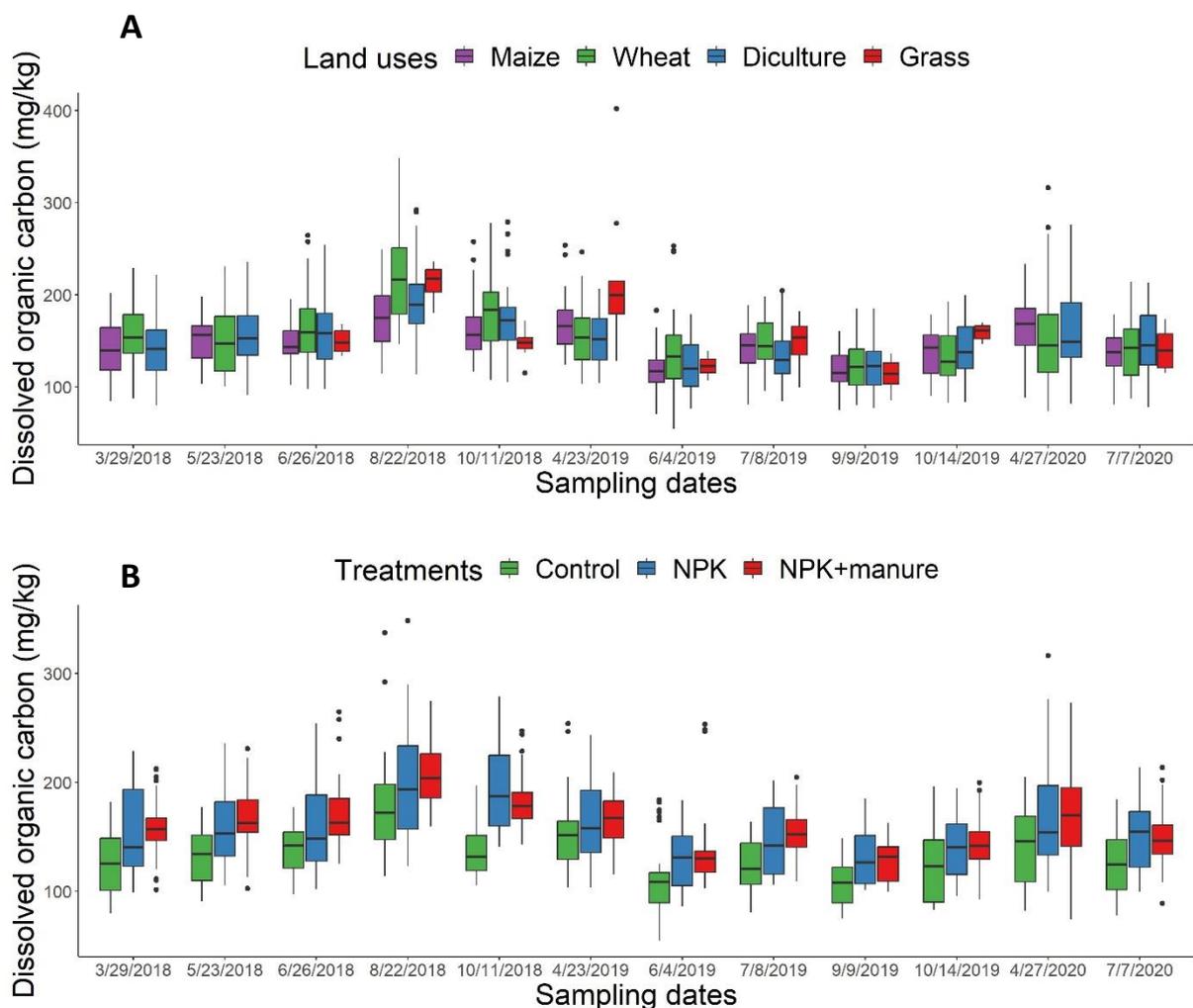
The principal component (PC) scores were classified based on their loading factor, and only values  $>0.65$  were further interpreted. Each classified component from DOM PCA results was correlated with environmental properties (rainfall, soil water content, and air temperature) using Spearman's correlation coefficient. Furthermore, a regression analysis was used for significant correlation coefficients with a sufficient correlation value ( $\rho \geq 0.7$ ).

A permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) followed by multilevel pairwise comparison using `adonis2` (Martinez, 2020) was used to study the interaction effects between sampling dates with plants and treatments on measured SOM pool variables. Spearman's correlation coefficient was used between each soil C pool (fast and slow pools) and bulk soil with DOM parameters. PCA was performed with the `rda` function for solid-phase-related SOM pools. Packages 'vegan', 'psych', and 'ggplot2' were used for correlation and visualization in R software, version 4.3.1 (R Core Team, Vienna, Austria).

## 5. Results

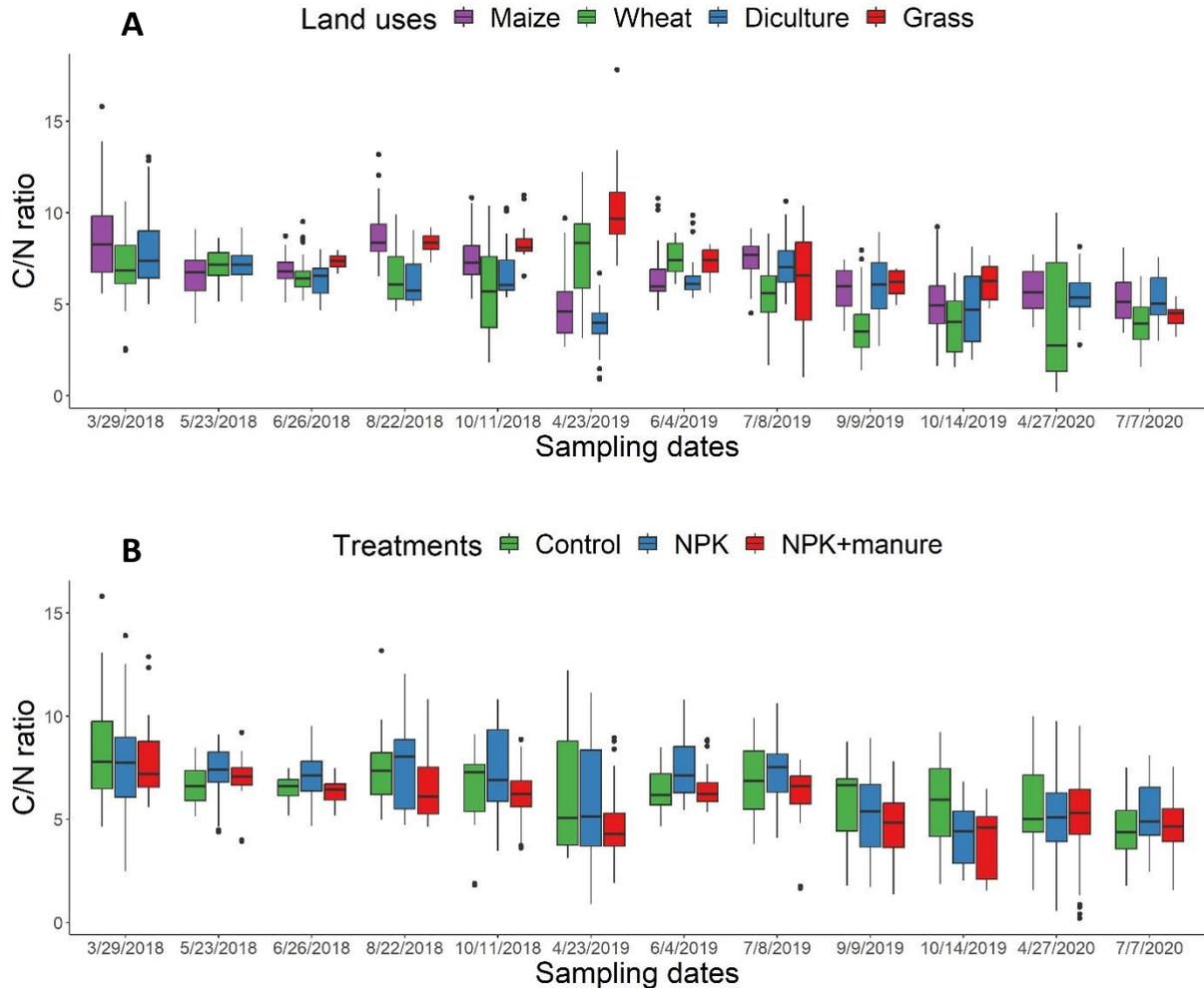
### 5.1. Seasonal concentration and composition of DOM under cropland

Within cropland, the highest DOC concentration was found in NPK+manure treated-plot soil under wheat plant while the lowest value was in control plot soil under maize plant ( $234.37\pm 30.46$  mg/kg and  $99.1\pm 18.95$  mg/kg, respectively, Figure 4). TDN concentration followed the same pattern as DOC concentration (Figure S1). The overall treatment effects (i.e., not on each date of the soil sampling) on DOM parameters were studied. DOC and TDN concentrations were significantly higher under NPK-manure-treated plot soil than NPK-treated-plot soil; the lowest was under control plot. At the same time, the C/N ratio was significantly higher in the NPK+manure-treated-plot soils than in the control and NPK treated-plot-soils, which did not differ from each other (Kruskal-Wallis test, section S2, supplementary).



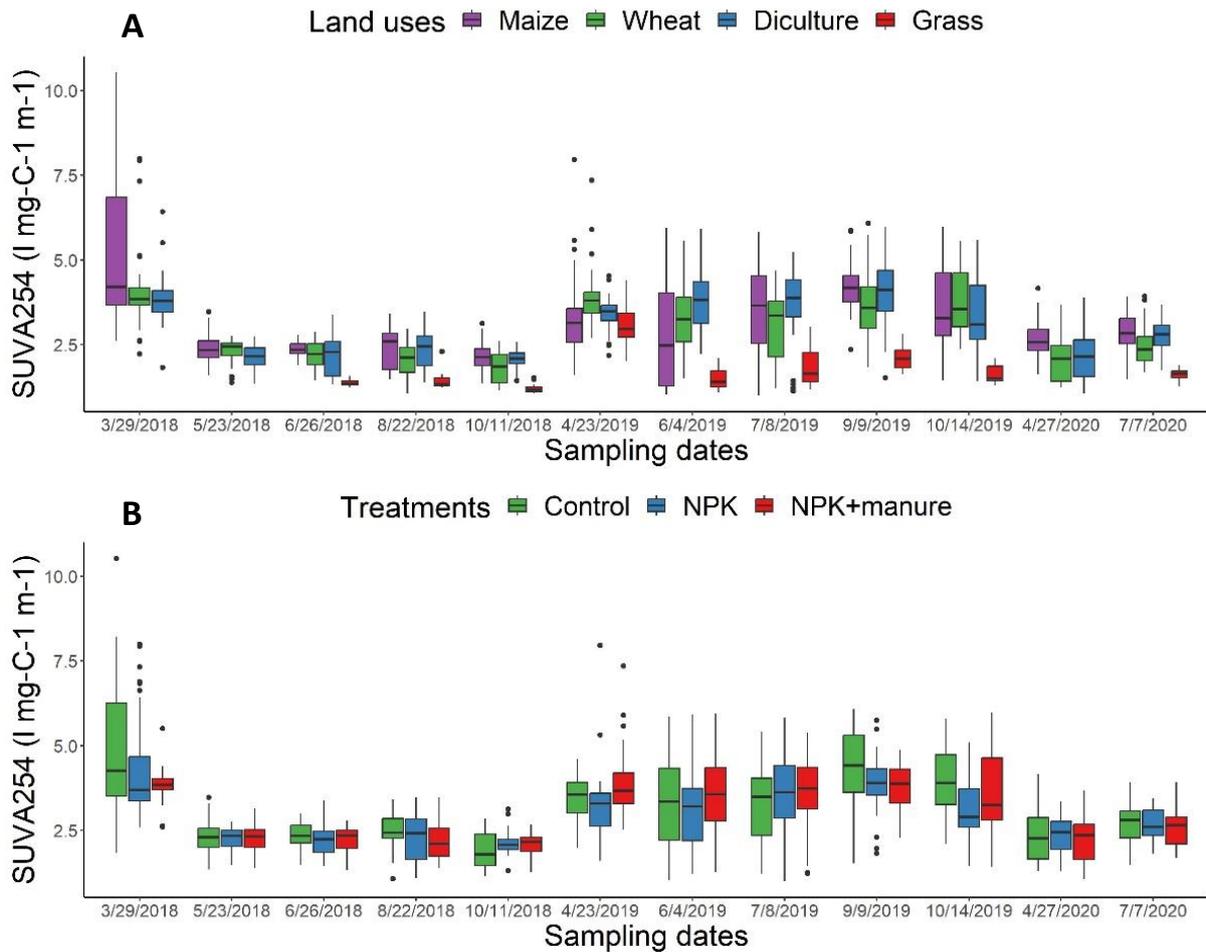
**Figure 4.** The dissolved organic carbon (DOC) concentration in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.

The C/N, referring to DOC/TDN, varied from 1 to 13 (Figure 5), with the lowest mean value ( $2.95\pm 3$ ) after NPK fertilization, indicating the influence of N fertilizers on the C/N ratio. Hence, the C/N ratio value was higher in grassland than in cropland (section 5.3). For example, the lowest C/N ratio value was in cropland under NPK-treated plot soils in April 2020, while the highest was under grassland in April 2019.



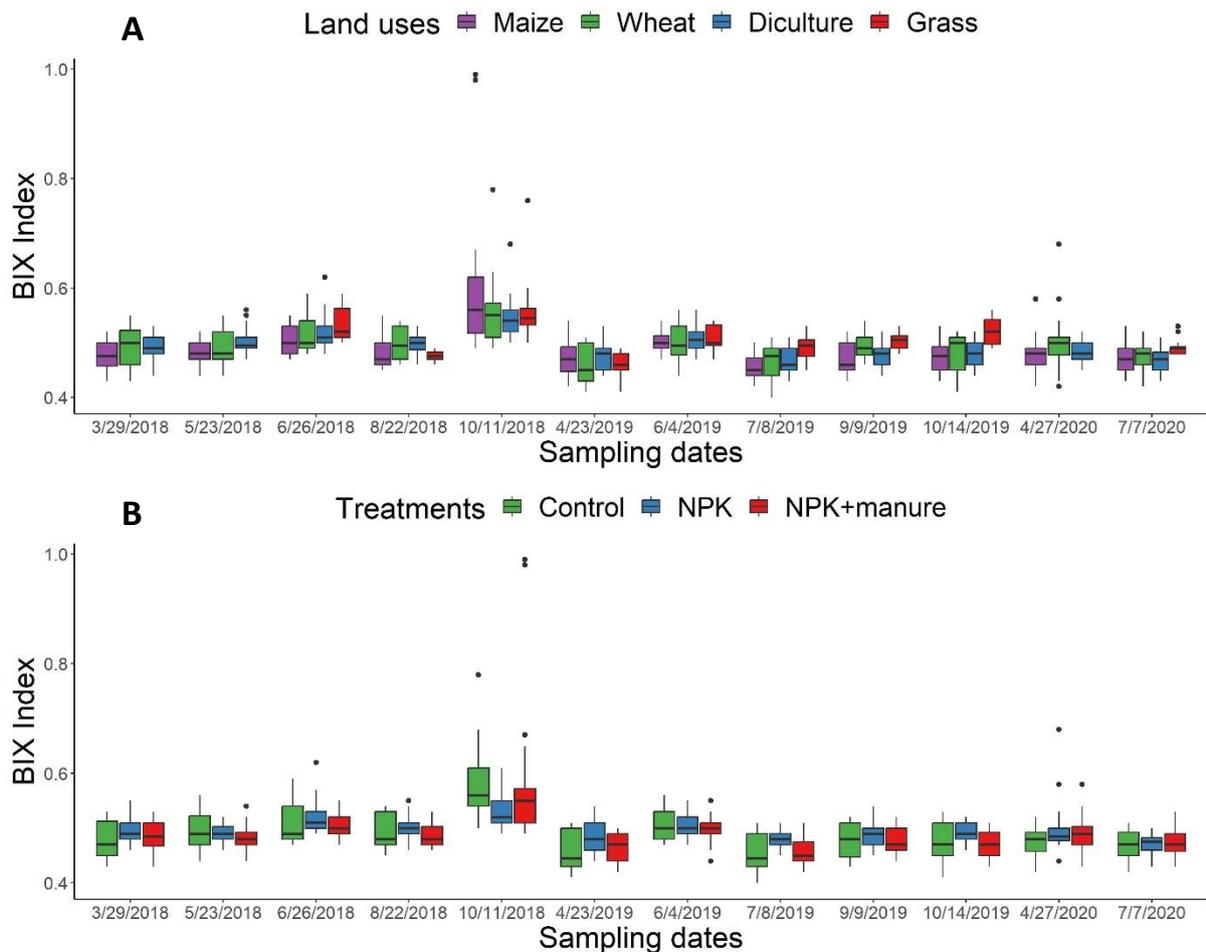
**Figure 5.** The C/N ratio values in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.

SUVA<sub>254</sub> value had a high variation in cropland across sampling times (Figure 6). Nevertheless, the highest SUVA<sub>254</sub> mean value was ( $7.15\pm 1.84$ ) in March 2018 (Maize, control plot), while the lowest mean value was ( $1.82\pm 0.42$ ) in October 2018 (NPK+manure treated plot soil, wheat). Furthermore, SUVA<sub>254</sub> was unaffected by treatments (Asymptotic Sig. (2-sided test)  $p=0.06$ , Kruskal-Wallis test, section S2, supplementary).



**Figure 6.** The specific absorbance (SUVA<sub>254</sub>) values in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.

Furthermore, in cropland DOM compositions, the BIX values had a narrow range variation seasonally (0.44–0.65) (Figure 7). The lowest BIX values were found in spring, while the highest values during the growing season were in line with the C/N ratio. Between treatments, the lowest BIX value was found in the control plot (April 2019, Maize), while the highest was in the NPK+manure plot (October 2018, Maize). BIX index did not significantly differ between the control plot and NPK+manure treated soil plots. Nevertheless, the BIX value was significantly low under NPK-treated soil plots ( $p < 0.05$ , Kruskal-Wallis test, section S2, supplementary).



**Figure 7.** The biological index (BIX) value in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.

The HIX values were up to 0.9, except all samples from all treatments had values  $>0.9$  in April 2019 (Figure S2). For example, the HIX index showed a high value in April 2019 (NPK-treated plot, wheat), while the lowest was in October 2018 (control plot, Maize). FI index also had the highest value in April 2019 and the lowest in September 2019 in grassland (Figure S3). HIX was significantly different between NPK+manure and control plots, while it was not different between NPK and NPK+manure treated plot soils. FI index did not significantly differ among cropland treatments ( $p < 0.05$ , Kruskal-Wallis test, section S2, supplementary).

There were apparent fluctuations in Coble's peaks A, B, T, M, and C and their ratio across sampling times (Figure S4-S11). For example, the highest value for coble's peak A was ( $2.18 \pm 0.23$ ) in April 2019 (NPK+manure treated soil plots, supplementary), while the lowest mean value was ( $0.92 \pm 0.12$ ) in September 2019 (Wheat, control plots). Coble's peak B showed an opposite trend to the A/C index (Figures S5 and S10). For example, the highest Coble's peak B mean value was ( $0.48 \pm 0.4$ ) in the control plot in October 2018 (Control plot, Maize), while the lowest mean value was ( $0.03 \pm 0.03$ ) in March 2018 (NPK+manure treated soil plots,

Maize). All coble peaks differed among treatments within cropland except T/A indices, and coble B was not significantly changed among treatments within cropland (Kruskal-Wallis test, section S2 (A), supplementary).

The DOM variable's seasonality in August 2018 compared to April 2019 was studied. There was a significant overall difference between the DOM parameters between these two sampling dates, except for the FI index and Coble's peak A (Table 5). These differences included higher DOC concentrations in August than in April and the opposite results for TDN concentrations. Furthermore, there were higher aromaticity, A/C, and HIX indices in April than in August. Nevertheless, opposite results were found for Coble's peak B, T, and their ratio.

**Table 5.** DOC concentration and DOM composition seasonal differences within the cropland site in August 2018 compared to April 2019. C and N: Carbon and nitrogen.

<i>Date</i>	<i>TDN (mg/kg)</i>	<i>DOC (mg/kg)</i>	<i>C/N ratio</i>	<i>SUVA<sub>254</sub></i>	<i>BIX index</i>
<i>August</i>	28.65±8.58 a	195.7±46.93 a	7.16±1.83 a	2.28±0.58 a	0.49±0.02 a
<i>April</i>	34.9±18.01 b*	159.56±31.67 b***	5.58±2.62 b***	3.54±0.95 b***	0.46±0.03 b***
<i>Date</i>	<i>HIX index</i>	<i>FI index</i>	<i>Coble's Peak B (A+C)/(B+T) index</i>	<i>Coble's peak T</i>	<i>Coble's peak A</i>
<i>August</i>	0.86±0.02 a	1.2±0.03	0.3±0.04 a	0.24±0.04 a	1.57±0.2
<i>April</i>	0.93±0.01 b***	1.21±0.05	0.16±0.07 b***	0.21±0.03 b***	1.63±0.39
<i>Date</i>	<i>T/A index</i>	<i>A/C index</i>	<i>Coble's peak M</i>	<i>Coble's peak C</i>	
<i>August</i>	0.15±0.03 a	1.73±0.13 a 2.08±0.27	4.65±0.93 a	1.16±0.18 a	0.91±0.14 a
<i>April</i>	0.14±0.03 b***	b***	6.73±2.43 b***	1.04±0.18 b***	0.78±0.14 b***

Different letters referred to the significant effects of the sampling date on each SOM variable. The sign (\*\*\*, \*\*, \*) referred to the test's significance and corresponding to each p-value < 0.001, < 0.01, < 0.05, respectively. Data was given a mean with standard deviation (n=216) for cropland. Further information was outlined (Mann-Whitney U test, section S2 (B), supplementary).

Further seasonal changes in DOM parameters were studied, including each sampling date, across plants and fertilization (section 5.5, Figure 7).

## 5.2. Seasonal concentration and composition of DOM under grassland

In grassland, the highest DOC concentration was (213.41±18.77 mg/kg) in August 2018, while the lowest value was (112.78±16.56 mg/kg) in September 2019 (Figure 4). DOC concentrations in August 2018 significantly differed from all other sampling dates except for

April 2019. TDN was significantly different between August 2018 and April 2019 and similar to other sampling dates, with exceptions that it did not significantly differ with July and October 2019 (Mann-Whitney, Asymp. Sig. (2-tailed) <0.05, section S3).

In grassland, the C/N ratio was significantly higher in spring than in the rest of the year's seasons (Figure 5). For example, the C/N ratio in April significantly differed from that detected at other sampling dates (Mann-Whitney, Asymp. Sig. (2-tailed) < 0.05, section S3, supplementary). The highest mean value was found in April 2019 ( $10.42 \pm 0.293$ ), and the lowest mean value was in July 2020 ( $4.35 \pm 0.64$ ). The C/N ratio was significantly different between August 2018 and April 2019. Comparing August 2018 with other sampling dates, there were significant differences in the C/N ratio except with July 2019 and June 2018 (Mann-Whitney, Asymp. Sig. (2-tailed) <0.05, section S3, supplementary).

The lowest BIX mean value ( $0.46 \pm 0.02$ ) in April 2019 compared to the highest mean value in October 2018 ( $0.56 \pm 0.06$ ) was found (Figure 7). BIX value did significantly differ between April 2019 and August 2018, while BIX value under August 2018 versus other sampling dates showed a similar result with all other DOM compositions (Mann-Whitney, Asymp. Sig. (2-tailed) <0.05, section S3, supplementary). Similar temporal dynamics were also observed for the cropland plots, but the differences were not significant (Mann-Whitney, Asymp. Sig. (2-tailed) >0.05, section S3, supplementary).

The highest HIX index was detected in June 2018 and April 2019 ( $0.9 \pm 0.01$  and  $0.91 \pm 0.01$ , respectively, Figure S2), while the lowest value was detected in July 2020 ( $0.87 \pm 0.01$ ). A significantly lower HIX index in August 2018 than in April 2019 and September 2019 was found (Mann-Whitney, Asymp. Sig. (2-tailed) >0.05, section S3).

Furthermore, the highest FI index mean values were detected in June 2018, while the lowest was in September 2019 ( $1.18 \pm 0.03$  and  $1.1 \pm 0.01$ , respectively, Figure S3). No significant difference in the FI index between August 2018 and April 2019, but August 2018 was significantly higher than July 2019, and September 2019 and July 2020 were found (Mann-Whitney, Asymp. Sig. (2-tailed) >0.05, section S3, supplementary).

The highest aromaticity index (SUVA<sub>254</sub>) mean values were in April 2019, while the lowest was in October 2018 ( $3.11 \pm 0.73$  and  $1.22 \pm 0.14$ , respectively, Figure 6). The aromaticity index was significantly higher in April 2019 than in August 2018, while August 2018 was significantly higher than October 2018. Nevertheless, it was significantly lower than in July 2019 and 2020 (Mann-Whitney, Asymp. Sig. (2-tailed) >0.05, section S3, supplementary). Coble peaks and their ratio did not differ between August versus April sampling dates, except

the T/A ratio was significantly higher in August than in April (Mann-Whitney, Asymp. Sig. (2-tailed) >0.05, section S3, supplementary).

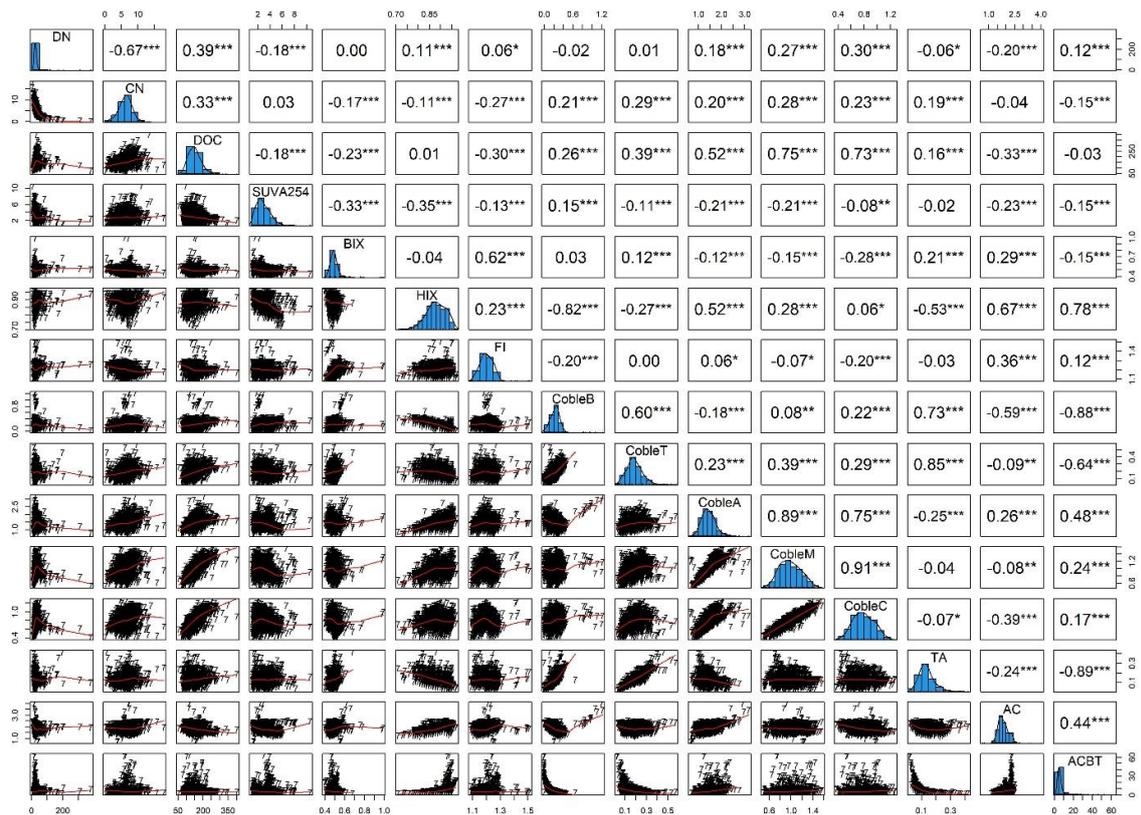
### **5.3. DOM property variations between grassland and cropland**

Comparing grassland to cropland, DOC and TDN concentrations and C/N ratios were significantly higher in grassland than in cropland (Figures 4, 5, S1). The SUVA<sub>254</sub> values ranged from <1 to 7.1, and it had a minimum variability for some sampling, while high variations in others were detected (Figure 6). The highest SUVA<sub>254</sub> value was found in cropland in March 2018 (Maize, control plot), while the lowest was in grassland in October 2018 (Figure 6). Similar results for DOM compositions except for coble peaks (Coble's peaks A, B, M, C and (A+C)/(B+T) indices) were lower in grassland compared to cropland. Furthermore, FI index and SUVA<sub>254</sub> were significantly lower in grassland than in cropland, while Coble peak T was at its highest value in grassland compared to cropland (diculture crops, control pot) (Mann-Whitney, Asymp. Sig. (2-tailed) <0.05, section S4, supplementary).

In the presented chapter, overall differences in DOM properties were analyzed among land uses (between sites) and within each site. A further investigation was done to explain DOM variability, especially over a long sampling campaign under various treatment inputs and plants (section 5.5).

### **5.4. Correlations between DOM parameters**

DOM parameters were significantly correlated with each other but with a low correlation value ( $\rho < 0.7$ ), except for Coble's peaks (Figure 8). For example, Coble's peak M and C were significantly and positively correlated ( $\rho = 0.91$ , p-value < 0.001), T/A index with (A+C)/(B+T) index ( $\rho = 0.89$ , p-value < 0.001), Coble's peak A with C ( $\rho = 0.75$ , p-value < 0.001), Coble's M with DOC concentrations ( $\rho = 0.75$ , p-value < 0.001), HIX index with Coble's peak B and (A+C)/(B+T) index ( $\rho = -0.82$ ,  $\rho = 0.78$ , p-value < 0.001, respectively), coble's peaks B and T with (A+C)/(B+T) index ( $\rho = -0.88$ ,  $\rho = -0.64$ , p-value < 0.001, respectively, supplementary).



**Figure 8.** The correlation plot shows the relationship between the measured DOM parameters data, including cropland and grassland (n=1404). The significance level was calculated using Spearman’s correlation coefficients ( $\rho$ ). The sign (\*\*\*, \*\*, \*) referred to the test’s significance and corresponding to each p-value  $< 0.001$ ,  $< 0.01$ ,  $< 0.05$ , respectively. DOM parameters started from the top: DN (total dissolved nitrogen), CN (C/N ratio); DOC: Dissolved organic carbon; SUVA<sub>254</sub>; HIX; Coble peak’s B, T, A, M, and C; TA (T/A index), AC (A/C index), and ACBT (A+C)/(B+T) respectively.

### 5.5. The effect of plant coverage, fertilization, and time on the DOM parameters

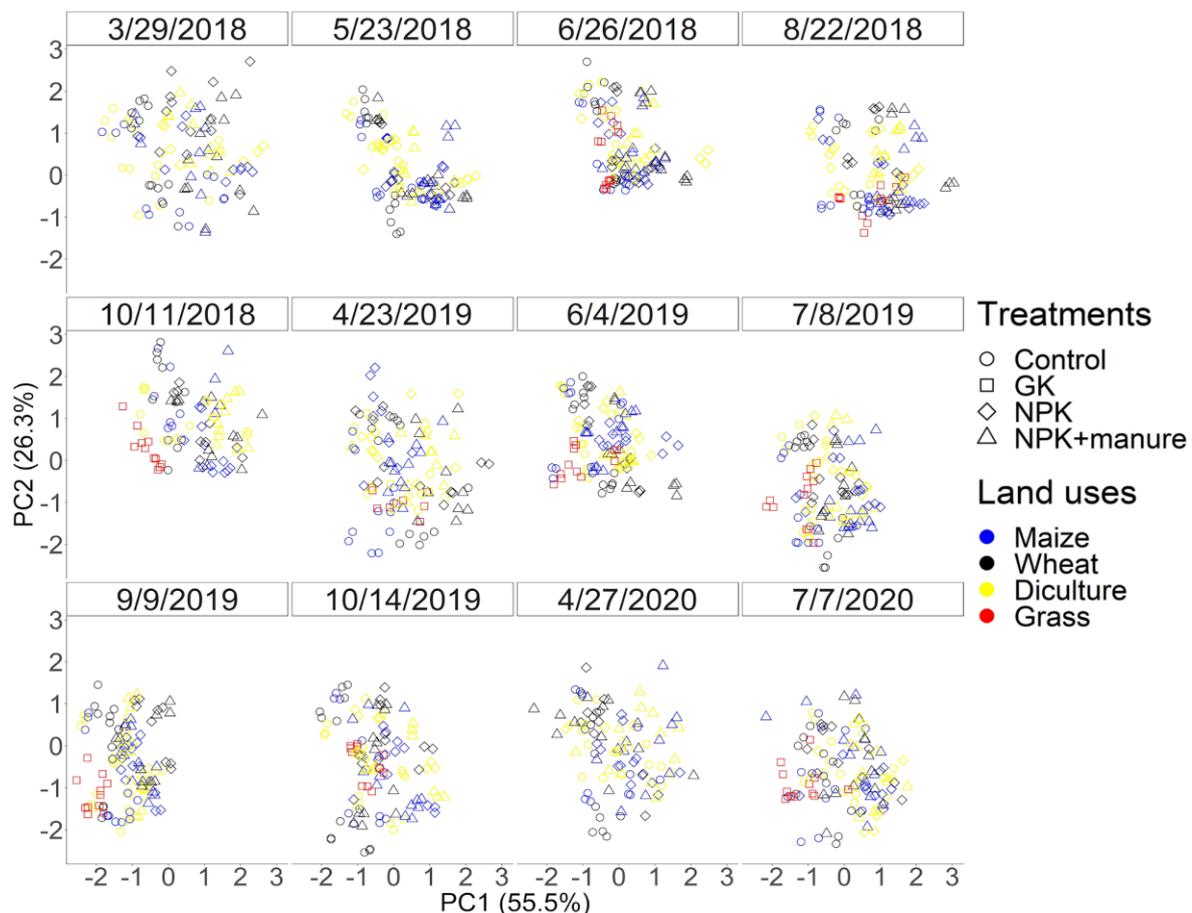
Seasonally fluctuations between sampling dates for DOM parameters were observed for some DOM properties, especially in grassland (section 5.2). 81.8% of the total DOM variance was explained in the first two principal components (PCs). DOC concentration with the humic material-related Coble peaks was in the first component (PC1), explaining 55.5% of DOM variation. In contrast, DOM compositions, indicated by BIX and FI indices, were contained in the second component (PC2) and explained an additional 26.3%. DOC and Coble’s peaks were in one direction in PC1. BIX and FI were also in one direction in PC2 (Table 6).

**Table 6.** The rotated principal component results highlighted the structure of the loading matrix correlation between the DOM parameters within the principal components (PCs).

<i>Variables</i>	<i>Principle Component</i>	
	<i>PC1</i>	<i>PC2</i>
<i>Dissolved organic carbon (DOC)</i>	0.784	-0.247
<i>Biological index (BIX)</i>	-0.135	0.867
<i>Fluorescent index (FI)</i>	-0.014	0.915
<i>Coble's peak A height</i>	0.873	0.087
<i>Coble's peak M height</i>	0.986	-0.015
<i>Coble's peak C height</i>	0.923	-0.177

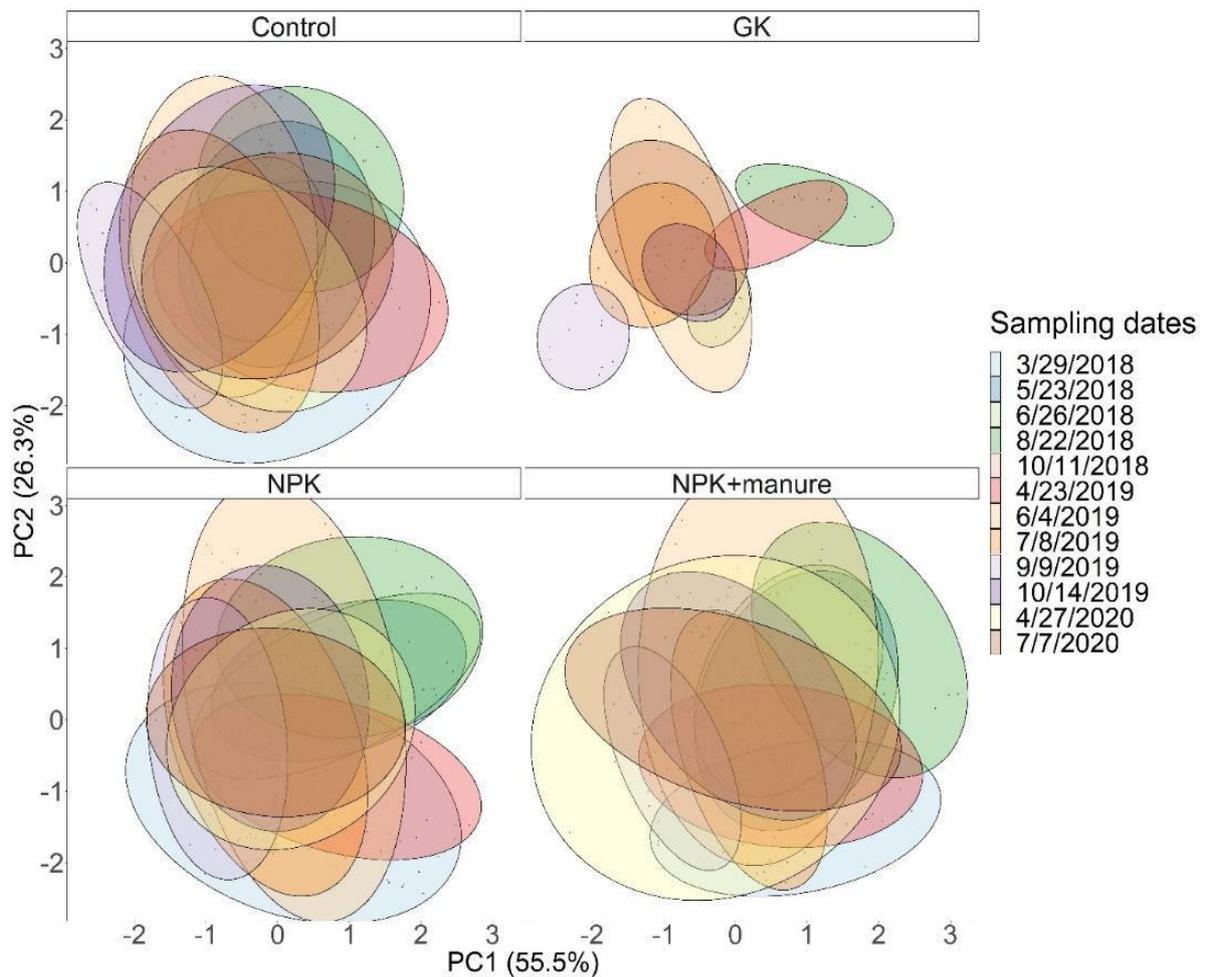
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization<sup>a</sup>. <sup>a</sup> Rotation converged in 3 iterations.

The results showed apparent interaction effects from sampling time and plants on DOM parameters (Figure 9). For example, grassland differed from cropland in October 2018. Nevertheless, there were no apparent effects from treatments and plants within each date on DOM parameters in cropland (Figure S12).



**Figure 9.** Principle component results for the measured DOM parameters for twelve sampling dates under various plants and treatments. The DOM variances were explained in brackets. NPK: nitrogen, phosphorus, and potassium.

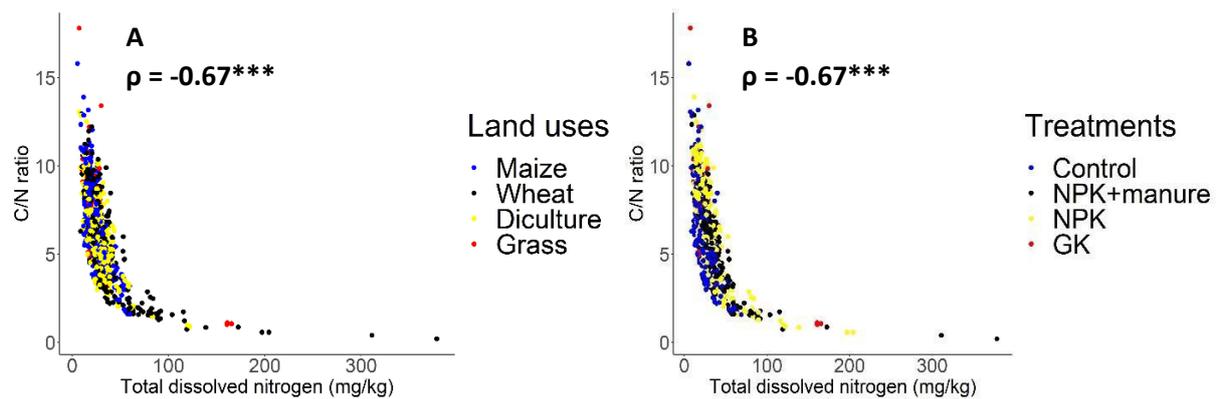
Furthermore, DOM parameters within cropland showed a seasonal pattern, especially between specific sampling dates (Figure 10). For example, October 2019 with August 2018 and April 2019 in control plots with minor overall between August 2018 and April 2019 in NPK and NPK+manure treated plots soils. Within grassland, DOM parameters were changed seasonally. DOM parameters in August 2018 differed from all other sampling dates except for partly overlapping with April 2019. Similar results were found for land use effects on DOM parameters (Figure S12). Furthermore, there were significant but weak correlations between each PC and weather variables (Spearman's correlation, p-value <0.001, Figure S13, supplementary, n=1404).



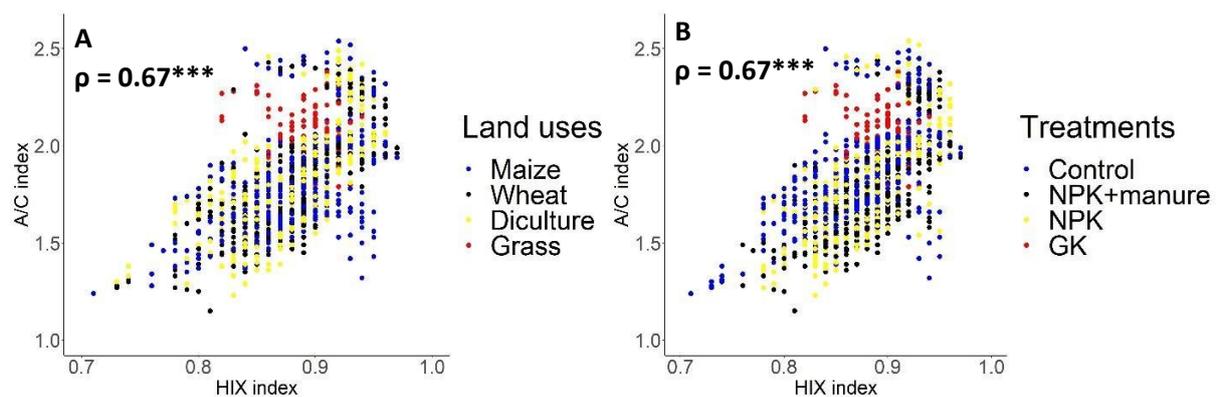
**Figure 10.** Principle component results highlighted the seasonality of treatments for both land uses for the measured DOM parameters. The DOM variances were explained in brackets. Oval cloud shapes highlighted the seasonal pattern of DOM parameters within grassland and each cropland treatment. NPK: nitrogen, phosphorus, and potassium.

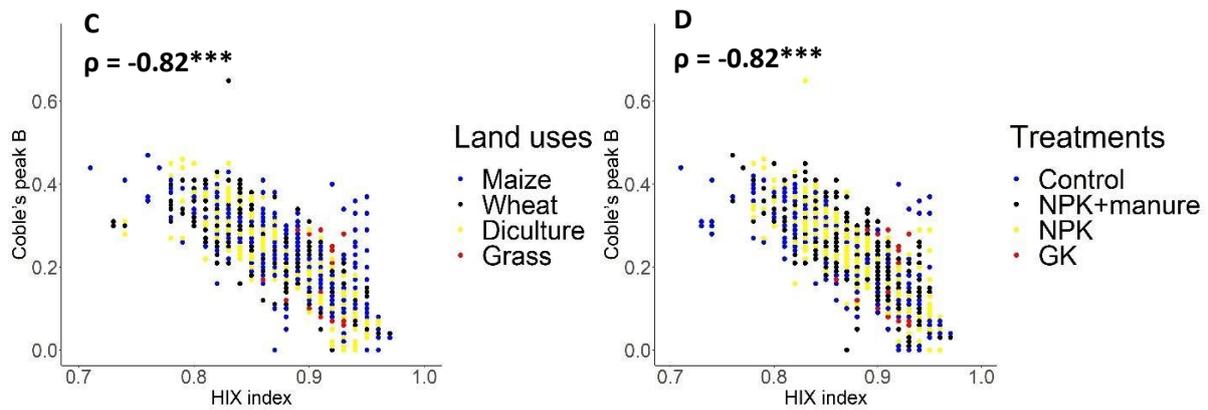
DOM parameters, which were not contained in PCA due to their low contribution to the analysis and reducing PCA validity (section 4.7), had significant correlations with weak correlation coefficient values with each other (Figure S14) and significantly correlated but

weakly with weather data (Spearman's correlation,  $p$ -value  $< 0.001$ , Figure S15, supplementary,  $n=1404$ ). The treatment and crop effects on these correlated DOM parameters also had a similar pattern to what was found for the former PCA results (Figure 10 and S12). For example, there was a significant correlation between TDN concentration and C/N ratio with no apparent effects from treatment or plants on them (Figure 11,  $n=1404$ ) and between HIX with A/C index or Coble B (Figure 12,  $n=1404$ ). The correlation between TDN and DOC was significant but low ( $\rho = 0.39$ ,  $p$ -value  $< 0.001$ , Figure 8).



**Figure 11.** The relationship between total dissolved nitrogen (TDN) concentration and the C/N ratio. Plot (A) referred to the correlation grouped with land uses. Plot (B) referred to the correlation grouped with treatment. The significance level was calculated using Spearman's correlation coefficients ( $\rho$ ). The sign (\*\*\*) referred to the test's significance and the  $p$ -value  $< 0.001$ .

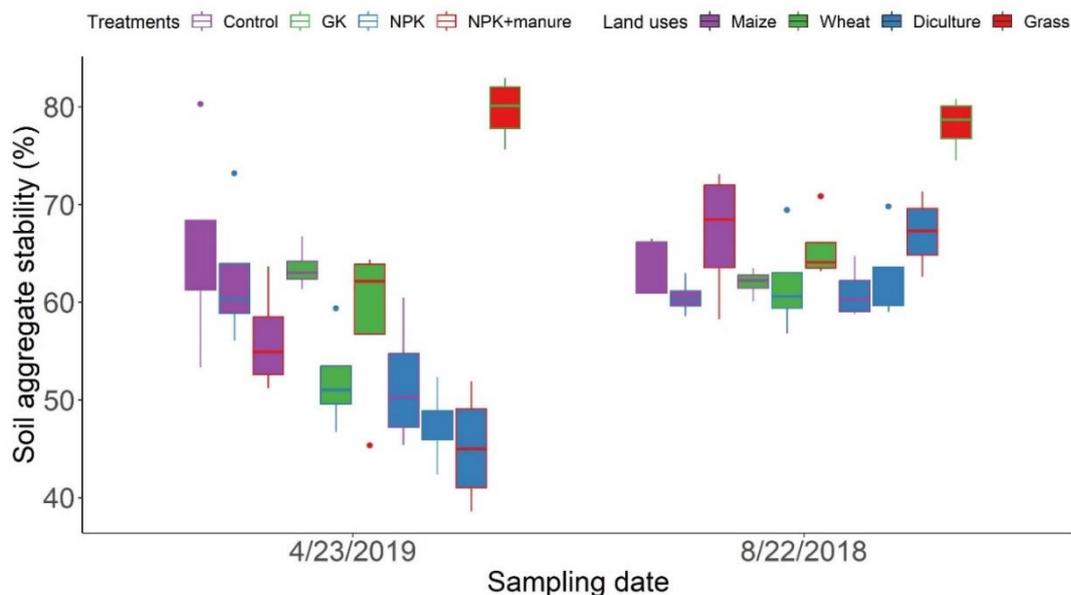




**Figure 12.** The relationship between the HIX index with the A/C index (Plots A and B) and Coble's peak B (Plots C and D), n=1404. Plot (A) referred to the correlation grouped with land uses. Plot (B) referred to the correlation grouped with treatments. A similar order was made for plots C and D. The significance level was calculated using Spearman's correlation coefficients ( $\rho$ ). The sign (\*\*\*) referred to the test's significance corresponding to the p-value < 0.001.

### 5.6. Aggregate stability across land uses

In August 2018, the value of soil aggregate stability (g) (<2 mm to >63 $\mu$ m) values were around 80% in grassland and 65% in cropland (Figure 13). It was significantly higher in grassland than in cropland. Within cropland, fertilization significantly affected aggregate stability (Kruskal-Wallis Test, section S6, A). Aggregate stability was significantly higher under the NPK+manure treated plot compared to NPK in August but did not differ from the control plot ( $p > 0.05$ ). Aggregate stability did not significantly differ among plants within cropland (Kruskal-Wallis Test, section S1, B; section S6, Mann-Whitney U test C).



**Figure 13.** Aggregate stability ratio in grassland and cropland under different treatments and plants across April and August dates. GK= grass and NPK: nitrogen, phosphorus, potassium.

Aggregate stability differed across plants in April 2019 (Figure 13). Like August, grassland had an aggregate stability value of around 80% while it was around 65% under maize and wheat compared to the diculture crops ( $\leq 50\%$ ). Furthermore, aggregate stability in grassland was higher than in cropland, while it was not significantly affected by treatments within the cropland (One-way ANOVA, Tukey HSD test, Section S7 (A, B, and C, supplementary)).

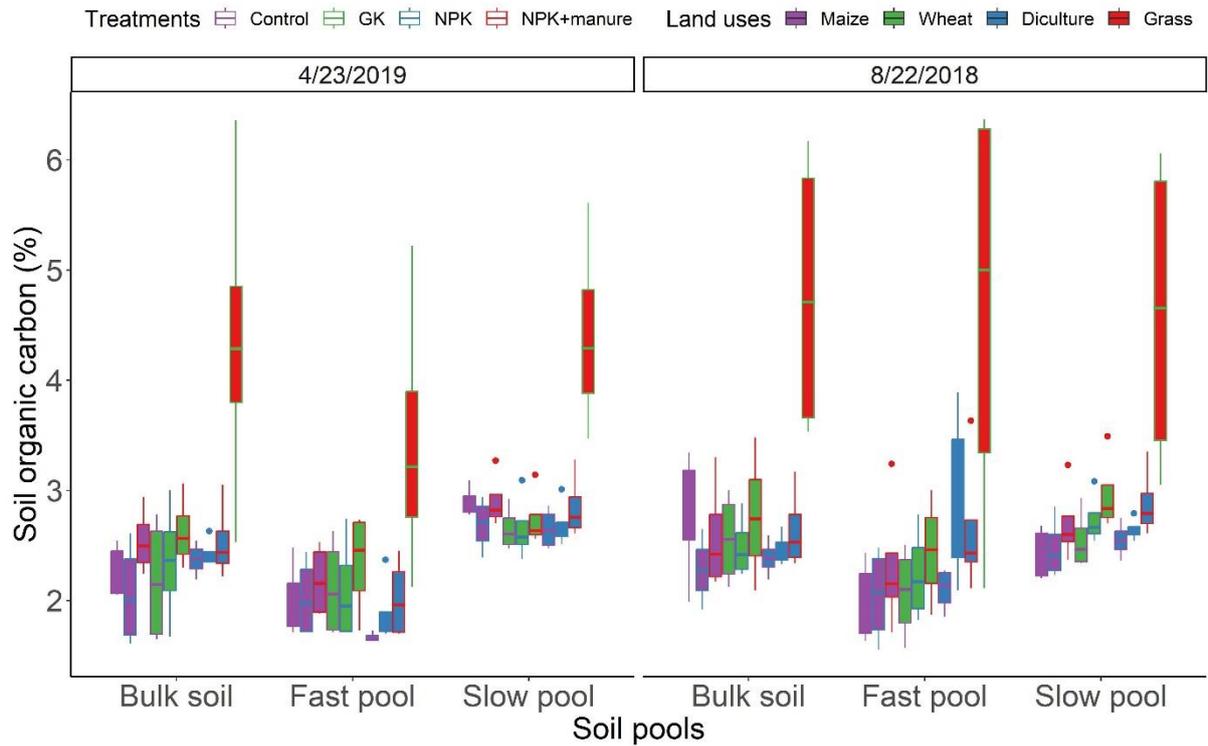
Comparing both sampling dates, aggregate stability in grassland has not changed seasonally (Figure 13). The aggregate stability value under grassland was significantly higher than in cropland ( $p < 0.001$ ). Furthermore, the control plot under maize in April showed a higher value than in August, but it was not significantly different ( $p > 0.05$ ). Nevertheless, aggregate stability in each NPK and NPK+manure plot was significantly higher in August than in April within cropland (section S8, supplementary).

## **5.7. Quantity and composition of solid-phase-related soil organic matter**

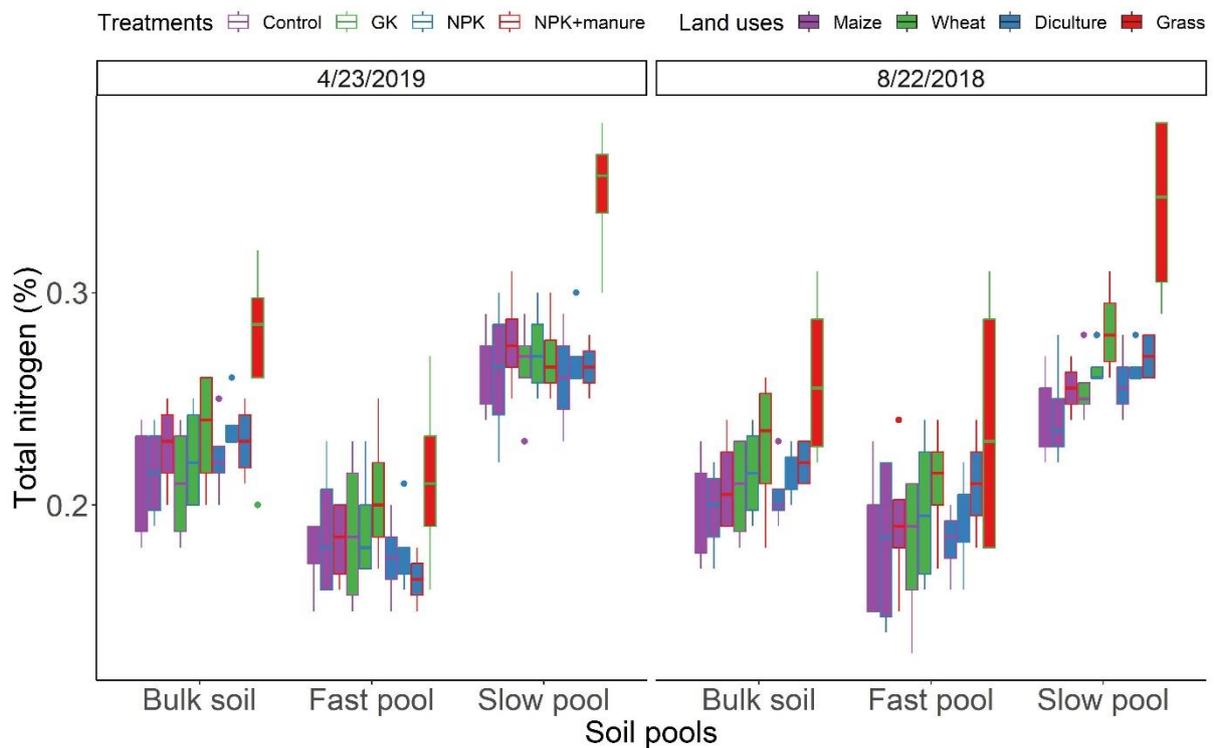
SOC and TN concentrations were higher in the slow pool than in the fast pool, with fluctuations between plants and treatments within cropland over both sampling dates (Figures 14 and 15). In both sampling dates, grassland showed a higher SOC and TN concentration than cropland.

### **5.7.1. SOC concentration and SOM compositions in summer**

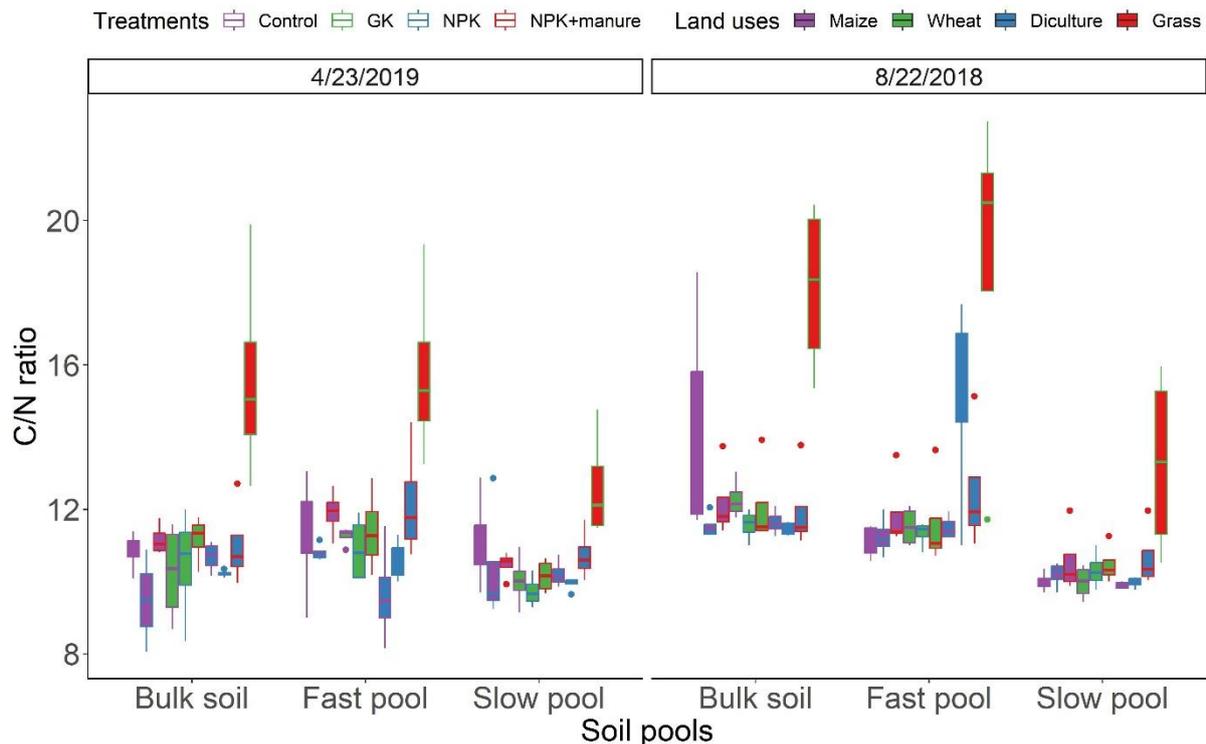
The highest values of SOC and total N concentration were ( $2.96 \pm 0.35$  and  $0.28 \pm 0.02$  respectively) in the slow pool (wheat, NPK+manure treatment) (Figures 14 and 15). The lowest mean values for SOC and total N were ( $1.99 \pm 0.37$  and  $0.18 \pm 0.03$ , respectively) fast pool (maize and control plot). In contrast, the highest C/N ratio was in the fast pool ( $15.21 \pm 2.93$ ) (diculture and NPK plot), while the lowest mean value was ( $9.9 \pm 0.09$ ) in the slow pool (diculture and control plot) (Figure 16).



**Figure 14.** The SOC concentration in grassland and cropland sites under different treatments and crops. GK= grass. NPK: nitrogen, phosphorus, potassium.



**Figure 15.** The total nitrogen concentration in cropland and grassland sites under different treatments and plants(crops). GK= grass. NPK: nitrogen, phosphorus, potassium.



**Figure 16.** The C/N ratio in grassland and cropland sites under different treatments and land uses (crops) across both sampling dates. GK= grass. NPK: nitrogen, phosphorus, potassium.

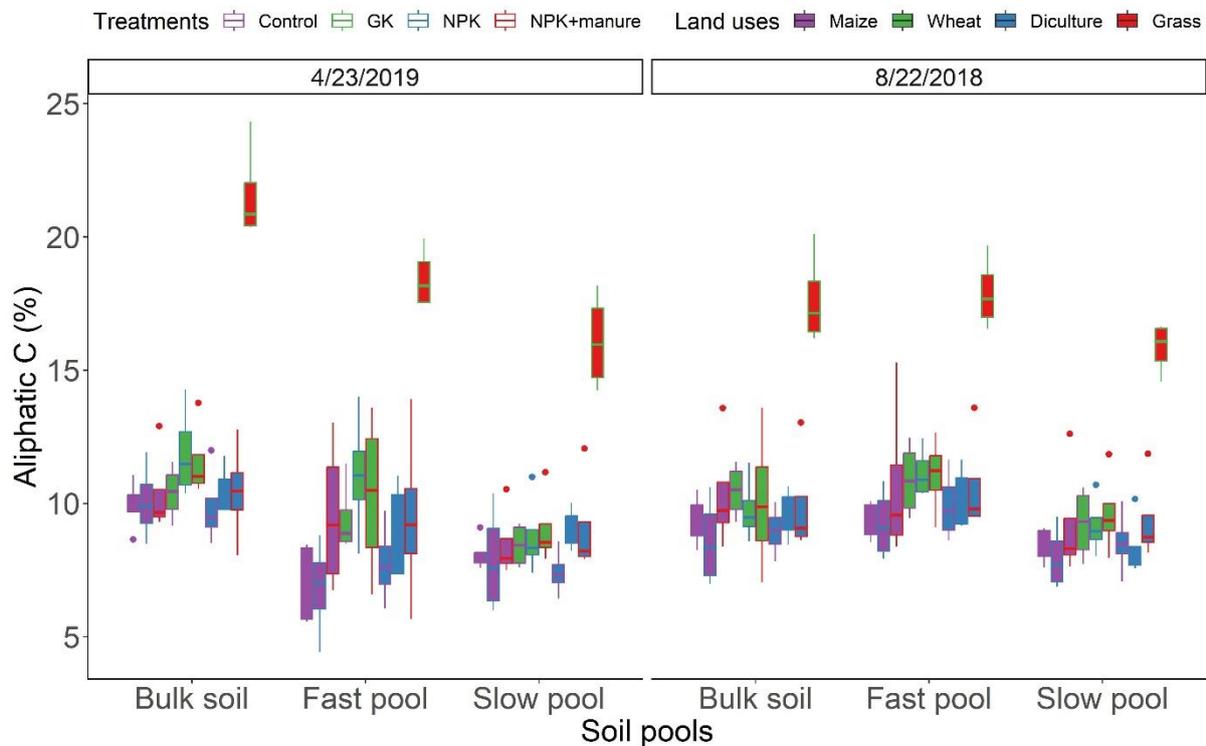
Within cropland, a lower relative amount of aliphatic C and high aromatic values were found in the slow pool compared to the fast pool (Figures 17 and S16). Amide N and polysaccharides ratios had similar patterns between soil C pools. Both were higher in the fast pool than in bulk soil and slow pool (Figures 18 and S17). The slow pool had the lowest polysaccharide value. Phenolic lignin was not different between soil pools, with a higher value in bulk soil (Figure 19). The lowest C/O ratio of functional group value was in the fast pool, while the highest was in the slow pool (Figure 20). Furthermore, a higher aromaticity ratio was found in the slow pool than in the fast pool and bulk soil (Figure S18, supplementary). These SOM variables significantly differed among soil C pools ( $p$ -value $<0.05$ , pairwise comparisons, Table S2 (A), supplementary).

In grassland, SOC concentration had similar values among soil pools (Figure 14). Nevertheless, the highest concentration of total N value was in the slow pool, while the lowest was in the fast pool ( $0.34\pm 0.04$ ,  $0.23\pm 0.06$ , respectively, Figure 15). Like the cropland, the highest C/N ratio was ( $18.86\pm 4.88$ ) in the fast pool, and the lowest was ( $13.27\pm 2.63$ ) in the slow pool (Figure 16).

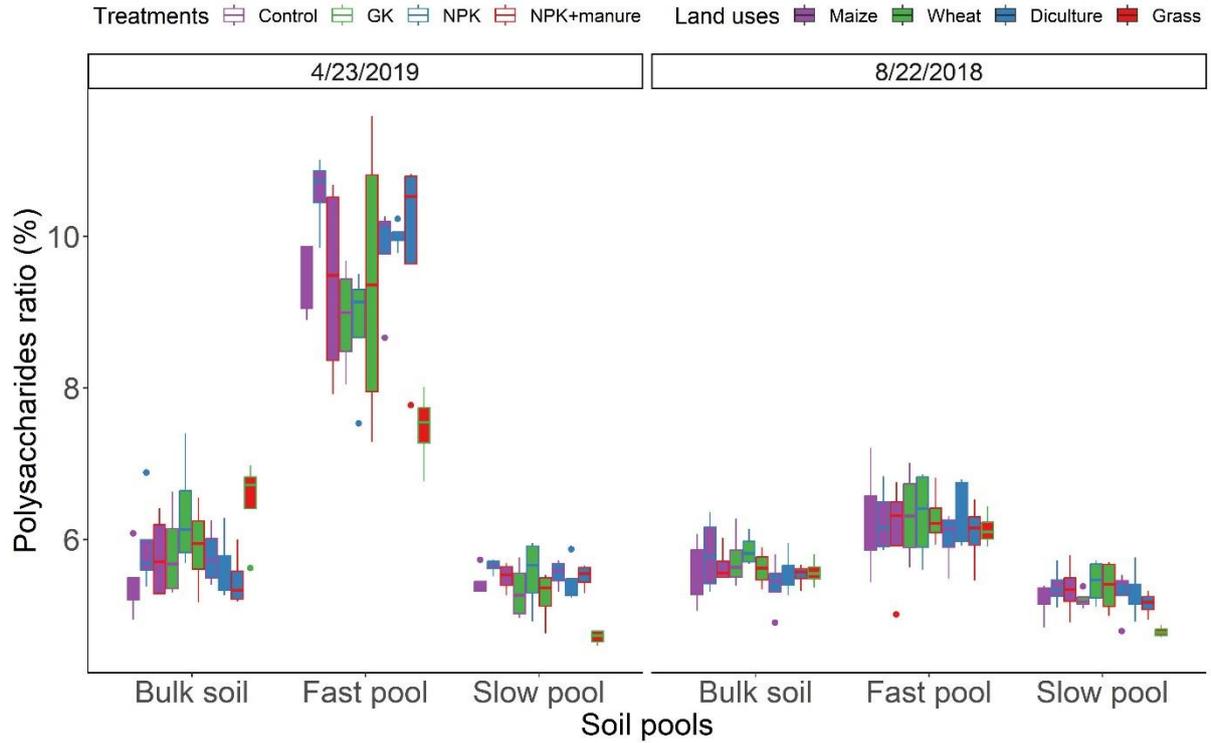
Furthermore, a similar SOM composition pattern to cropland was found in grassland but with different values. For example, the relative amount of aliphatic C was lower in the slow

pool than in the fast pool, while a high aromatic ratio was found in the slow pool (Figures 17 and S16, supplementary). The polysaccharides ratio was higher in the fast pool than in the bulk soil and slow pool (Figure 18).

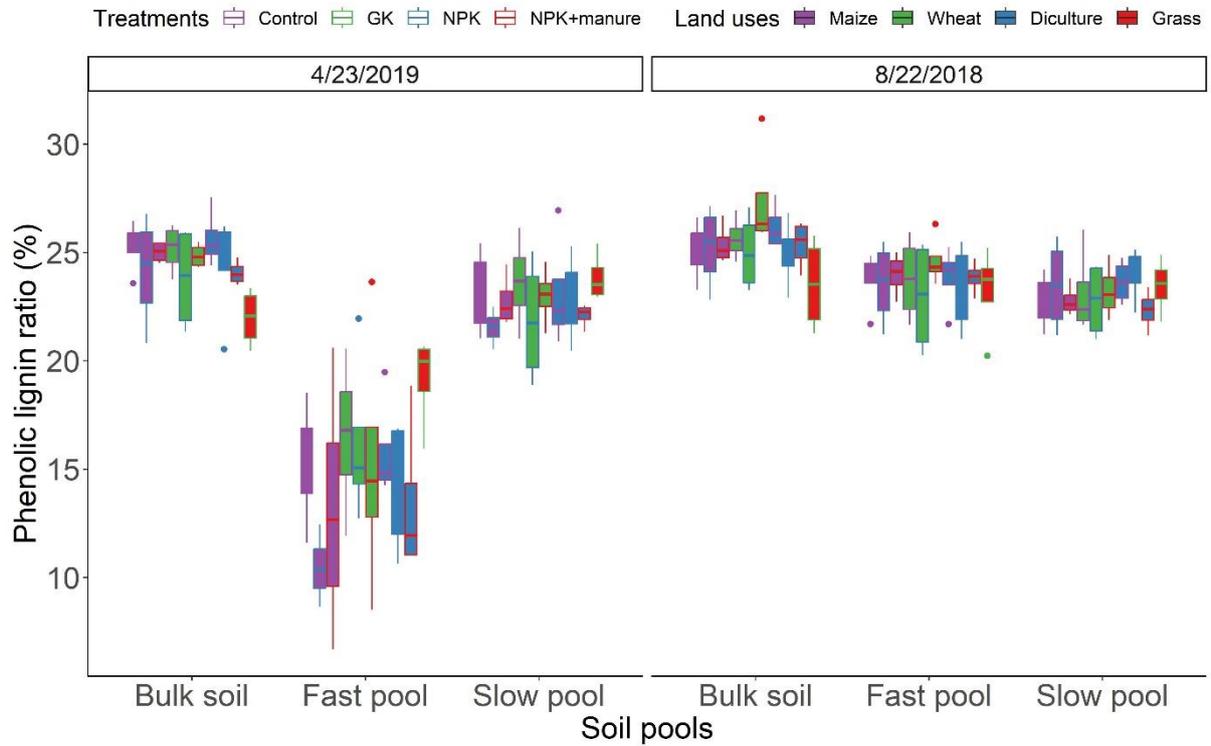
A lower amide N was in bulk soil than in slow and fast pools (Figure S17, supplementary). Phenolic lignin values were not different among soil pools (Figure 19). Furthermore, the lowest C/O ratio of functional groups was in the fast pool, while the highest value was in the slow pool (Figure 20). Furthermore, the aromaticity index was slightly lower in the fast pool than in the bulk soil and slow pool (Figure S18). The soil pool factor significantly affected these SOM variables (PERMANOVA,  $p$ -value $<0.05$ ). Nevertheless, SOM variables among soil pools were not significantly different ( $p>0.05$ ), pairwise comparisons, Table S2 (B), section S9).



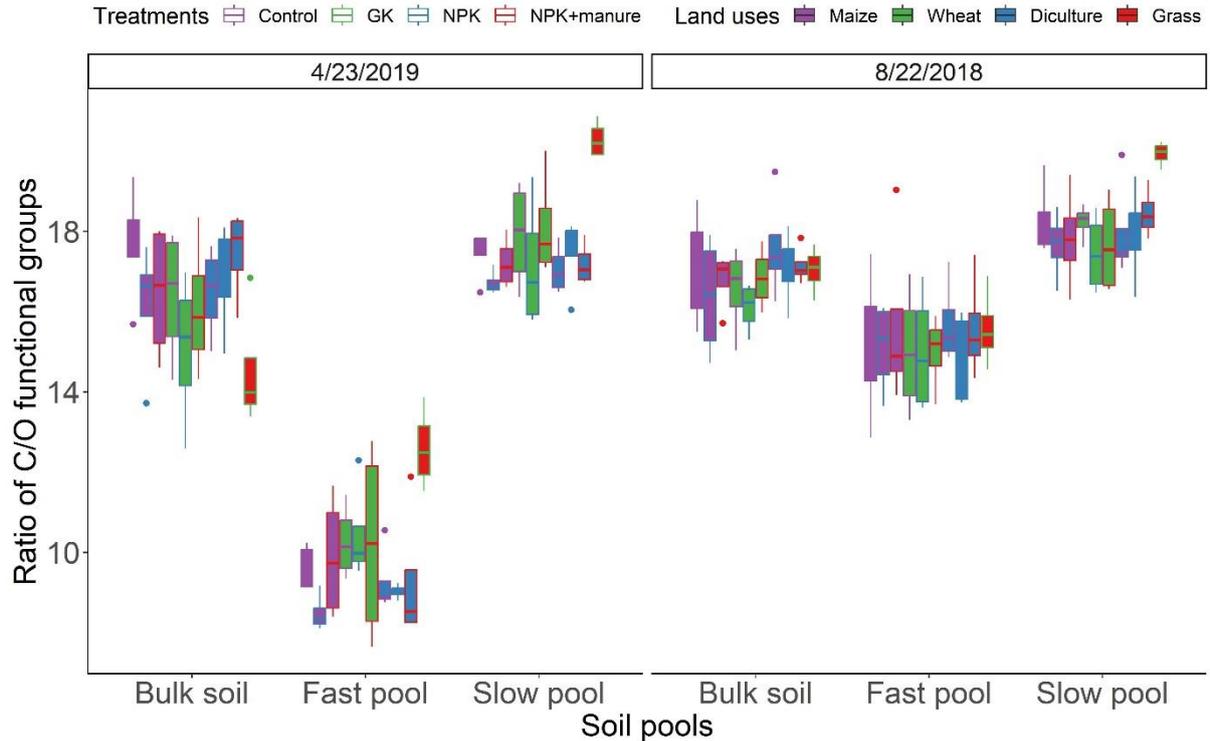
**Figure 17.** The relative amount of aliphatic C components in SOM under grassland and cropland sites with different treatments and plants(crops) and sampling dates. GK= grass. NPK: nitrogen, phosphorus, potassium.



**Figure 18.** The ratio of polysaccharides in SOM under grassland and cropland sites with different treatments and crops across both sampling dates. Slow pool (<math><63 \mu\text{m}</math>) and fast pool (>math>>63 \mu\text{m}</math>). GK= grass. NPK: nitrogen, phosphorus, potassium.



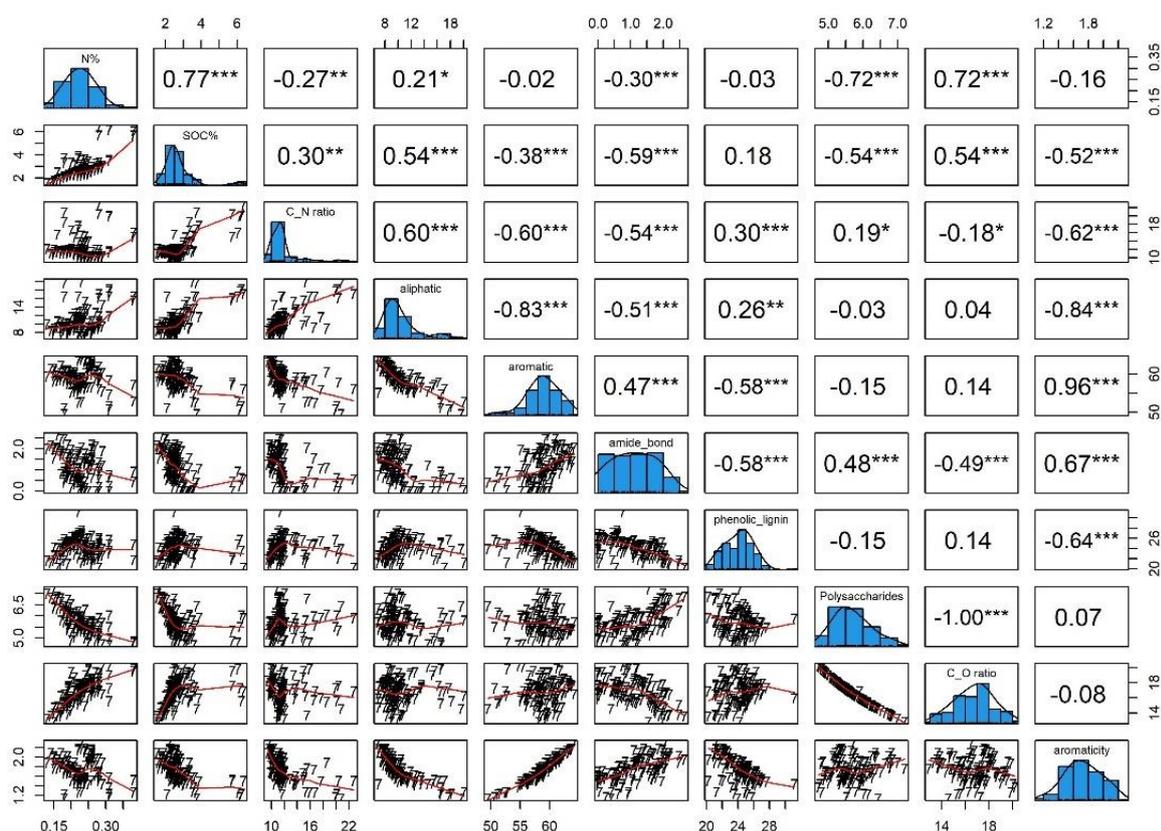
**Figure 19.** The ratio of phenolic lignin in grassland and cropland sites under different treatments and plants(crops) across both sampling dates. GK= grass. NPK: nitrogen, phosphorus, potassium.



**Figure 20.** The ratio of C/O functional groups in grassland and cropland sites under different treatments and plants(crops) across both sampling dates. GK= grass. NPK: nitrogen, phosphorus, potassium.

### 5.7.1.1. Correlations between solid-phase related SOM variables in summer

The measured SOC concentration and most SOM composition properties were significantly correlated (Figure 21,  $p$ -value  $< 0.05$ ). For example, total N was positively correlated with SOC concentration ( $\rho=0.77^{**}$ ,  $n=120$ ). SOC concentration was negatively related to aromaticity, polysaccharides, and amide N ( $\rho=-0.52^{**}$ ,  $\rho=-0.54^{**}$ , and  $\rho=-0.59^{**}$ , respectively,  $n=120$ ), while it was positively associated with C/O ratio and the relative amount of aliphatic C ( $\rho=0.54$ ). Furthermore, amide N was positively and moderately correlated with polysaccharides ( $\rho=0.48$ ), while the C/O ratio was negatively and strongly correlated with polysaccharides ( $\rho=-1$ ).



**Figure 21.** The correlation plot showing the relationship between the measured SOM parameters for the August sampling date (n=120). The significance level was calculated using Spearman’s correlation coefficients ( $\rho$ ). The sign (\*\*\*, \*\*, \*) referred to the test's significance and corresponding to each p-value < 0.001, < 0.01, < 0.05, respectively. C\_N (C/N ratio); amide\_bond (amide N); and C\_O (ratio of C/O functional groups).

### 5.7.1.2. Plants and fertilization effects on solid-phase SOM variables in summer

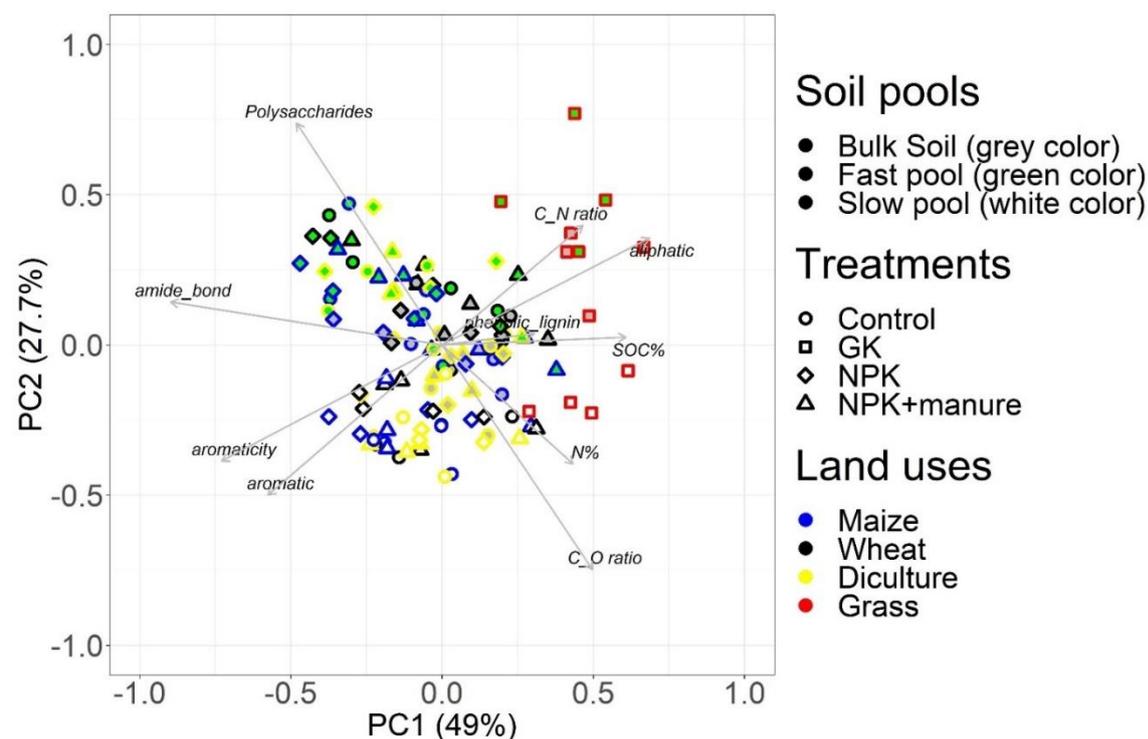
Including grassland and cropland data in PCA, in the first component (PC1), SOC concentration and the relative amount of aliphatic C component were in the opposite direction with amide N and aromaticity (Table 7). In the second component (PC2), the C/O ratio and polysaccharides were in opposite directions with a high loading value. PC1 explained 49% of SOM’s chemical properties variation compared to PC2 (27.7%).

**Table 7.** The principal component results highlighted the structure of the loading matrix correlation between the SOM variables within the principal components (PCs), including grassland and cropland data for the summer sampling date (August 2018).

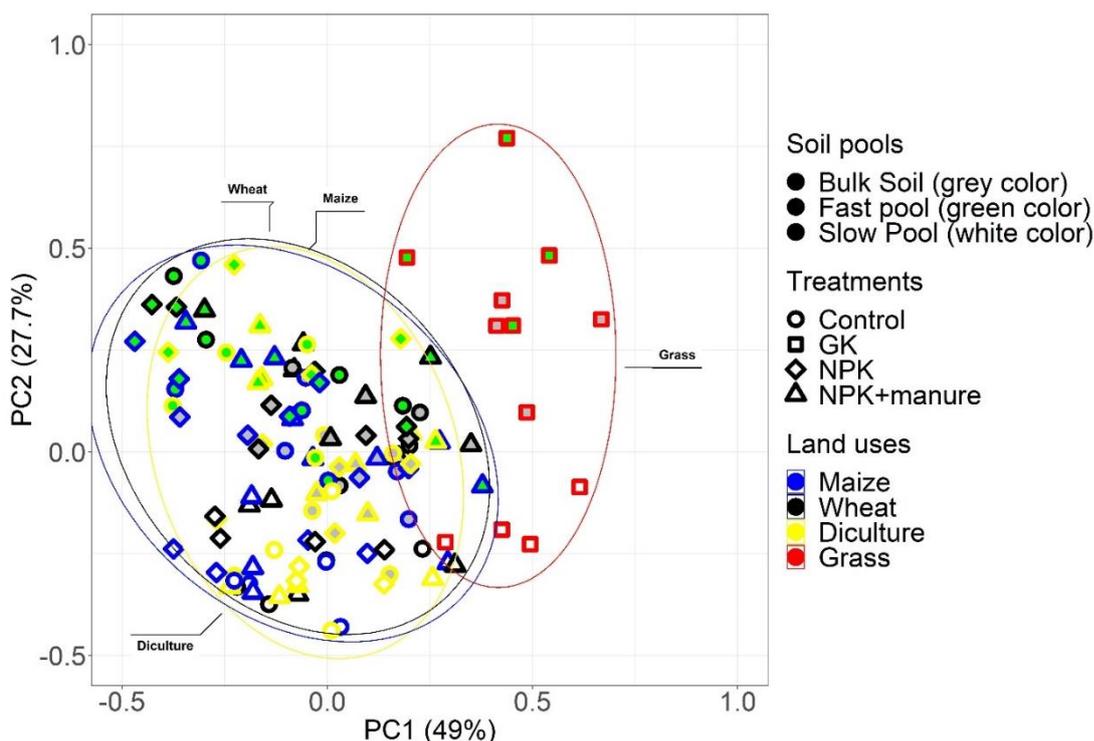
<i>Variables</i>	<i>Principle Component</i>	
	<i>PC1</i>	<i>PC2</i>
Total N	0.433	-0.396

Soil organic carbon	0.608	0.026
C/N ratio	0.465	0.398
Aliphatic C	0.686	0.356
Aromatic C	-0.576	-0.500
Amide N	-0.898	0.143
Phenolic lignin	0.304	0.037
Polysaccharides	-0.481	0.738
C/O ratio	0.497	-0.747
Aromaticity	-0.729	-0.386

The grass was scattered along both SOC and the relative amount of aliphatic C and was separated from the cropland in the first principal component (PC1) (Figure 22). SOM variables significantly differed among the plants (Figure 23). Furthermore, the soil pool effects on SOM variables, including both study sites, showed that the slow pool partly overlapped with the fast pool. SOM variables in the bulk soil were between the slow and fast pools (Figure S19, section S9, supplementary).



**Figure 22.** Biplot of the first two components showing the loading values in PC 1 and 2 within grassland and cropland in August (n=120). The measured SOM parameter variances were explained in brackets. NPK: nitrogen, phosphorus, and potassium. The following variables shortcut referred to (aromatic\_aliphatic\_C (aromaticity index), C\_O (C/O ratio), amide\_bond (amide N), SOC (soil organic carbon), N (total N), C\_N (C/N ratio)).



**Figure 23.** PCA results for the measured SOM variables, including grassland and cropland, in August. It highlighted the land use effects on SOM variables. The SOC variances were explained in brackets. NPK: nitrogen, phosphorus, and potassium. The circled cloud indicated the effects of land uses (crops) on the measured SOM parameters.

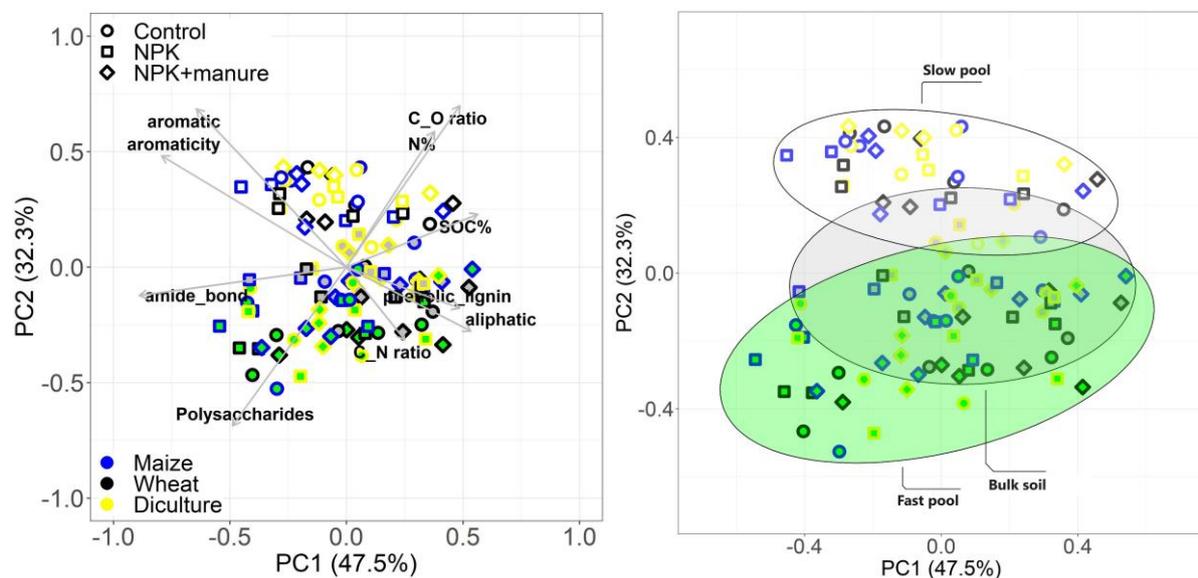
Because grassland had distinct SOM properties from cropland, further PCA was conducted for only cropland. Aliphatic SOM components were in the opposite direction from aromatic SOM components and aromaticity in PC1 (Table 8). In PC2, the C/O ratio and total N were in the opposite direction with polysaccharides. A total of (47.5%) of data variations were described in PC1 compared to PC2 (32.3%) (Figure 24). No apparent effects from treatments or plants on SOM variables were found within cropland (Figure S20 and S21, supplementary, section S9).

**Table 8.** The principal component results highlighted the structure of the loading matrix correlation between the SOM variables within the principal components (PCs) in the cropland for the summer sampling date (August 2018).

<i>Variables</i>	<i>Principle Component</i>	
	<i>PC1</i>	<i>PC2</i>
Total N	0.378	0.587
Soil organic carbon	0.563	0.229
C/N ratio	0.250	-0.314
Aliphatic C	0.534	-0.277

Aromatic C	-0.644	0.686
Amide N	-0.890	-0.122
Phenolic lignin	0.486	-0.177
Polysaccharides	-0.489	-0.687
C/O ratio	0.487	0.699
Aromaticity	-0.793	0.482

In contrast, the soil pools ruled SOM composition with minor overlaps, while bulk soil was in between (Figure 24). A higher contribution of phenolic lignin ratio, total N, SOC concentration, and C/O functional group ratio were contained within the slow pool compared to fresh OM in the fast pool, indicated by amide N and polysaccharides (Figures 24). These results were aligned with the observation of a higher SOC and total N concentration in the slow pool than in the fast pool (Figures 14 and 15).



**Figure 24.** Biplot of the first two components shows the loading values in PC 1 and 2 (left side) and PCA results for the measured SOM variables (right side). In August, it highlighted the soil pool's effects on SOM variables within cropland. The SOC variances were explained in brackets. The circled cloud indicated the soil pool's effects on the measured SOM parameters. The following variables shortcut referred to (aromatic\_aliphatic\_C (aromaticity index), C\_O (C/O ratio), amide\_bond (amide N), SOC (soil organic carbon), N (total N), C\_N (C/N ratio)).

Furthermore, the measured SOM variables were unaffected by the interaction effects between plants and treatments with soil pools, using PERMANOVA. It confirmed the PCA results about the significance of only soil pool as a predominant factor affecting SOM variables besides land use effects on SOM variables, which mostly come from the distinct characteristic

of SOM variables in cropland compared to grassland site ( $p$ -value $<0.05$ , Table S2 (C), section S9, supplementary).

### 5.7.2. SOC concentration and SOM composition in Spring

The highest SOC and total N concentrations were ( $2.9\pm 0.25$  and  $0.27\pm 0.02$ , respectively) in the slow pool (maize crop, NPK+manure plot) (Figures 14 and 15). The lowest values for SOC and total N were ( $1.66\pm 0.04$  and  $0.17\pm 0.02$ ) in the fast pool (diculture, NPK, and control plots, respectively) of cropland. In contrast, the highest C/N ratio was in the fast pool ( $12.17\pm 1.6$ ) (diculture, NPK+manure plot), while the lowest C/N ratio value was ( $9.48\pm 1.22$ ) in the bulk soil (maize, NPK plot) (Figure 16).

SOM compositions in cropland showed the highest relative amount of aliphatic C components in bulk soil, followed by the fast pool, and the lowest in the slow pool (Figure 17). In contrast, the aromatic C component was lower in the bulk soil compared to soil pools (Figure S16). Amide N and polysaccharides ratios were higher in the fast pool than in the slow pool and bulk soil (Figures 18 and S17). Both phenolic lignin and C/O ratios had lower values in the fast pool (Figures 19 and 20) compared to the slow pool and bulk soil. The aromaticity value was higher in the fast pool compared to the slow pool and bulk soil. These SOM variables significantly differed between soil pools (PERMANOVA,  $p$ -value $<0.05$ , pairwise comparisons, Table S3 (A), supplementary).

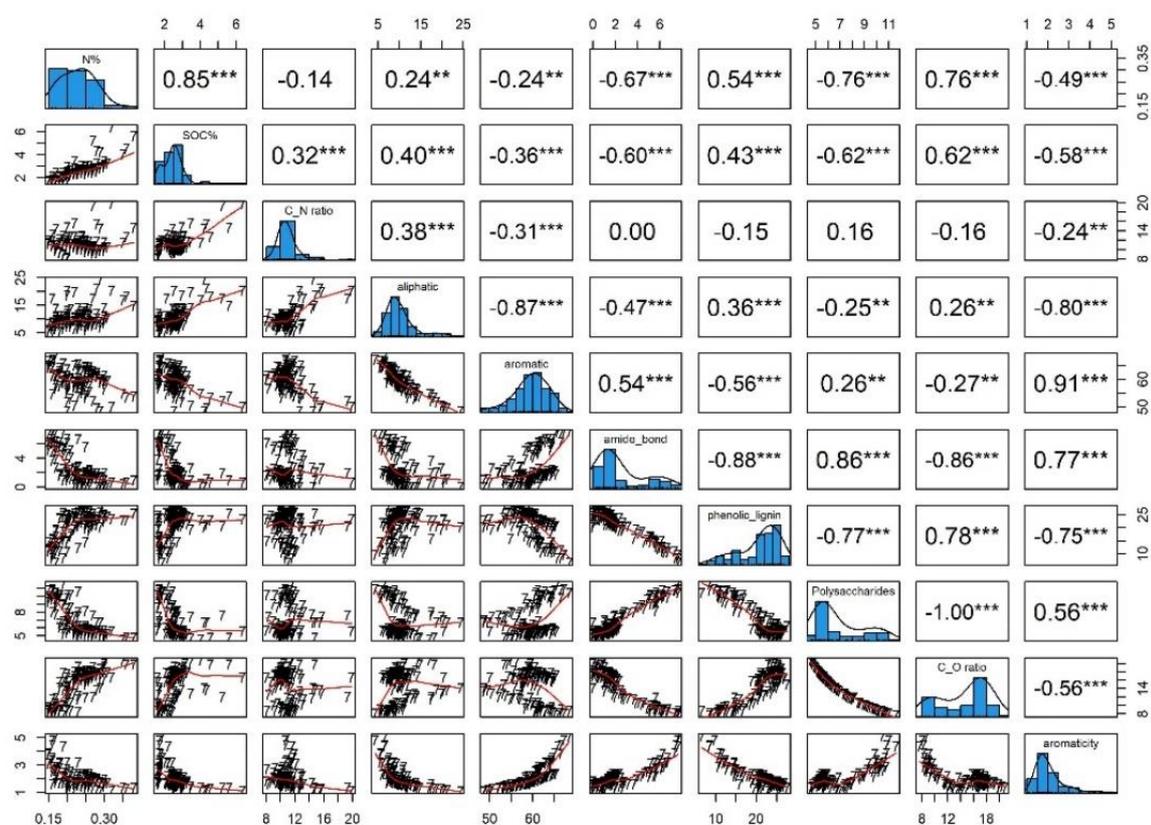
In grassland, the highest SOC concentration was in the slow pool ( $4.41\pm 0.91$ ), while the lowest mean value was ( $3.44\pm 1.3$ ) in the fast pool (Figure 14). The highest total N concentration in grassland was ( $0.34\pm 0.03$ ) in the slow pool, while the lowest value was ( $0.21\pm 0.04$ ) in the fast pool (Figure 15). Furthermore, the highest C/N ratio was ( $15.79\pm 2.57$ ) in the fast pool, and the lowest mean value was ( $12.62\pm 1.52$ ) in the slow pool (Figure 16).

The relative amount of aliphatic C component was higher in bulk soil with some variations across soil pools (Figure 17), while the aromatic ratio was in the opposite pattern (Figure S16). Amide N and polysaccharides were higher in the fast pool than in the slow pool (Figures 18 and S17), while an opposite finding was shown for phenolic lignin and C/O ratio (Figures 19 and 20). Furthermore, the aromaticity index was higher in the fast pool than in the slow pool and bulk soil (Figure S18). These SOM variables significantly differed between soil C pools (PERMANOVA,  $p$ -value $<0.05$ ). Nevertheless, SOM variables among soil pools have approached the borderline to be significantly different among bulk soil versus fast and slow pools ( $p$ -value= 0.07-0.09). Nevertheless, the SOM variable in the slow pool was close to being

significantly different from the fast pool ( $p=0.051$ ) (pairwise comparisons, Table S3 (B), section S9, supplementary).

### 5.7.2.1. Correlations between solid-phase related SOM variables in spring

Most of the measured SOC concentration and SOM compositions were significantly correlated (Figure 25,  $p$ -value  $< 0.05$ , section S9, supplementary). For example, SOC concentration was negatively related to aromaticity, polysaccharides, and amide N ( $\rho=-0.58$ ,  $\rho=-0.62$ , and  $\rho=-0.60$ , respectively). The ratios of amide N and polysaccharides were positively and strongly correlated with a correlation coefficient ( $\rho= 0.86$ ). The C/O functional group ratio was strongly negatively correlated with polysaccharides ( $\rho=-1$ ).



**Figure 25.** The correlation plot showing the relationship between the measured SOM parameters for the April sampling date ( $n=120$ ). The significance level was calculated using Spearman's correlation coefficients ( $\rho$ ). The sign (\*\*\*, \*\*, \*) referred to the test's significance and corresponding to each  $p$ -value  $< 0.001$ ,  $< 0.01$ ,  $< 0.05$ , respectively. C\_N (C/N ratio); amide\_bond (amide N); and C\_O (ratio of C/O functional groups).

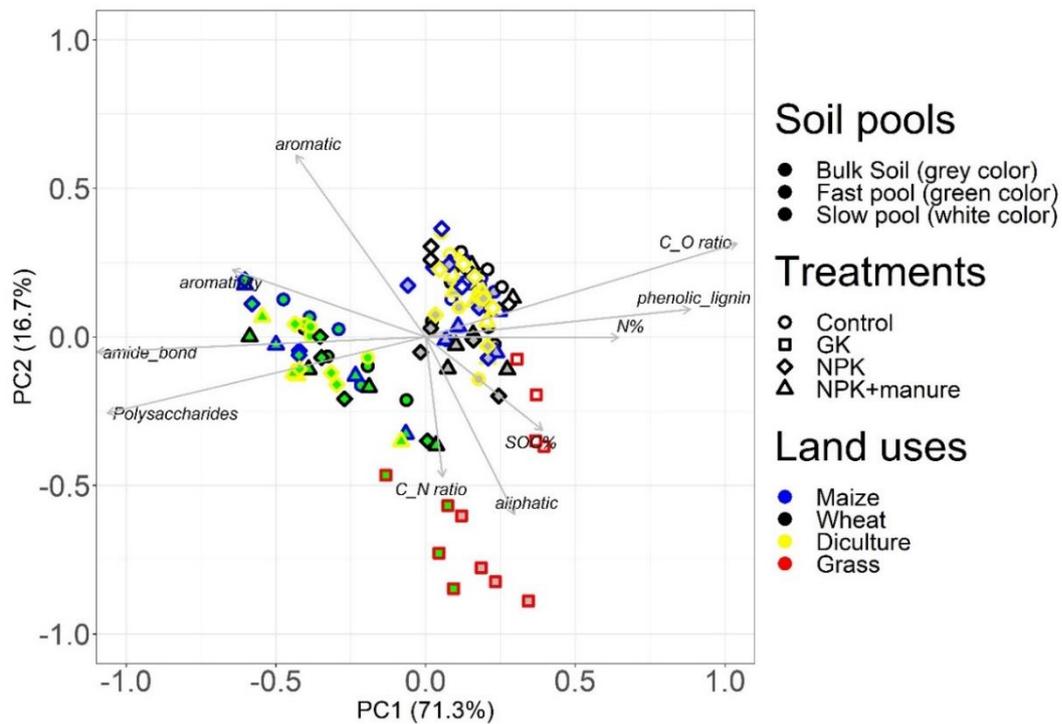
### 5.7.2.2. Plants and fertilization effects on solid-phase SOM variables in spring

Including grassland and cropland data, total N, C/O ratio, and phenolic lignin were in opposite directions with amide N, polysaccharides, and aromaticity (Table 9, Figure 26). All SOM variables significantly differed between soil pools (Figure S22). These SOM variables were contained in the first component (PC1). In the second component (PC2), aliphatic and aromatic C ratios were in opposite directions. PC1 explained 71.3% of the variation in chemical properties of SOM compared to PC2 (16.7%).

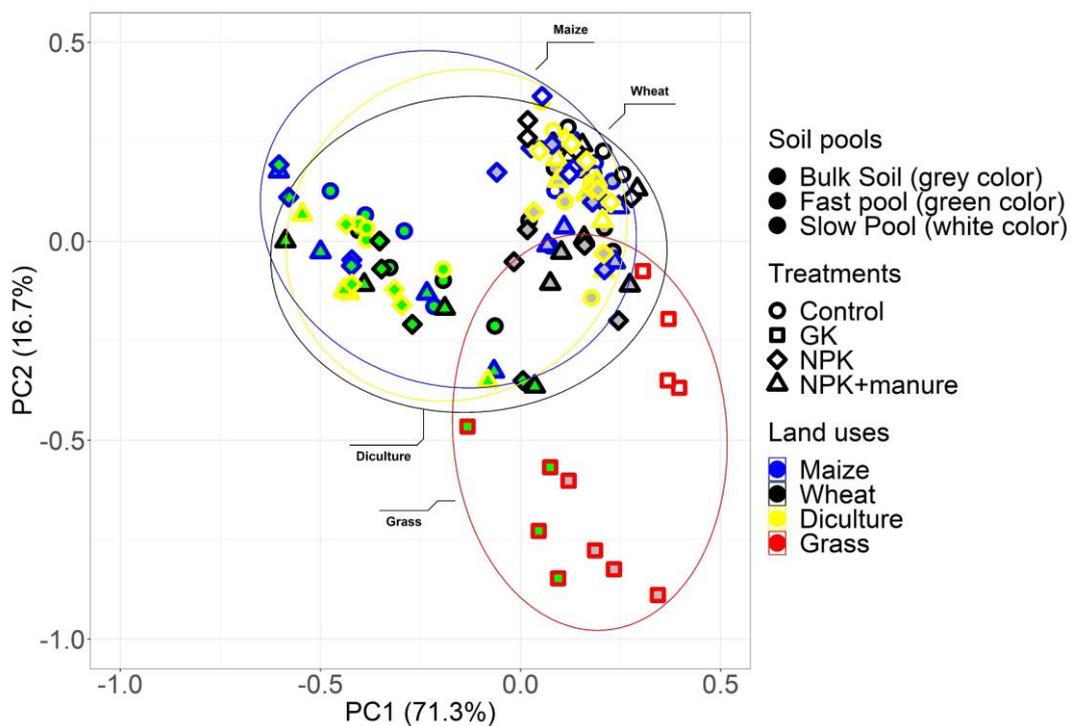
**Table 9.** The principal component results highlighted the structure of the loading matrix correlation between the SOM variables within the principal components (PCs), including grassland and cropland data for the spring sampling date (April 2019).

<i>Variables</i>	<i>Principle Component</i>	
	<i>PC1</i>	<i>PC2</i>
Total N	0.644	-0.002
Soil organic carbon	0.391	-0.313
C/N ratio	0.057	-0.471
Aliphatic C	0.297	-0.596
Aromatic C	-0.432	0.612
Amide N	-1.098	-0.051
Phenolic lignin	0.884	0.093
Polysaccharides	-1.063	-0.257
C/O ratio	1.041	0.314
Aromaticity	-0.645	0.225

Like August results, the grass is scattered along C/N, aliphatic, and SOC and was separated from the cropland (Figures 26 and 27). In contrast to August, the soil pool effects on SOM variables were evident in April (Figure S22). The fast pool was separated from the slow pool, and SOM variables in the bulk soil were between the slow and fast pools. Polysaccharides and C/O ratio were the main drivers between soil pools.



**Figure 26.** Biplot of the first two components showing the loading values in PC 1 and 2 within grassland and cropland in April and loading values in PC 1 and 2 for cropland and grassland in April. The explained SOM parameter variances were in brackets. NPK: nitrogen, phosphorus, and potassium. The following variables shortcut referred to (aromatic\_aliphatic\_C (aromaticity index), C\_O (C/O ratio), amide\_bond (amide N), SOC (soil organic carbon), N (total N), C\_N (C/N ratio)).



**Figure 27.** PCA results for the measured SOM variables, including grassland and cropland, in April. It highlighted the crop's effects on SOM variables. The SOC variances were explained

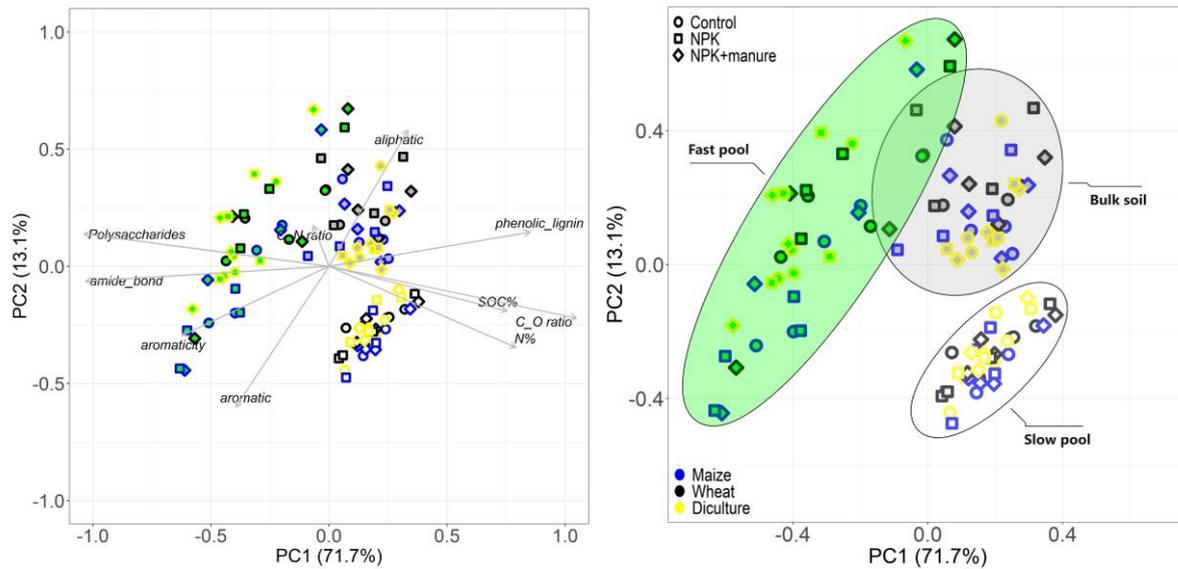
in brackets. The circled cloud indicated the effects of land uses (crops) on the measured SOM parameters.

Because grassland had distinct SOM properties from cropland (Figures 26 and 27), further PCA was done for only cropland data. The total N and SOC concentrations and C/O ratio were in opposite directions with aromaticity, amide N, and polysaccharides in PC1 (Table 10, Figure 28). The aromatic and the relative amount of aliphatic C components were in opposite directions in PC2.

**Table 10.** The principal component results highlighted the structure of the loading matrix correlation between the SOM variables within the principal components (PCs) in the cropland for the spring sampling date (April 2019).

<i>Variables</i>	<i>Principle Component</i>	
	<i>PC1</i>	<i>PC2</i>
Total N	0.790	-0.346
Soil organic carbon	0.753	-0.190
C/N ratio	-0.067	0.174
Aliphatic C	0.334	0.582
Aromatic C	-0.394	-0.599
Amide N	-1.032	-0.063
Phenolic lignin	0.851	0.146
Polysaccharides	-1.038	0.138
C/O ratio	1.046	-0.221
Aromaticity	-0.622	-0.295

No apparent effects from fertilization or plants on SOM variables were found (Figures S23 and S24). In contrast, the soil pool's effect was evident on SOM parameters (Figure 28). The slow and fast pools were wholly separated, while SOM variables in bulk soil were between the slow and fast pools. A total of (71.7%) of data variations were described in PC1 compared to PC2 (13.1%). Phenolic lignin, total N, SOC, and C/O ratio were scattered along the slow pool compared to fresh OM in the fast pool, as indicated by amide N and polysaccharides (Figure 28).



**Figure 28.** Biplot of the first two components showing the loading values in PC 1 and 2 (left side), and PCA results for the measured SOM variables (right side). It highlighted the soil pool's effects on SOM variables within cropland for April. The SOC variances were explained in brackets. The circled cloud indicated the soil pool's effects on the measured SOM parameters. The following variables shortcut referred to: (aromatic\_aliphatic\_C (aromaticity index), C\_O (C/O ratio), amide\_bond (amide N), SOC (soil organic carbon), N (total N), C\_N (C/N ratio)).

In parallel with PCA results, the interaction between plants, treatments, and soil pools did not significantly affect SOM variables. Nevertheless, grassland significantly differed from plants in cropland sites, using PERMANOVA. It confirmed the PCA results about the significance of only soil pool as a predominant factor affecting SOM variables. Furthermore, it emphasized the distinct characteristic of SOM variables in grassland compared to cropland sites ( $p$ -value $<0.05$ , Table S3 (C), section S9, supplementary).

### 5.7.3. The seasonal variations of solid-phase SOM compositions

In cropland, there was an increasing pattern in SOC concentration and C/N ratio toward summer sampling time compared to spring, especially in bulk soil and the fast pool (Table 11). In the slow pool, sampling time did not affect SOC total N concentration and the C/N ratio. In contrast, the C/N ratio and total N concentrations in the bulk soil were significantly affected by the sampling date. Furthermore, in the fast pool, SOC concentrations and C/N ratio were affected by sampling time. In grassland, SOC, total N concentrations, and C/N ratio were unaffected by the sampling date factor or did not change seasonally among soil C pools and bulk soil (Table 11).

**Table 11.** The SOC content and total N and C/N ratio in each soil pool and bulk soil over August and April sampling dates in grassland and cropland sites. SOC and total N: the soil organic carbon and total nitrogen percentage, respectively.

<i>Site</i>	<i>Date</i>	<i>Fraction</i>	<i>Total N (%)</i>	<i>SOC (%)</i>	<i>C:N ratio</i>
	August	Bulk soil	0.26±0.04	4.78±1.35	18.12±2.44
	April		0.27±0.05	4.36±1.56	15.65±3.06
Grassland	August	Fast pool	0.23±0.06	4.62±2.06	18.86±4.88
	April		0.21±0.04	3.44±1.3	15.79±2.57
	August	Slow pool	0.34±0.04	4.6±1.5	13.27±2.63
	April		0.34±0.03	4.41±0.91	12.62±1.52
	August	Bulk soil	0.21±0.02 a	2.55±0.39	12.11±1.4 a
	April		0.22±0.02 b*	2.37±0.37	10.59±0.99 b***
Cropland	August	Fast pool	0.19±0.03	2.31±0.55 a	11.99±1.68 a
	April		0.18±0.02	2.03±0.37 b*	11.11±1.18 b*
	August	Slow pool	0.25±0.01	2.65±0.29	10.23±0.55
	April		0.26±0.02	2.74±0.23	10.30±0.84

Different letters referred to the significant effects of the sampling date on each SOM variable. The sign (\*\*\*, \*\*, \*) referred to the test's significance and corresponding to each p-value < 0.001, < 0.01, < 0.05, respectively. Data was given a mean with standard deviation (n=8) for grassland and (n=72) for cropland. Total N and SOC: Soil organic carbon and total nitrogen concentration, respectively. Further information was outlined (section S10, supplementary).

For SOM composition in cropland, only polysaccharides and C/O ratio were seasonally changed in the slow pool (Table 12). The sampling time factor affected all the SOM compositions in the fast pool. In contrast, aliphatic C, amide N, and phenolic lignin were changed seasonally in bulk soil. In grassland, the SOM compositions stayed the same seasonally, especially in the slow pool. Furthermore, most SOM compositions were not changed seasonally in bulk soil and fast pools. For example, in the fast pool, aliphatic, aromatic C, and aromaticity were not changed seasonally, while in bulk soil, amide N, phenolic lignin, and aromaticity were not changed seasonally (Table 12).

**Table 12.** SOM compositions in each soil pool and bulk soil over August and April sampling dates in grassland and cropland sites.

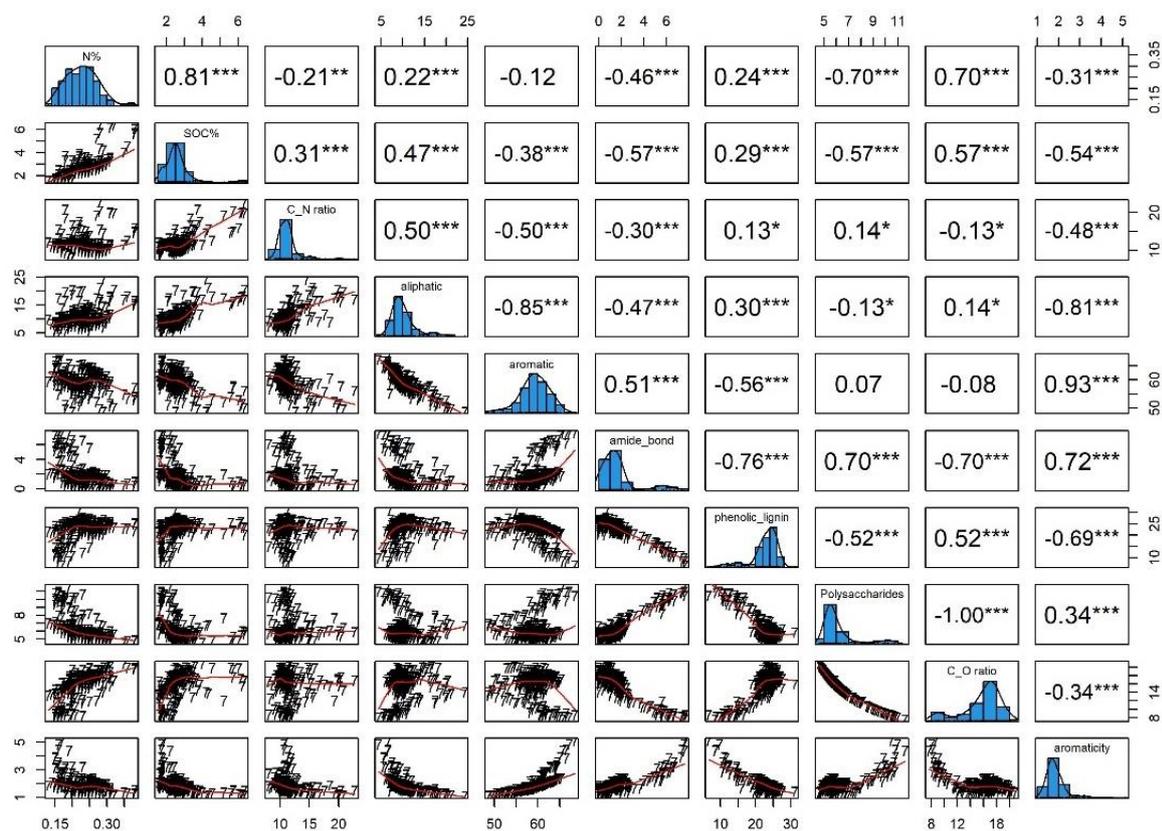
<i>Site</i>	<i>Date</i>	<i>Fraction</i>	<i>Aliphatic C</i>	<i>Aromatic C</i>	<i>Amide N</i>	<i>Polysaccharides</i>
	August	Bulk soil	17.64±1.76 a	53.45±3.05 a	0.17±0.18	5.56±0.18 a
	April		21.6±1.86 b*	49.27±0.66 b*	0.67±0.5	6.51±0.6 b*
Grassland	August	Fast pool	17.88±1.36	52.1±2.84	0.62±0.22 a	6.13±0.22 a
	April		18.45±1.14	52.36±1.78	2.58±0.8 b**	7.46±0.52 b**

	August	Slow pool	15.84±0.96	55.08±1.85	0.83±0.32	4.78±0.07
	April		16.08±1.83	54.64±1.22	0.71±0.22	4.71±0.09
	August	Bulk soil	9.68±1.58 a	58.63±1.66	0.63±0.41 a	5.63±0.32
	April		10.56±1.44 b**	57.92±1.81	1.02±0.62 b**	5.77±0.55
Cropland	August	Fast pool	10.35±1.6 a	58.12±1.79 a	1.57±0.67 a	6.21±0.5 a
	April		8.92±2.48 b**	61.55±3.63 b***	5.44±1.44 b***	9.58±1.08 b***
	August	Slow pool	8.87±1.38	61.4±1.8	1.3±0.58	5.29±0.27 a
	April		8.47±1.33	61.94±1.9	1.44±0.6	5.46±0.27 b*
<b>Site</b>	<b>Date</b>	<b>Fraction</b>	<b>Phenolic lignin</b>	<b>C:O ratio</b>	<b>Aromaticity</b>	
	August	Bulk soil	23.53±2.17	17.03±0.58 a	1.3±0.14	
	April		21.98±1.36	14.55±1.56 b*	1.13±0.03	
Grassland	August	Fast pool	23.23±2.12 a	15.57±0.97 a	1.28±0.15	
	April		19.14±2.19 b*	12.59±1.01 b**	1.4±0.16	
	August	Slow pool	23.46±1.3	19.93±0.29	1.4±0.1	
	April		23.85±1.12	20.28±0.46	1.37±0.07	
	August	Bulk soil	25.59±1.57 a	16.83±1.02	1.67±0.15	
	April		24.72±1.59 b*	16.52±1.57	1.65±0.15	
Cropland	August	Fast pool	23.74±1.56 a	15.22±1.36 a	1.72±0.18 a	
	April		14.47±3.96 b***	9.61±1.31 b***	2.86±0.9 b***	
	August	Slow pool	23.12±1.38	17.93±0.95 a	1.93±0.19	
	April		22.67±1.79	17.36±0.96 b*	2±0.22	

Different letters referred to the significant effects of the sampling date on each SOM variable. The sign (\*\*\*, \*\*, \*) referred to the test's significance and corresponding to each p-value < 0.001, < 0.01, < 0.05, respectively. Data was given a mean with standard deviation (n=8) for grassland and (n=72) for cropland. Further information was outlined (section S10, supplementary).

SOM variables were not significantly affected by the sampling date and their interaction effects with plants and treatment factors for the slow pool in cropland (Table S4, PERMANOVA, pairwise comparisons, section S10, supplementary). Nevertheless, the measured SOM variables were significantly affected by the sampling date for the fast pool and bulk soil (Table S5 and S6). In grassland, all SOM variables were not significantly affected by sampling time in the slow pool (Table S7), within a marginal significance of sampling date effects on the measured SOM variables in both fast pool and bulk soil (>0.05 p-value <0.07) (Table S8 and S9).

Including both summer and spring sampling dates, correlation results between the measured SOM variables were not considerably different from those on each date (Figure 29).



**Figure 29.** The relationship between the measured SOM parameters for both sampling dates (n=240). The significance level was calculated using Spearman's correlation coefficients ( $\rho$ ). The sign (\*\*\*, \*\*, \*) referred to the test's significance and corresponding to each p-value < 0.001, < 0.01, < 0.05, respectively. C\_N (C/N ratio); amide\_bond (amide N); and C\_O (ratio of C/O functional groups).

Using PCA, Grassland also showed a distinct SOM composition for summer and spring sampling dates (Figure S25). Total N and SOC concentrations, C/O ratio, and phenolic lignin were in opposite directions with amide N, polysaccharides, and aromaticity (Table 13). These SOM variables had a higher loading value in the first component (PC1). The second component (PC2) had a high loading for a relative amount of aliphatic and aromatic C components in opposite directions in PC2. PC1 explained 62.2% of the variation in chemical properties of SOM compared to PC2 (20.1%). The relative amount of aliphatic C component ratio and SOC concentration were scattered along grassland, separated from the cropland. Neither SOM variables were different between soil C pools (Figure S26) nor affected by sampling date (S27).

**Table 13.** The principal component results highlighted the structure of the loading matrix correlation between the SOM variables within the principal components (PCs) in both sites, including sampling dates.

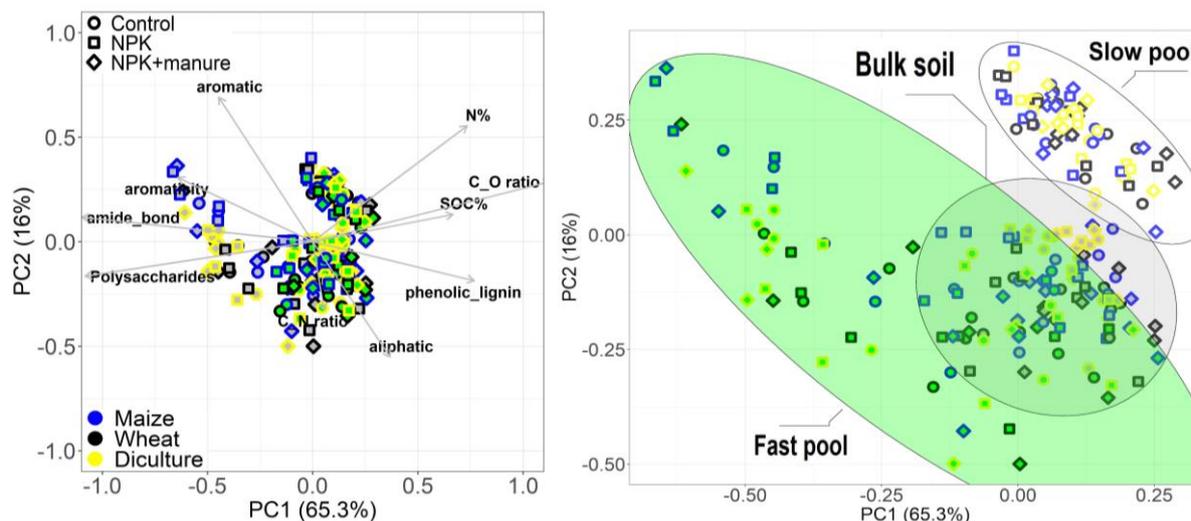
<i>Variables</i>	<i>Principle Component</i>	
	<i>PC1</i>	<i>PC2</i>
Total N	0.637	-0.054
Soil organic carbon	0.539	-0.525
C/N ratio	0.201	-0.607
Aliphatic C	0.375	-0.661
Aromatic C	-0.491	0.626
Amide N	-1.126	-0.131
Phenolic lignin	0.753	0.170
Polysaccharides	-1.064	-0.362
C/O ratio	1.082	0.401
Aromaticity	-0.648	0.154

Because grassland had distinct SOM properties from cropland, further PCA was conducted for cropland data only. Total N and SOC concentration, phenolic lignin, and C/O ratio were in opposite directions with aromaticity, amide N, and polysaccharides in PC1 (Table 14). At the same time, aromatic and aliphatic ratios were in opposite directions in PC2. A total of (65.3%) of data variations were described in PC1 compared to PC2 (16%). In contrast, SOM variables in the slow and fast pools were wholly separated, while SOM variables in the bulk soil were between the slow and fast pools (Figure 30). No apparent effects from fertilization, crops, or sampling time factor on the SOM variables were detected (Figures S27-S29). Total N and SOC concentrations and C/O ratio were the main drivers separating the slow pool, indicating fresh OM in the fast pool by containing amide N and polysaccharides with high aromaticity.

**Table 14.** The principal component results highlighted the structure of the loading matrix correlation between the SOM variables within the principal components (PCs) in the cropland, including both sampling dates.

<i>Variables</i>	<i>Principle Component</i>	
	<i>PC1</i>	<i>PC2</i>
Total N	0.734	0.555
Soil organic carbon	0.667	0.131
C/N ratio	0.054	-0.325
Aliphatic C	0.368	-0.551
Aromatic C	-0.446	0.691
Amide N	-1.098	0.117

Phenolic lignin	0.768	-0.186
Polysaccharides	-1.082	-0.165
C/O ratio	1.130	0.288
Aromaticity	-0.641	0.307



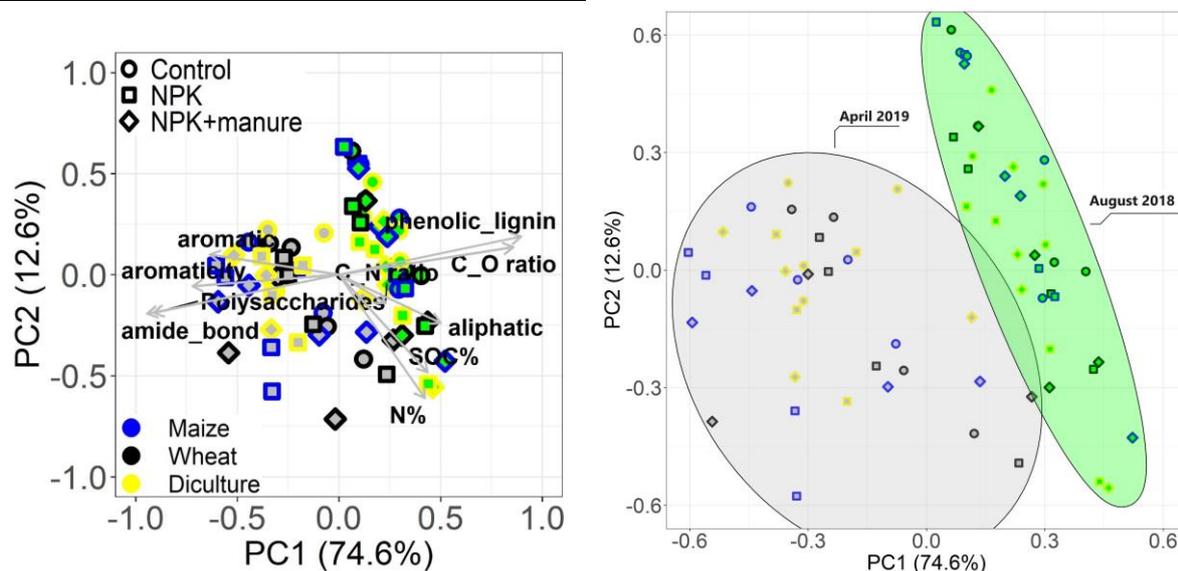
**Figure 30.** Biplot of the first two components showing the loading values in PC 1 and 2 (left side), and PCA results for the measured SOM variables (right side) for both sampling dates within cropland. The SOC variances were explained in brackets. NPK: nitrogen, phosphorus, and potassium. NPK: nitrogen, phosphorus, and potassium. The circled cloud indicated the soil pool’s effects on the measured SOM parameters.

Because there was a high variation in the fast pool (Figure 30), only the fast pool was further studied by an additional PCA analysis. PC1 contained phenolic lignin and C/O ratio, which were in opposite directions with the ratios of aromatic components, aromaticity, polysaccharides, and amide N, explaining 74.6% variance (Table 15, Figure 31). SOC and total N concentration were in PC2, with an additional 12.6% variance explained. The sampling date effects on the SOM variables in the fast pool were apparent. In contrast, no apparent effects from fertilization and plant factors on the SOM variables were found (Figures S30 and S31).

**Table 15.** The principal component results highlighted the structure of the loading matrix correlation between the SOM variables within the principal components (PCs) in the fast pool, including both sampling dates in cropland.

<i>Variables</i>	<i>Principle Component</i>	
	<i>PC1</i>	<i>PC2</i>
Total N	0.422	-0.610
Soil organic carbon	0.436	-0.482
C/N ratio	0.229	-0.140
Aliphatic C	0.500	-0.238
Aromatic C	-0.634	0.100

Amide N	-0.943	-0.193
Phenolic lignin	0.896	0.190
Polysaccharides	-0.912	-0.189
C/O ratio	0.857	0.137
Aromaticity	-0.720	-0.057



**Figure 31.** Biplot of the first two components showing the loading values in PC 1 and 2 (left side), and PCA results for the measured SOM variables (right side) for both sampling dates for the fast pool within cropland. The measured SOM parameter variances were explained in brackets. NPK: nitrogen, phosphorus, and potassium. The following variables shortcut referred to: (aromatic\_aliphatic\_C (aromaticity index), C\_O (C/O ratio), amide\_bond (amide N), SOC (soil organic carbon), N (total N), C\_N (C/N ratio)). The frame color for each geometrical shape referred to plant effects on SOM variables.

### 5.8. Relationships between DOM parameters and the solid-phase-related SOM pools

In the former section, there were differences in SOM compositions, especially in fast pool compositions, between the August 2018 and April 2019 sampling dates in cropland (Figure 31). These differences might result from differences in DOM contribution in the solid-phase SOM within each date.

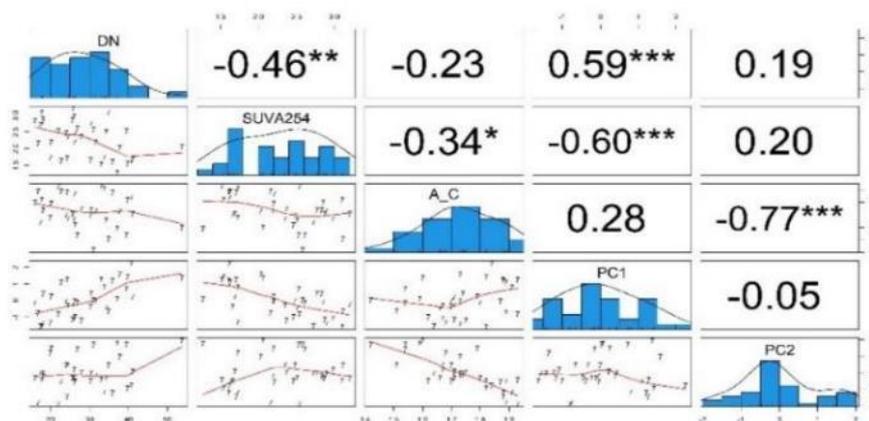
Because there were significant differences in DOM parameters between the August 2018 and April 2019 sampling dates (Table 5), a principal component analysis was made for August and April SOM-solid phase variables to correlate each PC with DOM parameters. In August, the slow pool had two PCs with a total explained variance (86.6%). SOC, total N, aromatic and the relative amount of aliphatic C ratio, and aromaticity were contained in the first PC, explaining about 69.2% of the data variance compared to PC2, which includes polysaccharides and phenolic lignin, explaining 17.4% of the data variation (Table 16). The highest correlation was reported between PC1 and SUVA index compared to PC2, which

correlated with the A/C index (Figure 32, Spearman's correlation,  $\rho = -0.60$ ,  $\rho = -0.77$ , respectively, p-value < 0.001).

**Table 16.** The rotated principal component results for (cropland, August sampling date). It highlighted the loading matrix correlation structure between the solid-phase SOM variables within the principal components (PCs) for the slow pool.

<i>Variables</i>	<i>Principle Component</i>	
	<i>PC1</i>	<i>PC2</i>
Total N	0.92	-0.027
SOC	0.894	-0.239
Aliphatic C	0.821	-0.373
Aromatic C	-0.912	0.283
Amide N	-0.476	0.783
Phenolic lignin	0.62	-0.528
Polysaccharides	-0.169	0.965
C/O ratio	0.176	-0.962
Aromaticity	-0.859	0.459

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization<sup>a</sup>. <sup>a</sup>Rotation converged in 3 iterations.



**Figure 32.** The correlation plot showing the relationship between the measured solid-phase SOM for the slow pool (cropland, August sampling date) within each PC with DOM parameters. The significance level was calculated using Spearman's correlation coefficients ( $\rho$ ). The sign (\*\*\*, \*\*, \*) referred to the test's significance and corresponding to each p-value < 0.001, < 0.01, < 0.05, respectively. PC1 (first principal component); PC2 (second principal component); DN (total dissolved nitrogen); A\_C (A/C index), sample size (n=36).

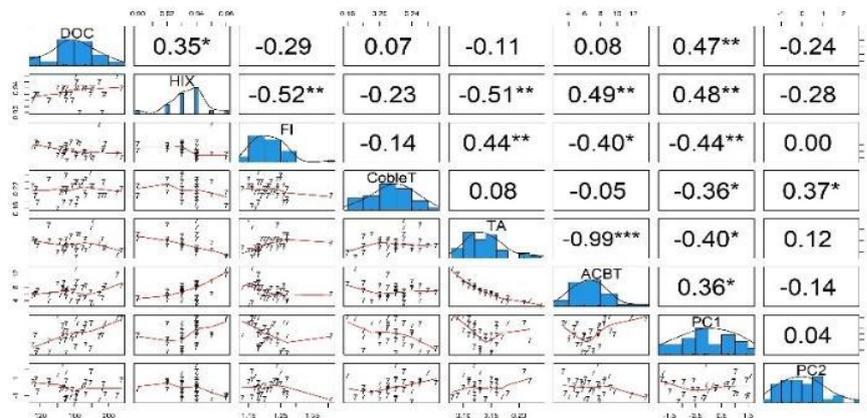
Similarly, PCA was done for the slow pool for April sampling dates, and a total explained variance in both PCs was (84.9%). All SOM compositions besides total N were contained in the first PC (Table 17), explaining about 67.6% of the data variance compared to PC2, which includes the C/N ratio, explaining 17.3% of the data variation. All DOM

parameters had a weak correlation with solid-phase SOM in April. For example, the highest correlation reported between PC1 and HIX was recorded ( $\rho = 0.48$ ) (Figure 33, Spearman's correlation).

**Table 17.** The rotated principal component results for (cropland, April sampling date). It highlighted the loading matrix correlation structure between the solid-phase SOM variables within the principal components (PCs) for the slow pool.

<i>Variables</i>	<i>Principle Component</i>	
	<i>PC1</i>	<i>PC2</i>
<i>Total N</i>	-0.869	-0.144
<i>Aromatic C</i>	0.962	0.056
<i>Amide N</i>	0.758	-0.556
<i>Phenolic lignin</i>	-0.894	-0.04
<i>Polysaccharides</i>	0.763	-0.508
<i>C/O ratio</i>	-0.761	0.507
<i>Aromaticity</i>	0.974	-0.083
<i>C/N ratio</i>	0.187	0.86

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization<sup>a</sup>. <sup>a</sup>Rotation converged in 3 iterations.



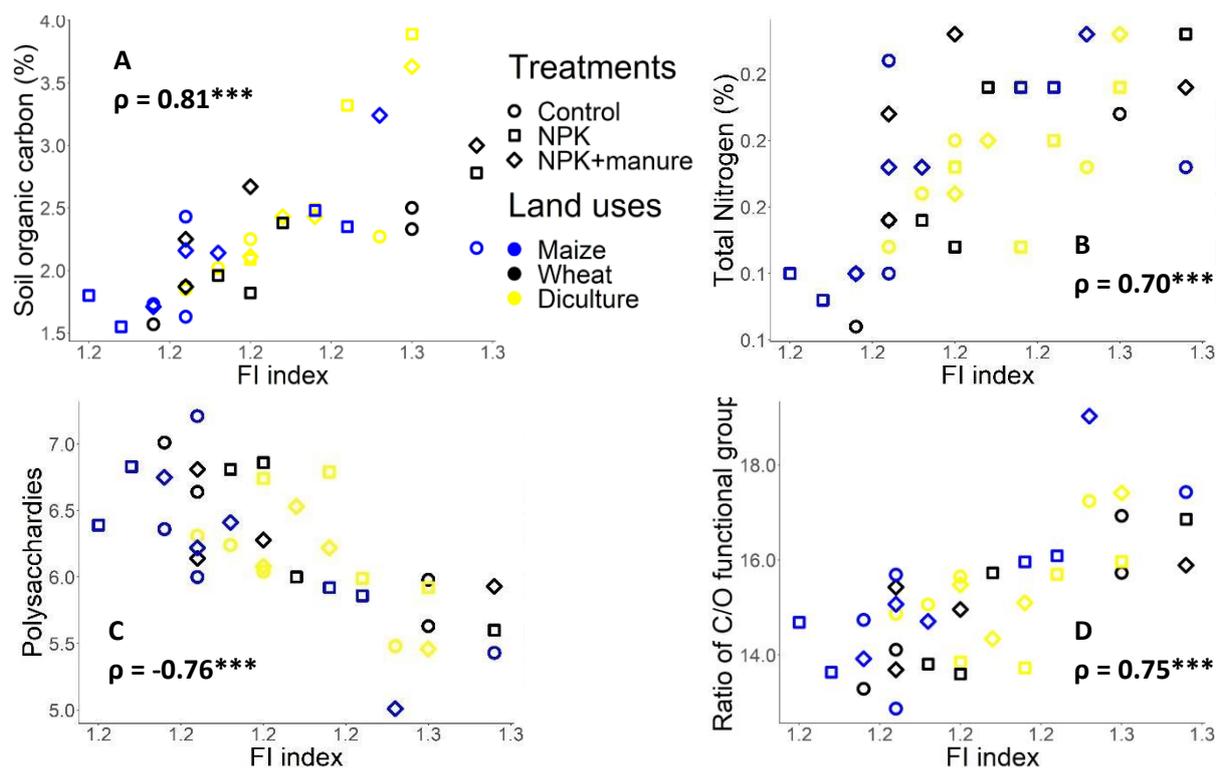
**Figure 33.** The correlation plot showing the relationship between the measured solid-phase SOM for the slow pool (cropland, April sampling date) within each PC with DOM parameters. The significance level was calculated using Spearman's correlation coefficients ( $\rho$ ). The sign (\*\*\*, \*\*, \*) referred to the test's significance and corresponding to each  $p$ -value  $< 0.001$ ,  $< 0.01$ ,  $< 0.05$ , respectively. PC1 (first principal component); PC2 (second principal component); DOC (dissolved organic carbon); FI (fluorescence index), TA (T/A index), and ACBT ((A+C)/(B+T)), sample size (n=36).

At the beginning of the current section, solid-phase SOM variables were reduced into two PCs and correlated with each DOM variable in the slow pool over each sampling date. No further investigations were done for the fast pool and bulk soil for each date since only August

2018 was proved to have a higher correlation value with DOM parameters than April 2019. Based on that, a new individual correlation was conducted to know which solid-phase SOM variable correlated with each DOM for only the August sampling date data.

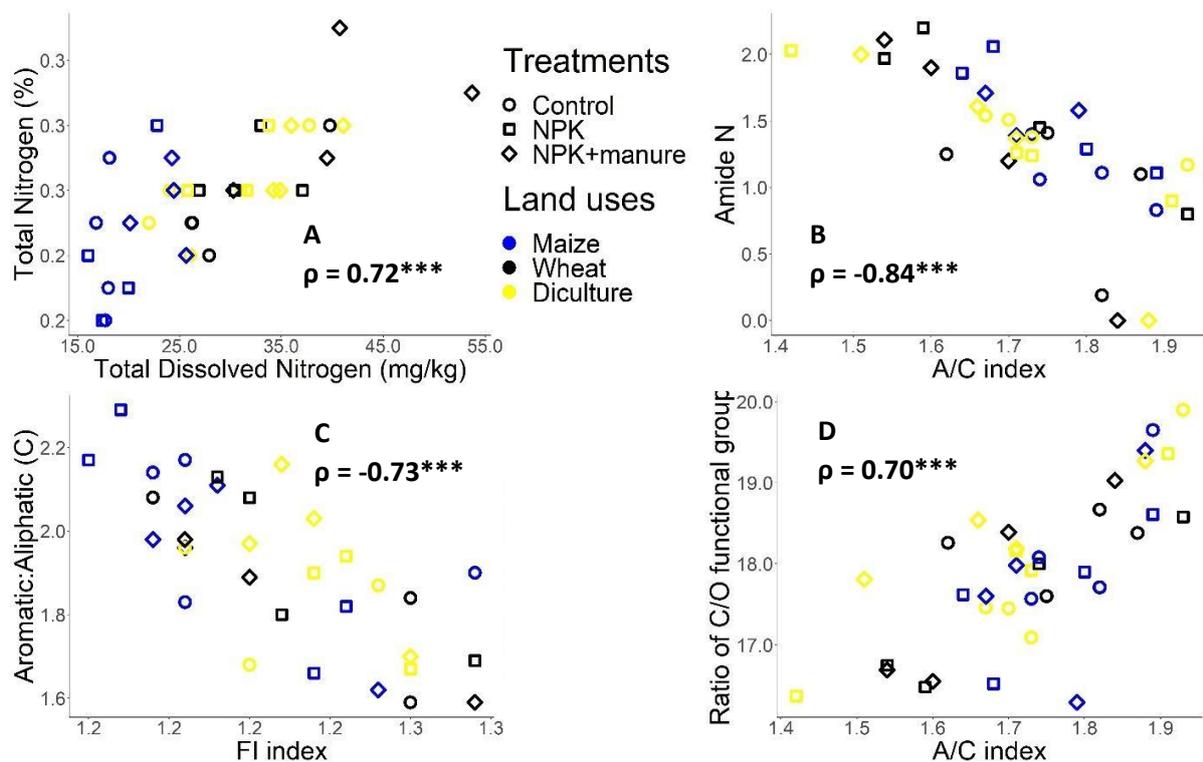
Significantly, most DOM compositions correlated, with considerable correlation coefficient values ( $>0.5$ ), with all soil C pools (slow and fast pools) and bulk soil in August, not April. These correlations were significant regardless of plants and treatments (Figure 34). A decision was made to show only correlation coefficient values at a threshold ( $\rho \geq 0.7$ ).

In the fast pool, total N and SOC concentrations significantly and positively correlated with the FI index (Figure 34 A and B). Polysaccharides were negatively correlated with the FI index, while the C/O ratio was positively correlated (Figures 34, C, and D). The relative amount of aliphatic C and aromaticity were significantly correlated with the FI index (Spearman's correlation,  $\rho = 0.71$ ,  $\rho = -0.70$ , respectively,  $p$ -value  $<0.001$ ). Furthermore, polysaccharides and C/O ratio were significantly correlated with the A/C index (Spearman's correlation,  $\rho = -0.71$ ,  $\rho = 0.71$ , respectively,  $p < 0.001$ ).



**Figure 34.** The correlation plots showing the relationship between the fluorescence index (FI) index with each soil organic carbon and total nitrogen concentrations (Plots A and B). Fluorescence index (FI) with polysaccharides and the C/O ratio (plots C and D). These correlations between SOM pools were for the August date. One legend was displayed for all in the plot (A). The significance level was calculated using Spearman's correlation coefficients ( $\rho$ ). The sign (\*\*\*) referred to the test's significance corresponding to the  $p$ -value  $< 0.001$ . Sample size ( $n=36$ ).

In the slow pool, the total N concentration was positively correlated with TDN. In contrast, amide N was negatively correlated with the A/C index (Figure 35 A and B, (Spearman's correlation,  $\rho = 0.72$ ,  $\rho = -0.84$ , respectively,  $p$ -value  $< 0.001$ ). Aromaticity was negatively correlated with the FI index, while the C/O ratio was positively correlated (Figure 35 C and D, (Spearman's correlation,  $\rho = -0.73$ ,  $\rho = -0.70$ , respectively,  $p$ -value  $< 0.001$ ). Furthermore, phenolic lignin and polysaccharides were significantly correlated with the A/C index of DOM (Spearman's correlation,  $\rho = 0.71$ ,  $\rho = -0.70$ , respectively,  $p$ -value  $< 0.001$ ). All correlations were significant, with no apparent effects from treatments or plants on these SOM variables, except maize, which had minor overlap with other plants ( Figure 35, A).



**Figure 35.** The correlation plots showing the relationship between total dissolved nitrogen of DOM with total nitrogen concentration and A/C index with amide N (Plots A and B). Aromaticity (aromatic:aliphatic ratio) with fluorescence (FI) index (plot C) and C/O ratio correlation with A/C index (plot D) in August. The significance level was calculated using Spearman's correlation coefficients ( $\rho$ ). The sign (\*\*\*) referred to the test's significance and the  $p$ -value  $< 0.001$ . Sample size ( $n=36$ ).

Similar correlation results were found between SOM variables in the bulk soil and DOM parameters in August, but with fewer correlated variables than slow and fast pools. Specifically, there was a positive significant correlation between SOC and FI index of DOM ( $\rho = 0.70$ ,  $p$ -value  $< 0.001$ ) while amide N was significantly and negatively correlated with FI and A/C indices (Spearman's correlation,  $\rho = -0.75$ ,  $\rho = -0.71$ , respectively,  $p$ -value  $< 0.001$ ).

## **6. Discussion**

### **6.1. Fertilization does not affect DOC concentration on each sampling date**

In contrast with the present hypotheses, DOM parameters were not statistically proven to be modified by either treatments or tillage within each sampling time (Figures 9, 11, 12, and S12). (Tiefenbacher et al., 2020) found that mineral (calcium ammonium nitrate) and organic fertilizations (pig slurry) decreased DOC concentration in the leachate, using lysimeters in sandy and silty loam soils. In the present study, the lack of considerable differences in DOC concentration between treatments can be related to tillage practice.

In a short timescale, tillage can reduce fertilization effects on DOC concentration. For example, tillage may cause a tradeoff between fertilization and soil structure to increase crop yield. Previous studies found that tillage systems changed soil properties, including DOM, but did not affect yield production (Cookson et al., 2008; Roper et al., 2010). For example, tillage can increase soil aeration and accelerate soil oxidation. In turn, decomposition processes can instantly respond to a change in soil condition and affect DOM compositions.

Tillage can expose labile OM or the applied fertilizers to decomposition. Organic fertilization was considered a sustainable practice (Lugato et al., 2014) to mitigate agricultural soil degradation by increasing SOC concentration. Nevertheless, the priming effects were also proposed to accelerate SOM decomposition due to fertilization, similar to the OM addition effects on SOM pools in forest soils (Lajtha et al., 2018).

Tillage effects on DOC concentration can be noted from high aromaticity ( $SUVA_{254}$  value) in cropland compared to grassland (Figure 6). It showed that decomposition had transformed DOM composition with no increase in concentration. For example, DOC concentration can be partly increased as a byproduct of decomposition. Nevertheless, DOC can also be a food source for microbial activities, which can lead to no net or overall effect on DOC concentration in each sampling date.

### **6.2. DOM parameters over three years of fertilization**

There were differences in DOC concentration after three years rather than on each sampling date. For example, DOC concentration in the NPK+manure treated-soil plot was approximately 19% higher than the control plot after three years. It partly agreed with an increase in DOC concentration in the O horizon, resulting from long-term fertilization in undisturbed soil (using a lysimeter in N-limited soils from Norway spruce forest) (Fröberg et

al., 2013). Furthermore, the applied mineral fertilization was changed seasonally, such as a doubled applied amount in August compared to April (Table 3). It can be proposed that the SOM decomposition can respond to these changes due to N input leading to a change in soil microbiome condition.

The soil condition was proposed to control DOC concentration in arable soil, similar to what was found about DOC concentration sensitivity to drought conditions rather than fertilization in sandy soil (Zsolnay & Gorlitz, 1994). The present study partly agreed that fertilization effects on the DOM pool could differ based on the amount and the applied quality (Ohno et al., 2007) besides soil cultivation practices, including tillage.

The present work partly agreed with previous work about the role of tillage in affecting DOM molecular composition. Most DOM parameters were unaffected by fertilization, with a more significant trend in HIX or complexity of DOM composition. A change in DOM composition was more related to a seasonal variation affecting the complex nature of OM in certain sampling times than others due to cultivation, including tillage (Figure S2). For example, higher aromaticity and humified SOM were found under conservation than conventional tillage practices (Jakab et al., 2022). Furthermore, the activity of soil microorganisms can be changed based on soil aggregate status (Bai et al., 2018). Hence, decomposition can respond to these changes in soil structure and affect DOM composition (Gmach et al., 2020).

An addition of organic fertilization can be adsorbed onto a mineral phase or another way to be mineralized based on soil microbiome condition. This assumption partly agreed with (Angers et al., 2006), finding that the DOM pool's seasonal variations were more predominant than manure additions. The present study proposed that fertilization effects on DOM composition were not straightforward due to intense cultivation. Furthermore, the applied manure was two years before the soil sampling campaign started (Table 3). Hence, manure can be decomposed by microorganisms and utilized by plants with no net increase in DOC concentration under arable soil conditions.

### **6.3. Land use affected DOM composition seasonally**

Seasonal fluctuation effects exceed the fertilization and plant effects on DOM parameters. Besides the sensitivity of DOM to a soil microbiome condition, in a chaotic soil system, DOM composition can have various molecular compositions ranging from easily decomposable sugars to complex phenolic lignin (Kalbitz et al., 2007). Such fertilizer (e.g.,

manure) addition can increase DOM nature to be more aromatic (Zsolnay et al., 1999) or complex molecular structure.

Due to various soil management in arable soil, DOM temporal variations were more detectable in grassland than in cropland. For example, the apparent seasonal differences in DOM parameters were detected between April and September 2019 and August 2018 (Figure 10). The highest increase in DOC concentrations was detected in August 2018, followed by April 2019, and the lowest was in September 2019 (Figure 4). A previous study found a higher DOC concentration in May and November (van den Berg et al., 2012). It showed the significance of seasonal climatical conditions controlling DOM variation. Nevertheless, (Wickland et al., 2007) found that DOM's biodegradability and chemical properties had no seasonal pattern in boreal regions. Further added, DOM had the lowest biodegradable content at not-drained sites.

There was a weak correlation between DOM parameters and weather data (Figure S15). The weak correlation can come from the weather data related to the soil's nature as a chaotic and heterogeneous system. It can stress the importance of considering soil microbiome conditions in studying the DOM pool (Al-Graiti et al., 2022).

Previous works had contradictory results regarding soil temperature effects on DOC concentration. For example, DOC production was increased by 20–135% in warmer conditions than at colder temperatures in Gelisol (using an incubation experiment in Alaska) (Neff & Hooper, 2002; O'Donnell et al., 2016). Nevertheless, warming conditions did not affect DOC concentrations in O and B horizons in Norway spruce forests (Fröberg et al., 2013).

Furthermore, Embacher et al., 2007 found that seasonality was the primary factor affecting DOC variations in three arable soils (Cambisol, Luvisol, and Chernozem). Still, that study worked on a high spatial difference between the studied sites (>600 km). In the present work, a higher biological production or contribution in DOM composition was in the summer season (August 2018) than in springtime (April 2019) (Figure 7 and Table 5). For example, as a change in biological production and decomposition or transformation degree of DOM acted in the same direction (Table 6), it showed that a change in microbiological activity could affect DOC concentration and their humic-related OM indicators seasonally.

#### **6.4. Soil management affected DOM properties by nitrogen input**

The C/N in the present work varied from 1 to 13 (Figure 5). Based on the C/N ratio within the soil microbiome, decomposition processes can be changed and affect the OM

stability. For example, OM with a high C/N ratio tends to be slowly decomposed compared to OM with a high N content or low C/N ratio. The C/N ratio was reported to range between 5 and 8 in the case of microbial biomass (Vance et al., 1987), while 12 and 30 for terrestrial humus (Aitkenhead & McDowell, 2000). Furthermore, when C/N ratio values ranged between 20 and 200, it indicated plant litter (Sardans et al., 2012).

There were seasonal differences in the amount of applied mineral fertilization in cropland (Table 3). Even though the soil C/N ratio was reported previously to range between 10:1–12:1 (Sparks, 2003) with an ideal averaged C/N ratio of 10:1 for agricultural soil systems, the lowest C/N ratio values (<5) were found after NPK fertilization in cropland (Figure 5). It indicated the influence of N fertilizers on the C/N ratio in arable Chernozem soils. Hence, a seasonal fluctuation of the C/N ratio in grassland was more evident than in cropland due to fertilization. For example, the C/N ratio was higher in springtime (April 2019) than in growing seasons, which may indicate a change in DOM composition.

A higher DOM complex molecular composition in spring 2019 than the microbial activities in growing seasons was detected (Figure S2). For example, the HIX value across all plants and treatments in spring 2019 was >0.9 and more than other dates. It explained a change in DOM aromaticity and complexity seasonally. It further proposed that soil microbiome conditions can be changed seasonally in responding to cultivation, including the seasonal applied mineral fertilization, affecting the DOM decomposability and composition.

Hence, the present work suggested that the C/N ratio was not only a significant indicator for soil C gain and loss (Kindler et al., 2011; Toosi et al., 2012; USDA-NRCS, 2011) but also for a change in DOM composition. It suggested that DOM complexity can be affected by C/N ratio ranges controlling microbiota and OM degradation.

In line with the C/N ratio results, a low biological production index in spring 2019 compared to a high biological production of DOM value during autumn (September 2018) followed by the summer times was detected (Figure 7)—the present results indicate seasonal microbial contribution to DOM production. A lower BIX value in spring indicated a complex organic matter. A more complex DOM molecular composition in the spring than the prior summer (Table 5) aligned with Coble's peak as a lower Coble's peak B with a higher A/C index was found (Figures S5 and S10, supplementary).

The variations in DOM parameters could come from a great extent of seasonal climate effects. For example, the complexity of riverine DOM might be reduced by a period of soil dryness (Wilson & Xenopoulos, 2009) or a change in DOC concentration due to a rainfall event

(van den Berg et al., 2012). Hence, the present results proposed that temporal patterns drove DOM composition in certain sampling times than others by affecting microbiological activities.

N fertilization significantly reduces the C/N ratio (Figure 11), which might affect the decomposition state of SOM. For example, the C/N ratio was an indicator for a stoichiometric correlation in which microbial activity controls C-N balance, describing the OM degradation (Manzoni & Porporato, 2009). Hence, decomposition processes can immobilize N to decompose OM with high C/N or OM quality, and TDN concentration can respond instantly to a change in C/N ratio in arable Chernozem soil.

A weak correlation between TDN and DOC concentrations was found. It proposed that TDN concentration can be subjected to mineralization and immobilization processes based on soil microbiome conditions and due to cultivation practice (e.g., N fertilization). Furthermore, the negative relationship between Coble's peak B and HIX index (Figure 12) was found. It highlighted that decomposition or microbial-related DOM contents (amino acid or protein derivatives) can be increased with a decrease in DOM complexity due to high microbial activity, accelerating the degradation of large biomolecules.

## **6.5. Changes in soil aggregate stability**

Across both sampling dates, tillage pressure decreased aggregate stability in cropland compared to grassland (Figure 13). In cropland, the topsoil layer was tilled up to 20 cm (section 4.1). In contrast, intact grassland soils might preserve permanent roots and earthworm activities, improving soil structure. Previous works found that SOC, TN, phenol oxidase and dehydrogenase activity increased under native perennial plants and decreased under intensive tillage (Bai et al., 2018; Veum et al., 2014).

In the present work, tillage effects on soil structure in arable soil were evident as soil aggregate stability was changed between land uses. Previous work also found that tillage negatively affected soil aggregation (Kahlon et al., 2013) in agricultural soil characterized by Stagnic Luvisol. A change in soil aggregate stability reflected a change in cultivation practice.

Soil aggregate stability was affected by fertilization rather than crops within cropland in August 2018, and opposite results were found in April 2019 (Figure 13). For example, soil aggregate stability was increased under NPK+manure treated plot soil in August, not April. It can come from the doubled fertilizer amount applied in August compared to April, especially under dicutlure soil plots (Table 3). In Central Europe, macroaggregate stability was increased from a doubling detrital input compared with regular litter inputs (Juhos et al., 2021). Hence,

these results further showed the importance of fertilization plants, which seasonally changed soil aggregate stability in arable Chernozem soil.

Furthermore, soil aggregate stability was seasonally changed in cropland, not grassland (Figure 13). In general, coarser soil aggregates were more susceptible to cultivation and environmental conditions (wet and dry) than soil microaggregates (Tisdall & Oades, 1982). In the present work, the seasonality of soil aggregate stability might come from a different N fertilization plan in August compared to April (Table 3).

## **6.6. Relationship between SOC concentration and SOM composition**

In the present work, a considerable positive correlation existed between SOC and total N concentration (Figures 21 and 25) compared to a weak relationship between TDN and DOC concentration (Figure 8). It can indicate that a stoichiometric dynamic of SOC with total nitrogen in OM was more apparent in solid-phase-related SOM than in the DOM pool.

Furthermore, amide N was positively correlated with polysaccharides and negatively with phenolic lignin. The negative correlation can indicate the recalcitrance of the organic structure of lignin compared to hydrolyzable microbial and faunal-based components in polysaccharides (Lützow et al., 2006). It can propose an increase of microbial-based compounds in a soil microbiome with declining recalcitrance organic compounds in the arable Chernozem soil. Generally, a biological amide bond can be created in ribosomes as complex proteins gather amino acids. Nevertheless, many enzymes, such as alcalase, could also create amides (Pitzer & Steiner, 2016).

These associations between SOM variables can further indicate a potential change in SOC concentration and SOM composition, which requires further work to study the amount of necromass in SOM pools in arable soil seasonally. Necromass refers to fragments of the cell envelope, enzymes, ribosomes, and other dead materials of microbial cells (Liang et al., 2019). For example, an enrichment of the  $N^{15}$  isotope in the occluded POM pool was suggested to be related to fungal hyphae contributing to the POM pool during fractionation (Mueller et al., 2009), while protein was found in organic-mineral silt and clay pools (Bol et al., 2009). Hence, the present findings highlighted the nature of SOM, which can be enriched with necromass or microbial remains.

## 6.7. Land use affects SOM stability and C storage

More SOC and total N concentrations were in grassland than in cropland on each sampling date, in soil pools (slow and fast pools) and bulk soil (Figures 14 and 15 ). The present results partly agreed with a change in land use from native forest to cropland, which decreased soil C stocks by 42% (Guo & Gifford, 2002). It further demonstrated why a change of 5% of grassland to cropland leads to a C loss of 300 Mt CO<sub>2</sub> eq. over a 50-year timescale. C loss negatively affected alternative soil management, including fertilization (Lugato et al., 2014).

The following previous works did not specifically study SOC in slow and fast pools but instead soil aggregate formation and stability related to C stability. For example, tillage was found to reduce iPOM and destroy stable microaggregate, a basic unit of macroaggregate formation (Six et al., 1998). That leads to a C loss (Balesdent et al., 2000; Six et al., 1999; Verchot et al., 2011; Weidhuner et al., 2021). Furthermore, the no-tillage practice had increased SOM stabilization compared to CT due to increased crop-derived C in microaggregate, reducing the macroaggregate turnover rate (Six et al., 1999). In the present work, when SOC concentration within the slow pool was significantly low in cropland compared to grassland, it showed the extent of tillage effects on C storage. Tillage destroyed the soil matrix and negatively affected SOM stability, and the secured C inside the slow pool was reduced (soil microaggregate scale, Figures 1 and 2).

The high extent of tillage effects on SOM variables in the present work can be explained by the C/N ratio (Figure 16). The C/N ratio of SOM varied from 12 to 18 in grassland and 10-12 in cropland. A change in the C/N ratio can indicate tillage effects on soil microbiome conditions and OM stability, as the meaning of C/N ratio ranges was outlined (section 6.4). For example, C/N ratio significance was stated as an indicator for a stoichiometric correlation in which microbial activity controls C-N balance and OM degradation (Manzoni & Porporato, 2009).

Related to the present work, (Jakab et al., 2023) found that conventional tillage negatively affected SOC concentration in fast and slow pools. Hence, a high soil aggregate stability in grassland compared to cropland revealed a significant tillage effect on soil structure and C storage (Figure 14). In contrast, intact soil in grassland soil can be proposed to maintain plant-derived OM residues. It might increase soil microaggregate formation and coarser aggregate (e.g., macroaggregates) stabilization and C storage, which was slated to be affected by soil aggregate dynamics (Six et al., 2000).

## **6.8. SOM compositions changed among land uses**

The lowest aromatic C ratio and highest relative amount of aliphatic C component in grassland than cropland showed that these SOM compositions were the most sensitive SOM parameters to tillage. For example, the relative amounts of aliphatic and aromatic C components parameters largely differed between land uses (i.e., cropland versus grassland) than other SOM compositions (Figures 17 and S16). The present finding further explained tillage's role in changing SOM's functional C groups in a soil matrix. For example, when the relative amount of aliphatic C was associated with mineral surfaces (Lehmann et al., 2007), high tillage intensity destroyed the soil matrix in cropland and affected SOM composition. Various tillage techniques also affected aliphatic and aromatic C ratios (Masoudi et al., 2023).

Tillage can potentially increase OM decomposition in cropland. A higher labile SOM composition in cropland than in the intact soils in grassland can show why there was a change in the SOM compositions between land uses. For example, amide N and polysaccharides with high aromaticity were all scattered along cropland, not grassland (Figures 22 and 26).

Cropland had a higher SOM decomposition degree (aromaticity index) than grassland (Figure S18). As the aromaticity index was previously defined (Margenot et al., 2015; Veum et al., 2014), the present results aligned with DOM findings when a high SUVA<sub>254</sub> value was found in cropland, not grassland (Figure 6).

## **6.9. SOC concentration and SOM composition differed between soil C pools**

In the present study, more C and N were stored in the slow pool than the fast pool and bulk soil in all land uses, plants, and fertilization-treated plot soils (Figures 14 and 15). The findings showed the significance of fine soil fraction (silt and clay) in accelerating C storage regardless of soil management practice (e.g., plants and fertilization) and land uses. Hence, the stabilized C in a slow pool was the primary way for OC storage and protection due to the high SSA of fine soil minerals with a high possibility of increasing organo-mineral complexes (Kögel-Knabner et al., 2008).

Differences in SOM compositions between slow and fast pools were found in cropland on each sampling date (Figures 24 and 28). A higher recalcitrance SOM composition (e.g., phenolic lignin and C/O ratios), SOC, and total N concentration were found in the slow pool compared to labile SOM composition in the fast pool, indicated by amide N and polysaccharides (Figures 18 and S17). Free and bound lipids were found to have different

preferential adsorption to different soil aggregate sizes based on their compositions (Angst et al., 2018). Such OM compositions were suggested to control hydrophobic DOM to outcompete or replace hydrophilic DOM by its strong sorption into a soil mineral (Kaiser & Zech, 1997). Hence, in the present work, recalcitrant SOM compositions were found in the slow pool (e.g., phenolic compounds within lignin-derived aromatic C signals). It explained the unique OM compositions of the slow pool in Chernozem soils.

The present work partly agreed with a previous work that studied OM composition within microaggregate. They found more decomposed and older OM in microaggregate than macroaggregate (von Lützow et al., 2007). More related to the present work, (Yeasmin et al., 2020) found a higher C/O ratio containing functional groups in the slow pool than in the POM pool. It explained a complex SOM composition in the slow pool with an advanced decomposition stage compared to the early stage in the POM pool (high aliphatic or more polysaccharides (O-rich functional groups)).

SOM compositions in the slow pool were not similar to bulk soil in cropland, but they were in grassland. (Schöning et al., 2005) found that clay fractions had a similar SOM composition to bulk soils (O/N alkyl C and alkyl C compounds), with a variation between particle size fractions (200–2000  $\mu\text{m}$ , 2–20  $\mu\text{m}$  and  $<2 \mu\text{m}$ ), using a radiocarbon method. Hence, in the present work, tillage also changed the SOM composition pattern between soil pools and bulk soil in arable Chernozem soil.

The slow pool fraction has a much lower C/N ratio than the fast pool (Figure 16). The present findings partly agreed with (Yeasmin et al., 2017), who studied the slow and POM pools. It also agreed with (Schöning et al., 2005) that clay fractions had a lower C/N ratio than sand and silt fractions. It indicated a distinctive SOM composition in the slow pool, as a high stored nitrogen and a low biological reactivity and recalcitrance OM composition were found in the present work (Figures 15 and 20). Furthermore, a high C:N ratio of plant litter inputs (e.g., waxes and lignin) can restrain microbial activities from metabolizing POM derived from plant residues. In contrast, a low C:N ratio or lignin/N ratio with abundant reactive minerals (e.g., clay) can increase the slow pool formation (Cyle et al., 2016).

## 6.10. Fertilization effects on SOC concentration

Fertilization effects on SOC concentration were not apparent during the April or August sampling dates (Figures S20 and S23). It was partly agreed with (Rui et al., 2022), who found that manure addition did not increase the stored SOC storage in the slow pool and microbial necromass in the Plano silt loam system. Another study found that fertilization increased SOC concentration in the POM pool (Liang et al., 2019). In line with DOM results within each sampling date, the positive results of the applied fertilizer on SOC and total N concentrations among slow and fast pools beside bulk soils might be reduced due to the priming effects (Lajtha et al., 2018). For example, using detrital input and removal treatment (DIRT) in forests, there is no effect from doubling aboveground litter input on SOM pools due to the priming effect (Lajtha et al., 2018).

The present work proposed that deep plowing can destroy soil structure, expose a fresh SOM to microbial activities, and enhance priming effects. Priming effects can increase SOM decomposition due to the acceleration of microbial activities as a response to the availability of easily decomposable organic substances (Azam et al., 1989; Kuzyakov et al., 2000).

In Martonvásár, fertilization was suggested to increase the amount of OC in fPOM, oPOM, and OM in the slow pool over several decades (Sleutel et al., 2006). Furthermore, tillage was found to have a higher effect on GHG flux than fertilization by impacting soil properties such as soil water content in a similar studied site (Dencső et al., 2020). As soil physical properties (e.g., bulk density) and C storage were degraded by tillage (Cookson et al., 2008; Kahlon et al., 2013; Osunbitan et al., 2005), (Jakab et al., 2023) found that the SOC concentration of slow and fast pools was negatively affected by tillage. The present study suggested revising soil management plans to increase C storage in arable lands.

If there was no tillage for a long time, fertilization effects might be more evident in SOC concentration. For example, (Alvarez, 2005) found that 79% of the nitrogen fertilizer's effects on SOC concentrations were positive when there were high soil crop residues, agreed with (Skadell et al., 2023). Soil aggregate stability was changed in the short term due to a change in agrotechniques (section 6.5). It showed the importance of adopting more effective fertilization plans to preserve soil aggregation and SOC concentration long-term.

The lack of effect from fertilization on SOC concentration raises the importance of using more conservative agricultural practices (e.g., NT) instead of conventional tillage (e.g., deep plowing). In the present work, plowing decreased soil aggregate stability, potentially increasing SOM decomposition and reducing SOC concentration. Hence, it was recommended

to decrease the cultivation intensity, particularly plowing, and add more organic fertilizers instead of every four years (Table 3) while considering n-potential, which was indicated as the proportion of non-complexed clay (NCC) to the SOC concentration (Merante et al., 2017).

### **6.11. Fertilization effects on SOM compositions**

No apparent effects from fertilization on SOM compositions in each date were found (Figures S20 and S23). Fertilization effects on SOM compositions in slow and fast pools beside bulk soil are not fully understood.

Previous works were provided here to compare different topics with a relatively close objective to the present work. Using XANES spectroscopy, more proteinaceous compounds were found after N addition than soil that did not receive N, studied Gleysol (Gillespie et al., 2014). Furthermore, adding straw was found to increase lignin. In contrast, cellulose was lost by this addition in upland and subtropical upland paddy soils (Chen et al., 2018). O-alkyl groups (labile OC) were positively affected by fertilizers. Nevertheless, the ratio of alkyl C/O-alkyl C functional groups (SOM decomposition index) was negatively affected by fertilization in the bulk soil (He et al., 2018) using the solid-state  $^{13}\text{C}$  NMR spectroscopy.

Tillage can reduce fertilization outcomes on SOM compositions by accelerating priming effects. Furthermore, part of fertilization can be an essential source of nutrient uptake for plant growth and ecosystem productivity. Such a significant ecosystem process related to arable soil was net primary production (NPP) (Bolinder et al., 2020). Hence, fertilization effects on SOM compositions were not straightforward and could continuously be subject to various changes in soil microbiome conditions.

Based on soil microbiome conditions, the same soil mineral may function as a sorbent, catalyst, and chemical reactant for OM association processes (Kleber et al., 2021). It showed the complex nature of SOM under a chaotic soil system. It further showed an extensive portfolio of debating and opposite results about SOM compositions responding to varied soil managements in a different soil system.

### **6.12. Plant effects on SOM variables**

Plants did not change SOC concentrations and SOM composition on each sampling date (Figures S21 and S24). Based on litter contents, some plant residues were soluble and had a lower molecular weight than other constituents with a complex structure or resistance to

degradation (Berg & McClaugherty, 2014). It might explain why the plant effects on the relative amount of aliphatic C component were relatively noticeable without strong evidence. For example, a relatively higher amount of aliphatic C component in wheat than in maize plants was found (Figure 17), which partly agreed with (Yeasmin et al., 2020). Conversely, another study found no effects from vegetation species on carbohydrate contents (Verchot et al., 2011).

The present findings partly disagreed with (Córdova et al., 2018), who studied different plant types and found cellulose and hemicellulose were increased under maize and soybean plants than oat and alfalfa plants in the subsoil of the accumulated slow pool at mesic Typic Hapludoll and Hapludalf. Furthermore, (Angeletti et al., 2021) also found that the oPOM pool had a lower decomposition degree in the legumes-based system than in the cereal system. Changes between the legumes and the cereal plants can reflect a more notable change in SOM composition in contrast to the present results when both maize and wheat crops showed no differences in SOM composition.

The present findings agreed with a study on Chernozem, which found that the access amount of stabilized SOM from plant residues did not change SOM composition in the slow and fast pools (Jakab et al., 2023). Nevertheless, high litter inputs in cropland under reduced or no tillage increased aggregate formation and oPOM persistence within a soil aggregate (Angst et al., 2023). If no-tillage practice existed, plant fragments would be kept on the soil surface for a long time, and in turn, SOM compositions in each soil C pool might vary based on plant litter quality and quantity.

### **6.13. Correlation of SOM in the slow and fast pools with DOM parameters in August**

In August, not April, there was a relatively considerable correlation between DOM parameters with solid-phase-related SOM in the fast and the slow pools and bulk soil ( $\rho > 0.7$ , Figures 34 and 35, respectively). For example, TDN (of the DOM pool) positively correlated with the total N concentration (of the slow pool) (Figure 35,  $\rho = 0.72$ ). It can indicate the DOM pool's seasonal role in affecting other SOM C pools.

SOM in the slow pool can be protected through occlusion within soil microaggregate and chemically protected by organo-mineral association within a fine soil mineral fraction (e.g., clay) (Kögel-Knabner et al., 2008). Nevertheless, the slow pool capacity to securely store C and make it inaccessible to soil biota can be subjected to soil microbiome conditions.

The emerging findings suggest a destabilization of the slow pool. It emphasized the multiple environmental conditions' roles in SOM cycling (Schmidt et al., 2011). For example,

plant root-deposited "low-molecular-weight exudates" (e.g., degradative enzymes from plant roots) can increase the solubilization and bioavailability of OM in the slow pool for feeding of soil-living forms in a soil system (Jilling et al., 2018).

#### **6.14. Seasonal changes in SOM composition in the slow pool**

There were minor seasonal changes in the slow pool by only increasing SOM recalcitrance composition in summer rather than spring (Table 12). It can be proposed that an OM exchange between soil C pools occurred in the soil microbiome under arable soil conditions. The DOM pool can affect OM exchange as TDN was positively correlated with TN (section 6.13). Furthermore, it was proposed that microbial activities can continuously break down OM into tiny fragments and change SOM composition. These fragments can be stabilized by interacting with soil mineral content and occluded into soil aggregates (Lehmann & Kleber, 2015). Hence, based on soil microbiome conditions, OM composition can be continuously exchanged, and these changes in soil microbiome can reflect a change in DOM composition.

In spring, SOM composition had a labile SOM composition (high polysaccharides ratio) than in the summer season (Table 12). Such a seasonal change in SOM composition could also be explained by seasonal cultivation effects (e.g., harvesting and plowing before soil sampling in the spring rather than summer). For example, tiny plant fragments can be physically secured by being a part of soil aggregate through the occlusion or chemically complexed with soil minerals (Lehmann & Kleber, 2015).

Plowing can mix tiny plant fragments in the soil matrix and change SOM composition. The present assumption was partly agreed with (Masoudi et al., 2023), showing that different tillage systems had changed SOM compositions (e.g., aliphatic C and aromatic C-H). Under suitable soil conditions (e.g., O<sub>2</sub> availability), microbial necromass can be formed, a central formation unit of the slow pool (Angst et al., 2023). Hence, necromass can change SOM compositions depending on soil microbiome or seasonality in arable Chernozem soil.

#### **6.15. Seasonal changes in SOM parameters in the fast pool**

All SOC contents and SOM composition were changed seasonally in the fast pool (Tables 11 and 12). SOC concentration and C/N ratio in the fast pool increased in summer than in spring. In the present study, harvesting and cultivation can particularly affect SOM composition by largely changing soil microbiome conditions, especially soil aggregate.

A high amide N and polysaccharides ratios were found in April compared to phenolic lignin and a high C/O ratio in August (Figure 31). It can indicate tillage effects on soil microbiome seasonally transforming the fast pool SOM composition. Particularly, it explained plowing effects in November before soil sampling in spring (section 4.1) in changing soil aggregate stability seasonally (Figure 13). SOM composition in the fast pool was considered sensitive to land use changes (Poeplau & Don, 2013), while an earlier finding stated that SOM in the fast pool was physically protected and stable (Zimmermann et al., 2007). Furthermore, the high aromaticity index (increase aromatic:aliphatic C functional groups ratio) showed an increase in decomposition with increased biological reactivity (decrease in C/O ratio) of SOM in April, not August, due to tillage effects.

A high lignin-derived aromatic C signal leads to an accumulation of SOM in the fast pool in summer, not spring (Figure 31). Based on a soil microbiome condition, microbial transformation and depolymerization of the SOM in the POM pool can appear (Angst et al., 2022). Previous works found that organic functional C groups (e.g., phenol, alcohol, and carboxyl) played an essential role in the adsorbing abilities of OM (Morris, 2004) and SOC biochemical stability based on their distributions and changes in soils (He et al., 2018; Margenot et al., 2015). Nevertheless, OC functional group functionalities may differ with soil heterogeneity and microbiome conditions (Audette et al., 2021; Smith et al., 2015).

Lignin, cellulose, and hemicelluloses were the most abundant plant residues, contributing to soil structure stabilization and nutrient cycling. Besides the net primary production significance in C flux in a soil system (Bolinder et al., 2020), once crop residues were left in the field, there was an increase in SOC concentration (Alvarez, 2005).

Even though the overall plant effects on SOM variables were not detectable, there might be a seasonal change in the amount of tiny fragments or decomposed plant materials in the soil microbiome due to cultivation and harvesting. For example, plant litter quality had a higher effect on C mineralization and slow pool accumulation than nutrient addition or soil type (Córdova et al., 2018), which further suggested POM as a precursor of the slow pool (Angst et al., 2023). It explained why there was a negligible amount of POM in soil samples during the fractionation in the present study for both dates. It can indicate that POM fractions can be occluded within the soil aggregate, decomposed, and adsorbed with fine soil minerals.

As organic matter, straw, and maize stalks were retained in crop field soil plots (section 4.1), these tiny fragments of plant residues may dramatically change SOM composition by tillage. For example, the last plowing practice was in November, meaning there was a soil

disturbance before the April 2019 soil sampling, and these plant fragments can be changed and mixed within the soil matrix in April (after plowing) rather than August.

SOM composition changes in the fast pool were accompanied by seasonal changes in the C/N ratio (Table 11). For example, a slightly higher C/N ratio was observed in the fast pool in summer than in spring. Hence, seasonal differences in the C/N ratio can indicate a change in soil microbiome condition, especially decomposition. For example, a low C/N ratio and a high relative amount of aliphatic C component showed that most partially decomposed plant litter could be further transformed and incorporated into the slow pool (Angst et al., 2023). Likewise, in the present work, it can be a feasible assumption that tiny plant fragments can affect the fast pool composition and stability.

The coexistence of microorganisms, suitable conditions (e.g., O<sub>2</sub> availability), and the availability of soil substrate can affect SOM decomposition under chaotic and heterogeneous arable soil systems. For example, soil functional complexity was stated in previous works. The co-location of OM molecular diversity temporally suggested the importance of both biotic (e.g., microbial products, rhizosphere, and plant roots) and abiotic factors (e.g., soil moisture and aggregate formation) to control SOM stability (Doetterl et al., 2015; Paul, 2016; Schmidt et al., 2011). Hence, a high OC molecular diversity may suppress the decomposition processes due to an increase in the metabolic demand of microbial communities and their energy fitness to decompose OM within a soil microbiome (Lehmann et al., 2020).

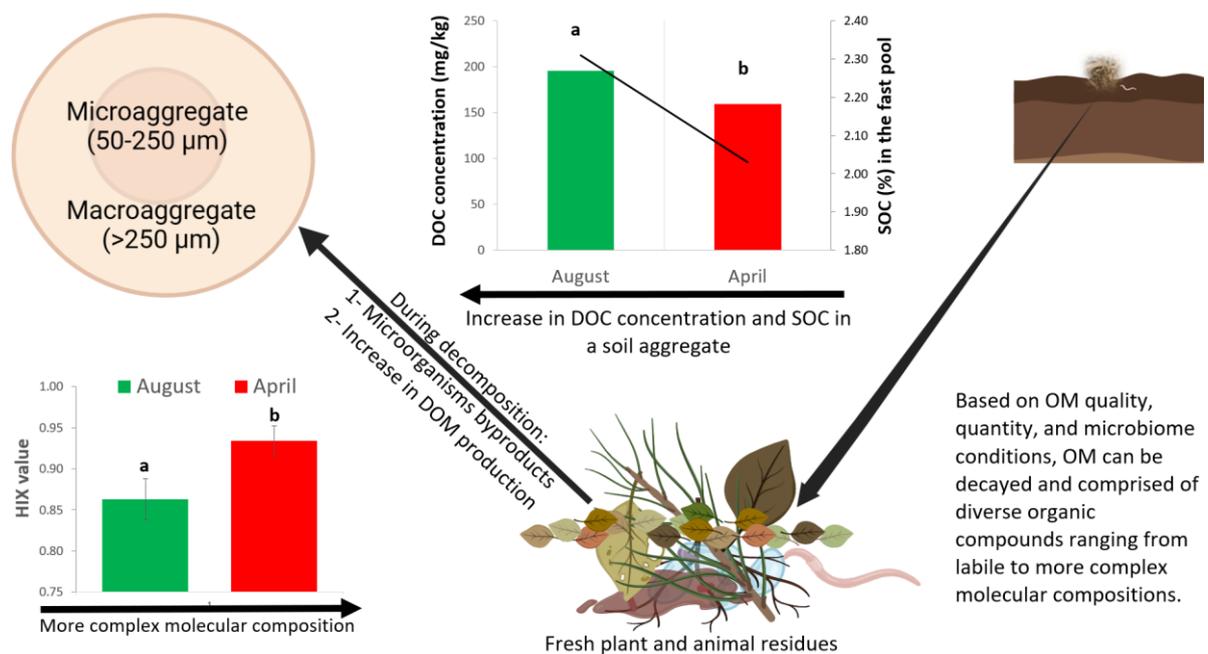
In the present work, a combination of tillage and changing soil microbiome conditions can seasonally affect soil oxidation state and OM fragments distribution in a soil matrix. Hence, it derived seasonal differences in a solid-phase-related SOM, more evidently in the fast pool. Further work is recommended to study plant litter residue quality (e.g., C/N ratio) change effects on soil C pool composition and formation.

#### **6.16. Relationship between DOM and solid-phase-related SOM parameters**

The present results suggested that the DOM pool, as a mobile and active pool, can be exchanged between SOM pools seasonally, affecting their formation and stability (Figure 1). It can also explain why there was a considerable correlation between DOM parameters, especially with FI index or DOM degradation degree (McKnight, Boyer, et al., 2001), and SOM variables in the fast pool, especially in summer, not spring (Figure 34, section 5.8). It suggested that OM composition can be continuously changed in arable Chernozem soil.

The DOM pool comprises thousands of organic molecules so that microbial necromass can be part of it. The necromass was proposed to play a role in the present result by affecting OM stability and the fast pool seasonality in Chernozem soil. In particular, Chernozem soil had more favorable microbial growth conditions than Podzols (containing more plant compounds, e.g., lignin) (Angst et al., 2021). For example, a microbial necromass contributed to more than half of SOC concentrations (Liang et al., 2019).

More labile DOM composition can affect SOC concentration in the fast pool during the summer season than in the following spring in cropland (Figure 36). A change in DOM composition can come from a biochemical link with POM fraction in Hungarian forest soils (Filep et al., 2022). Likewise, the DOM pool can be enriched with necromass or microbial remains, contributing to seasonal changes in SOM related to the fast pool. For example, DOM's composition in a soil system can have various molecular compositions ranging from easily decomposable sugars to complex phenolic lignin (Kalbitz et al., 2007).



**Figure 36.** Seasonally, the variation in dissolved organic matter (DOC) was in line with a change in soil organic carbon (SOC) in the fast pool in cropland (secondary axis). The humification index was referred to by (HIX). The figure was created with BioRender.com. Different letters ( a and b) referred to the significant effects of the sampling date on each SOM variable ( $p$ -value $<$ 0.05). Data was given a mean with standard deviation. The sample size was ( $n=216$ ) for the DOM variable and ( $n=72$ ) for solid-phase related soil organic matter. Further information was outlined (sections S2 and S10, supplementary).

Even though plants did not significantly affect SOM variables per each sampling date, it is still a feasible assumption that, due to harvesting and plowing, the priming effects might

accelerate the OM decomposition and change DOM composition in a particular date than others. A change in the biological production of DOM can also highlight a change in the soil microbiome affecting DOM pattern seasonally (Figure 7). Specifically, a higher BIX index in August than in April can indicate seasonal changes in the biological production of DOM, while opposite results for HIX values were found between each sampling date (Figure S2).

In the summer, there were significantly more DOC concentrations with seasonal differences in the biological contribution of DOM compositions (Figure 7). In contrast, complex DOM composition (high HIX index) was detected in spring. Those changes aligned with the seasonality of fast pool SOM composition. Based on soil microbiome conditions, including O<sub>2</sub> availability, the chemistry of OM inputs, pH, and the reactive properties of mineral surfaces, decomposition can instantly increase or decrease, affecting SOM composition. Regarding that, a labile OM composition might be further decomposed and incorporated into the soil aggregate due to rapid decomposition (Baldrian, 2014; Cyle et al., 2016; Kögel-Knabner & Amelung, 2021; Verrone et al., 2024). Hence, a labile DOM composition was proposed to increase SOC concentration in the fast pool during the summer compared to the following spring in cropland.

The present results further suggested that the DOM pool can drive SOM formation and stability in the fast pool, and, in turn, OM composition can be continuously changed in arable Chernozem soil.

## 7. Conclusion

Higher SOC and total N concentrations were found in grassland than in cropland at the slow and the fast pools and bulk soil and relatively not in the DOM pool. It suggested varied tillage effects on soil C pools. Furthermore, a change in SOC concentration between land uses accompanied a change in SOM compositions. Specifically, SOM compositions had higher aromaticity and decomposition degrees in cropland than in grassland. A higher aromatic C with a lower relative amount of aliphatic C component in cropland than grassland indicated that tillage or land use negatively affected SOM stability in arable soil.

A high SOC concentration and recalcitrant SOM composition (e.g., phenolic compounds within lignin-derived aromatic C signals) were found in the slow pool than in the fast pool. A labile SOM composition, indicated by amide N and polysaccharides with high aromaticity, was detected in the fast pool. It proved the slow pool's importance as the primary C protection route at Chernozem soils. It further explains the importance of fine soil mineral fractions in accelerating C storage regardless of fertilization and land use.

Continuous changes in SOM compositions are proposed in Chernozem arable soil. Specifically, considerable correlations were found between DOM pool and SOM variables in fast and slow pools and bulk soil in summer, not the following spring. These seasonal correlations affected SOM composition seasonally, especially in the fast pool. It explained the following: (1) An increase in DOC concentration was proposed to increase SOC concentration in the fast pool during the summer compared to the following spring in cropland. (2) The temporal dynamics of DOM compositions were in line with solid-phase-related SOM composition. Specifically, a labile DOM composition was compatible with an increased SOC concentration in the fast pool during the summer, not spring. (3) A high aromaticity index (increase in the ratio of aromatic:aliphatic C functional groups) indicated an increase in decomposition degree in cropland in spring, not summer. These seasonal differences in SOM compositions elucidate a seasonal change in SOM stability due to a potential change in soil microbiome conditions. It elaborates on the importance of the DOM pattern in driving seasonal changes in solid-phase-related SOM composition.

Hence, the OM compositions in the fast and the DOM pools were sensitive to the instant change in a soil system. These findings highlight the importance of monitoring SOM composition seasonally for an effective soil management plan in arable Chernozem soil.

## Thesis points

The present study contributed to scientific knowledge by studying SOC concentration and SOM compositions in three soil C pools and bulk soil seasonally at two sites (cropland and grassland) characterized by Chernozem. Previous works studied different soil systems ranging from forest to peaty gley soils, with limited work studying Chernozem in arable lands. Furthermore, sampling time and fertilization effects on solid-phase-related SOM compositions in the slow and fast pools and bulk soil are not fully understood. The list of findings of the present thesis is as follows:

- 1- SOM compositions related to DOM and fast pools were affected by the sampling time factor. Specifically, SOM compositions were changed over a short time in the fast pool. Hence, SOM composition in the fast pools was not only affected by land use but also by sampling time. It suggested that both fast and DOM pools are sensitive soil C pools to the instant change in arable Chernozem soil (Al-Graiti, under publication).
- 2- Aliphatic and aromatic C ratios were potent indicators of tillage effects on SOM composition. Tillage dramatically shifts SOM composition toward less SOM stability in cropland with a higher decomposition indicator than grassland.
- 3- Regardless of land uses and fertilization, the slow pool had higher SOC concentration and recalcitrant SOM than the fast pool. It shows the importance of the slow pool as the primary C protection route at Chernozem soil. Furthermore, SOM compositions in the slow pool were also changed based on land use. For example, SOM composition in the slow pool was not similar to bulk soil in cropland, but they were in grassland.
- 4- Plants and fertilization did not affect SOM compositions and SOC concentration in each soil C pool and bulk soil. It suggested that SOM patterns can be triggered by a spatially and temporally heterogeneous complex system in arable lands under varied agrotechniques, as revealed by a high variance of some SOM variables.
- 5- The local weather parameters were significantly correlated with DOM parameters but with a low correlation value. A low correlation value can be related to the soil's nature as a chaotic system. Hence, it was suggested that including microbial data is necessary for studying the DOM pool in arable soil (Al-Graiti et al., 2022).
- 6- A considerable correlation was found between parameters related to the solid-phase SOM and the DOM. These phenomena suggest a biochemical link exists between the DOM and other SOM pools. Hence, it indicated that OM can be constantly exchanged between soil C pools, affecting SOM pattern in arable Chernozem soil.

## Summary

Soil organic matter (SOM) is a valuable soil property, controlling its fertility. Studies suggest that SOM is being lost due to cultivation practices. This study aimed to investigate whether soil organic carbon (SOC) concentration and soil organic matter (SOM) compositions are affected by soil management practices (e.g., fertilization, crop rotation, and tillage) at two sites: Cultivated topsoil in cropland and nearby intact grassland soil at Martonvásár (47.331196 N, 18.789660 E), Hungary, characterized by Chernozem soil reference group. They were part of a long-term experiment comparing fertilization effects on soil quality under different plants. In this PhD research, soil samples were collected seasonally between 2018 and 2020. This study aimed to study SOC and total nitrogen concentration besides SOM composition in bulk soil and three soil C pools. Soil C pools were dissolved organic matter (DOM) and solid-phase-related SOM in fractionated soils. Fractionated soils included mineral phase-associated organic matter (MPAOM or slow pool) and aggregate-associated organic matter (AAOM or fast pool).

Both solid-phase-related SOM pools and the DOM pool were unaffected by fertilization or plants. Nevertheless, a higher SOC and total N concentration were found in grassland than in cropland. These results were more evident in solid-phase-related SOM results than in DOM. It explained varied tillage effects on soil C pools. It further showed the importance of soil aggregate stability in increasing SOC concentration and SOM stability. For example, tillage dramatically shifts SOM composition to be unstable in cropland compared to intact soil in grassland. Furthermore, SOM compositions in cropland had a higher decomposition indicator than in grassland. Both aliphatic and aromatic C ratios were found to be potent indicators of tillage effects on SOM composition.

DOC concentration was proposed to affect SOC concentration in the fast pool during the summer rather than in the following spring. It came from seasonally varying DOM composition, especially labile DOM composition in summer than in the following spring. Hence, DOM can be a direct property of soil organic matter in the short term. Furthermore, the solid phase related to SOM in the fast pool was not only affected by land use but also by the sampling time factor. Regardless of land use and fertilization, the slow pool had higher SOC concentration and recalcitrant SOM than the fast pool. It shows the importance of the slow pool as the primary C protection route. The present findings highlight the importance of studying SOM patterns seasonally for an effective soil management plan in arable soil.

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## Supplementary

This is a supplementary section for the thesis. It had ten sections named in order from section S1 to section S10.

**Section S1:** Figures S1-S11 were shown beside Table S1 in this section.

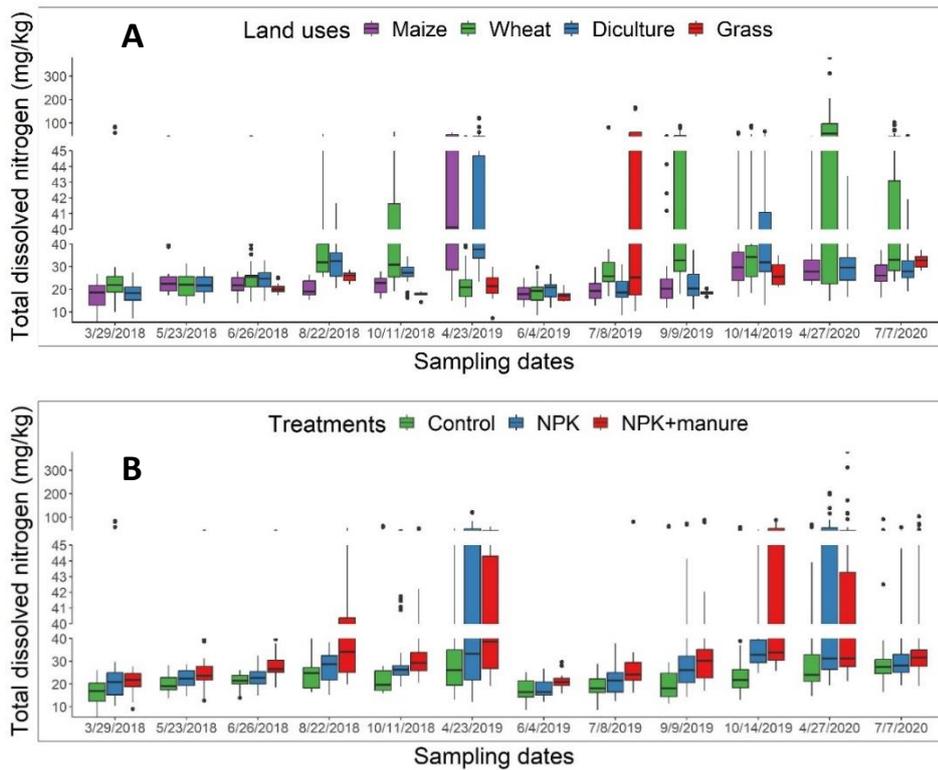
**Table S1:** The chemical and physical properties of manure were outlined in the study site, Martonvásár, Hungary.

**Istállótrágyaminták összetétele Martonvásári Növendék Szarvasmarha Telep, Erdőhát.**  
Mélyalmos, 6 hónapig istállóban érlelt  
Mintavétel 2003. május és október; 5-5 rész minta homogenizálásával

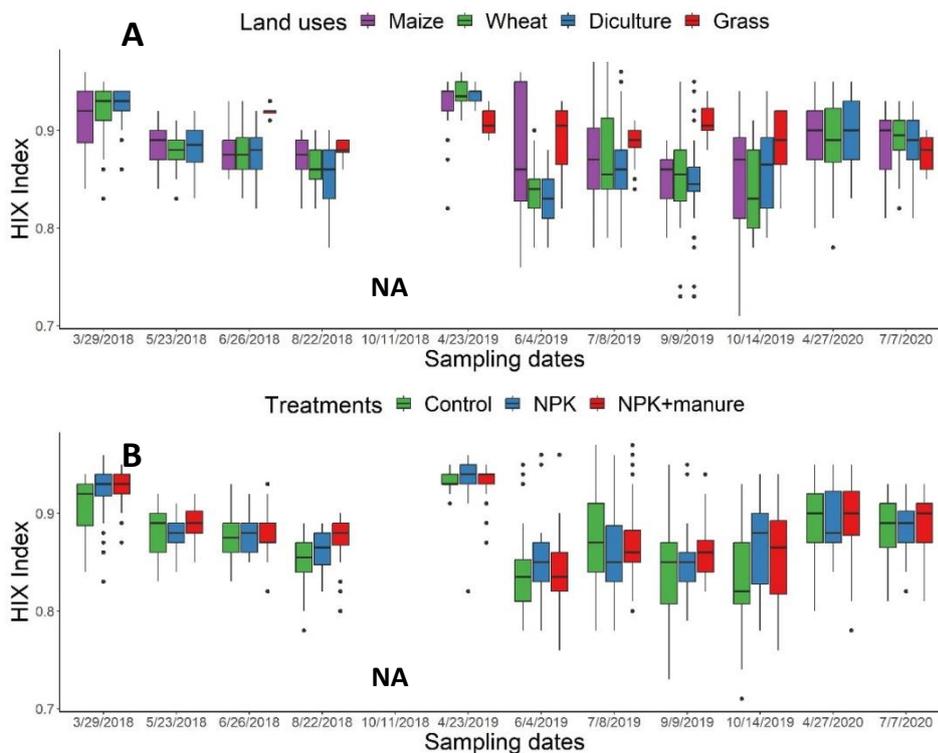
Vizsgált jellemzők	Mintavétel 2003. május			Mintavétel 2003. október			
	1	2	3	1	2	3	4
Száranyag %	20	28	24	26	27	24	25
Szervesanyag %	56	48	60	49	48	50	45
Szerves-C %	32	28	35	28	28	29	26
C/N arány	11	12	14	9	11	11	12
N %	2.99	2.29	2.48	3.02	2.45	2.60	2.19
K %	3.04	2.74	2.76	2.57	2.98	3.05	2.92
Ca %	2.41	2.52	2.34	2.67	2.97	3.47	3.51
Mg %	0.87	0.84	0.89	1.01	1.07	1.24	1.21
P %	0.83	0.65	0.69	1.27	1.19	1.18	1.10
Al %	0.52	0.91	0.46	1.05	3.30	1.16	3.67
S %	0.55	0.47	0.53	0.53	0.49	0.49	0.51
Fe %	0.27	0.52	0.10	0.20	0.35	0.20	0.43
Na %	0.28	0.25	0.25	0.29	0.38	0.26	0.33
Mn mg/kg	259	268	255	333	334	304	338
Zn mg/kg	163	155	180	204	202	176	202
Cu mg/kg	76	95	102	67	61	57	62
Ba mg/kg	52	66	54	26	36	25	41
Sr mg/kg	48	105	46	93	98	104	97
B mg/kg	32	36	33	31	34	36	34
Cr mg/kg	13	20	11	4	8	5	22
Pb mg/kg	14	14	22	13	10	5	9
Ni mg/kg	7	11	7	4	7	5	15
As mg/kg	1.44	3.02	1.50	0.60	1.75	0.79	1.26
Mo mg/kg	0.99	0.83	0.90	0.75	0.74	0.71	0.91
Se mg/kg	0.74	0.10	0.65	KH	KH	KH	KH
Co mg/kg	0.67	1.54	0.61	1.39	1.91	1.34	2.35
Cd mg/kg	0.09	0.09	0.09	0.22	0.22	0.19	0.23
NH <sub>4</sub> -N mg/kg	221	170	194	150	314	86	85
NO <sub>3</sub> -N mg/kg	155	104	274	627	109	174	286

Megjegyzés: légszáraz minták átlagosan 10% nedvességtartalommal.

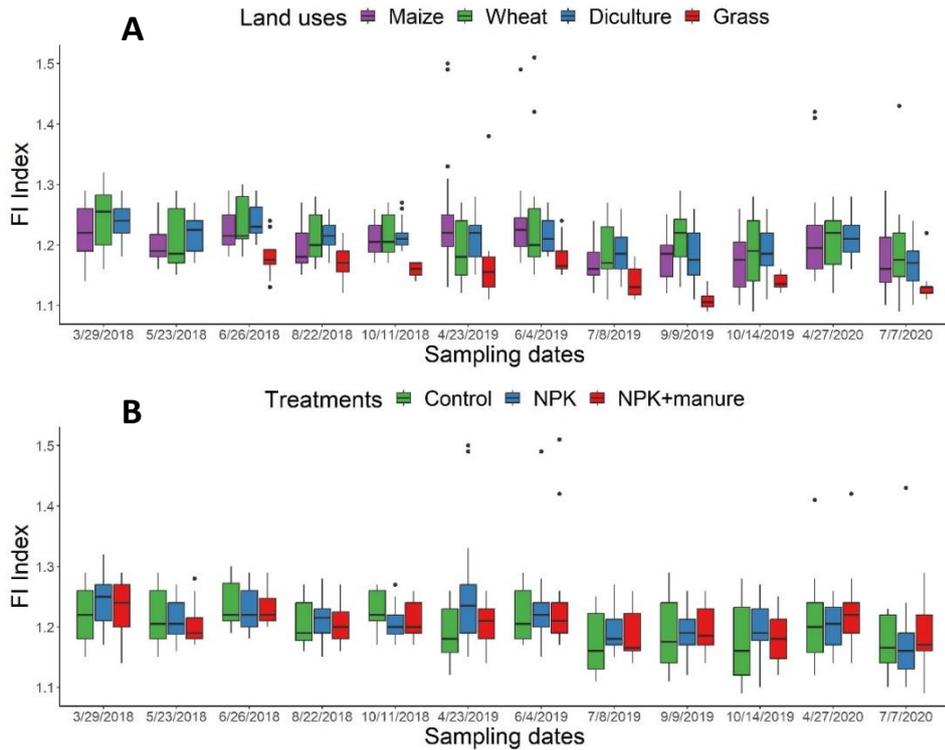
A Sn, Hg 0.1 mg/kg kimutatási határ (KH) alatt.



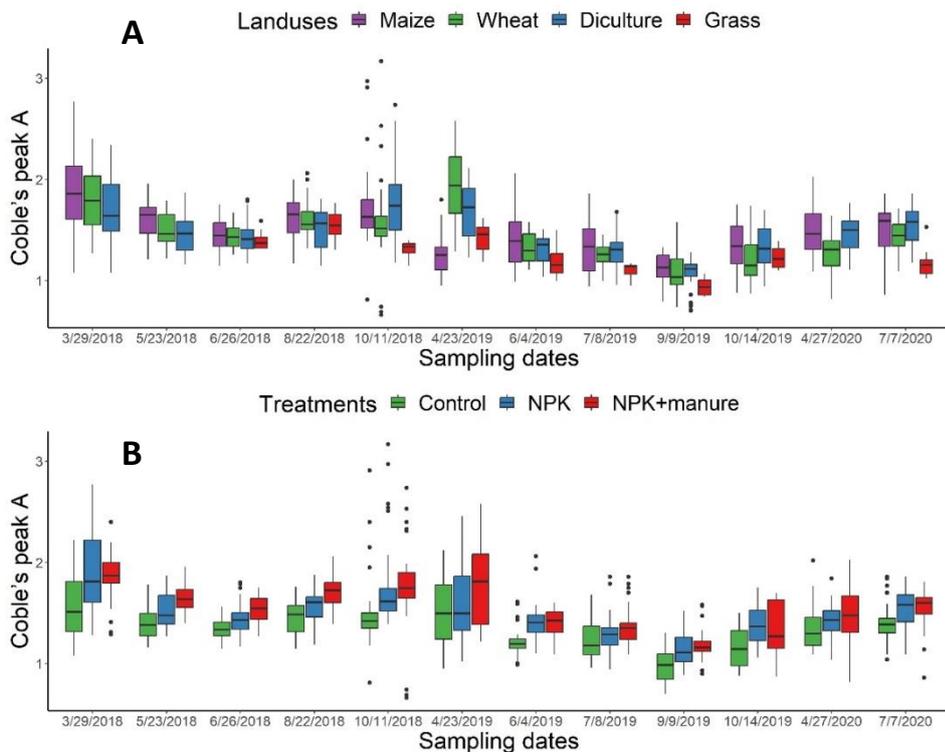
**Figure S1.** Total dissolved nitrogen (TDN) concentration in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium. A gap was set in the y-axis scale to enhance boxplot presentations.



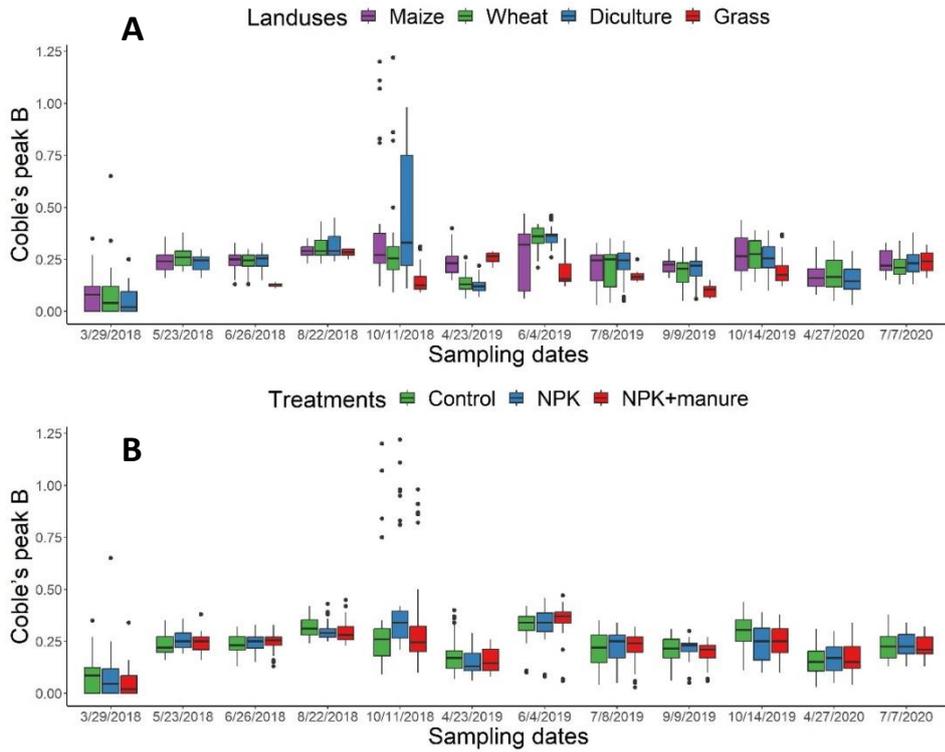
**Figure S2.** The humification index (HIX) in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.



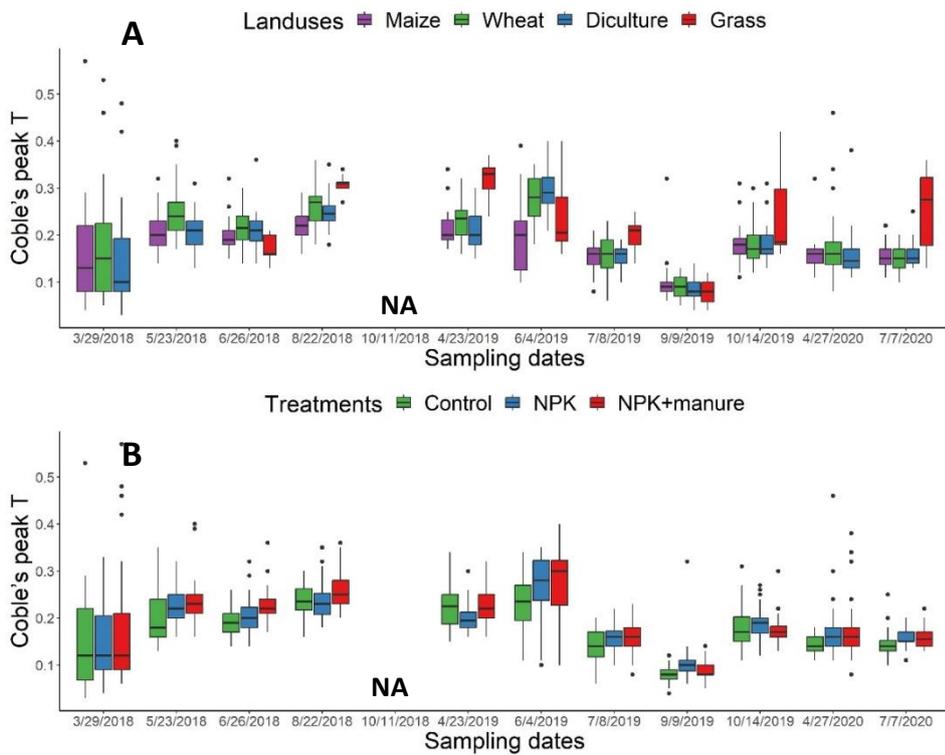
**Figure S3:** The fluorescence index (FI) in grassland and cropland sites under different crops or land uses (A) and treatments (B) across sampling dates. NPK: nitrogen, phosphorus, potassium.



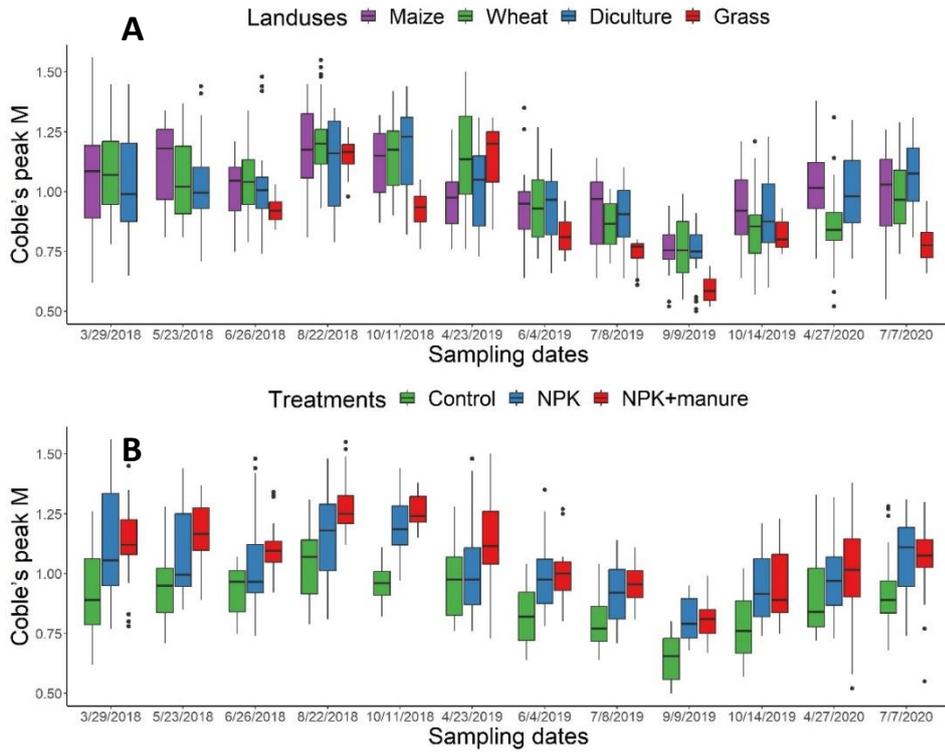
**Figure S4.** Coble's peak A values in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.



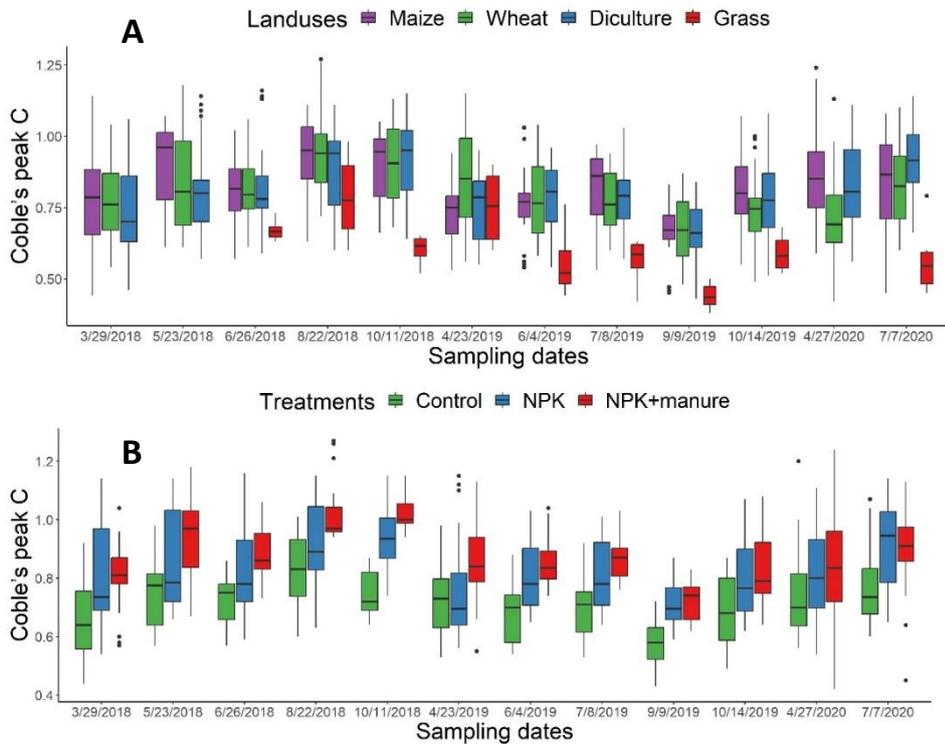
**Figure S5.** Coble's peak B values in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.



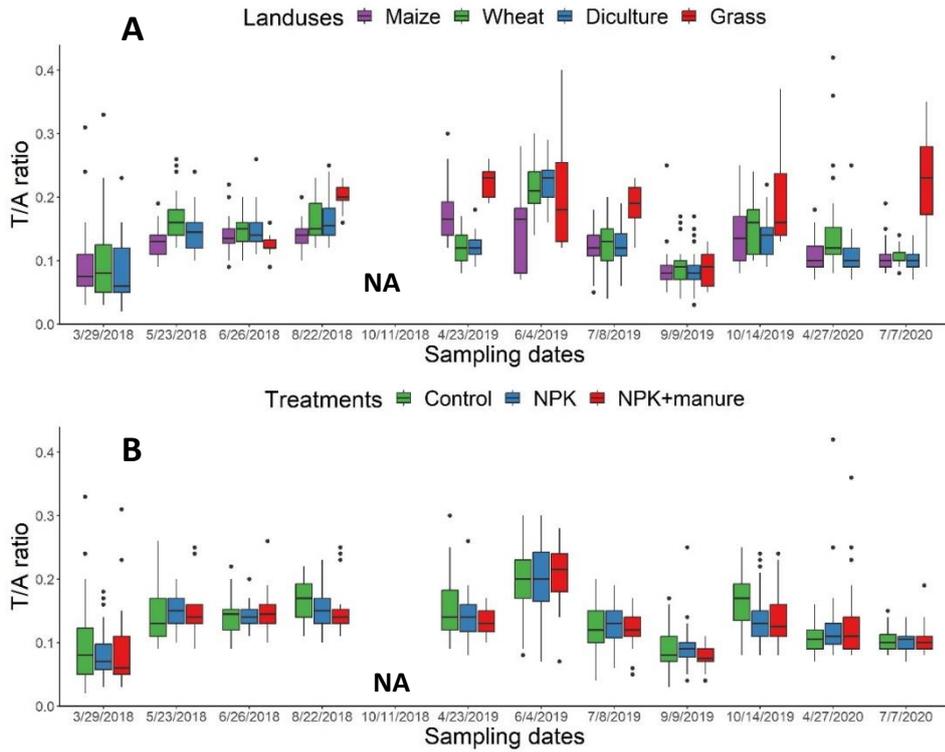
**Figure S6.** Coble's peak T values in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.



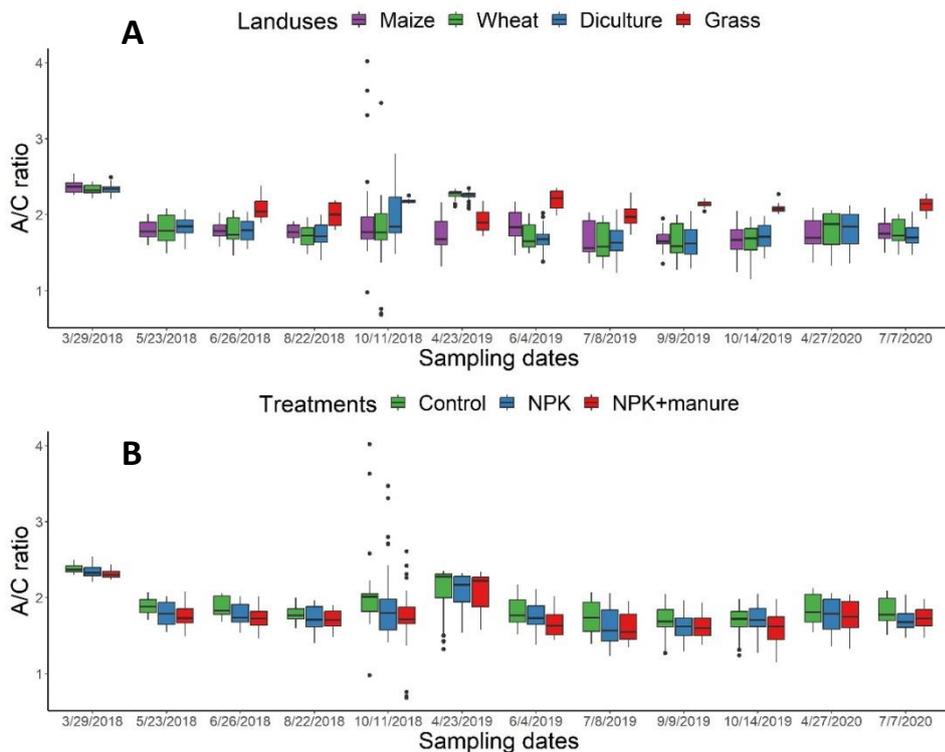
**Figure S7.** Coble's peak M values in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.



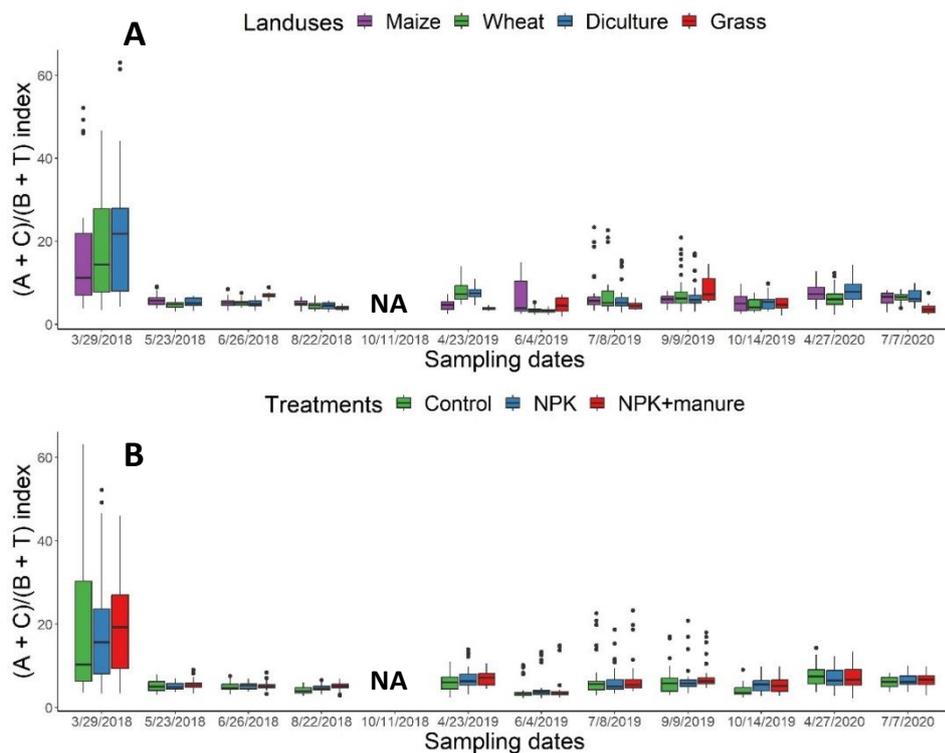
**Figure S8.** Coble's peak C values in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.



**Figure S9.** T/A index values in grassland and cropland sites under different crops or land uses (A) and treatments (B). NPK: nitrogen, phosphorus, potassium.



**Figure S10.** A/C index values in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.



**Figure S11.** (A+C)/(B+T) index values in grassland and cropland sites under different crops or land uses (A) and treatments (B) across all sampling dates. NPK: nitrogen, phosphorus, potassium.

**Section S2: A-** Kruskal-Wallis test and Pairwise Comparisons results for the overall treatment effects (for all sampling dates) on each DOM composition and DOC concentration within cropland.

**Nonparametric Tests**

**Independent-Samples Kruskal-Wallis Test**

**TDN (mg/kg) across Treatments**

**Independent-Samples Kruskal-Wallis Test Summary**

Total N	1293
Test Statistic	145.759 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

**Pairwise Comparisons of Treatments**

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
No fertilizer-NPK	185.497	25.451	7.288	<.001	.000
No fertilizer-NPK+manure	304.970	25.451	11.983	.000	.000
NPK-NPK+manure	-119.473	25.407	-4.702	<.001	.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

### C/N ratio across Treatments

#### Independent-Samples Kruskal-Wallis Test Summary

Total N	1293
Test Statistic	21.480 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	<.001

a. The test statistic is adjusted for ties.

#### Pairwise Comparisons of Treatments

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
NPK+manure-No fertilizer	-99.852	25.451	-3.923	<.001	.000
NPK+manure-NPK	104.072	25.407	4.096	<.001	.000
No fertilizer-NPK	4.220	25.451	.166	.868	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

### DOC (mg/kg) across Treatments

#### Independent-Samples Kruskal-Wallis Test Summary

Total N	1293
Test Statistic	138.464 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

#### Pairwise Comparisons of Treatments

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
No fertilizer-NPK	222.055	25.451	8.725	.000	.000
No fertilizer-NPK+manure	285.243	25.451	11.207	.000	.000
NPK-NPK+manure	-63.187	25.407	-2.487	.013	.039

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

**SUVA<sub>254</sub> across Treatments**

**Independent-Samples Kruskal-Wallis Test Summary**

Total N	1287
Test Statistic	5.424 <sup>a,b</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.066

a. The test statistic is adjusted for ties.

b. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

**BIX across Treatments**

**Independent-Samples Kruskal-Wallis Test Summary**

Total N	1293
Test Statistic	27.675 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	<.001

a. The test statistic is adjusted for ties.

**Pairwise Comparisons of Treatments**

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
NPK+manure-No fertilizer	-2.020	25.344	-.080	.936	1.000
NPK+manure-NPK	116.321	25.299	4.598	<.001	.000
No fertilizer-NPK	114.301	25.344	4.510	<.001	.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

**HIX across Treatments**

**Independent-Samples Kruskal-Wallis Test Summary**

Total N	1188
Test Statistic	8.239 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.016

a. The test statistic is adjusted for ties.

**Pairwise Comparisons of Treatments**

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
No fertilizer-NPK	36.898	24.326	1.517	.129	.388
No fertilizer-NPK+manure	69.784	24.326	2.869	.004	.012

NPK-NPK+manure	-32.886	24.326	-1.352	.176	.529
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Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

#### FI across Treatments

##### Independent-Samples Kruskal-Wallis Test Summary

Total N	1293
Test Statistic	3.998 <sup>a,b</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.135

a. The test statistic is adjusted for ties.

b. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

#### Coble B across Treatments

##### Independent-Samples Kruskal-Wallis Test Summary

Total N	1292
Test Statistic	2.865 <sup>a,b</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.239

a. The test statistic is adjusted for ties.

b. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

#### Coble T across Treatments

##### Independent-Samples Kruskal-Wallis Test Summary

Total N	1187
Test Statistic	16.361 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	<.001

a. The test statistic is adjusted for ties.

##### Pairwise Comparisons of Treatments

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
No fertilizer-NPK	63.167	24.334	2.596	.009	.028
No fertilizer-NPK+manure	97.012	24.350	3.984	<.001	.000
NPK-NPK+manure	-33.845	24.350	-1.390	.165	.494

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

### Coble A across Treatments

#### Independent-Samples Kruskal-Wallis Test Summary

Total N	1292
Test Statistic	124.822 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

#### Pairwise Comparisons of Treatments

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
No fertilizer-NPK	192.724	25.430	7.579	<.001	.000
No fertilizer-NPK+manure	277.408	25.444	10.903	.000	.000
NPK-NPK+manure	-84.684	25.400	-3.334	<.001	.003

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

### Coble M across Treatments

#### Independent-Samples Kruskal-Wallis Test Summary

Total N	1292
Test Statistic	209.222 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

#### Pairwise Comparisons of Treatments

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
No fertilizer-NPK	256.840	25.428	10.101	.000	.000
No fertilizer-NPK+manure	356.785	25.443	14.023	.000	.000
NPK-NPK+manure	-99.945	25.399	-3.935	<.001	.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

**Coble C across Treatments**

**Independent-Samples Kruskal-Wallis Test Summary**

Total N	1292
Test Statistic	263.901 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

**Pairwise Comparisons of Treatments**

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
No fertilizer-NPK	271.287	25.426	10.670	.000	.000
No fertilizer-NPK+manure	405.751	25.441	15.949	.000	.000
NPK-NPK+manure	-134.463	25.397	-5.295	<.001	.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

**T/A index across Treatments**

**Independent-Samples Kruskal-Wallis Test Summary**

Total N	1187
Test Statistic	1.793 <sup>ab</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.408

a. The test statistic is adjusted for ties.

b. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

**A/C index across Treatments**

**Independent-Samples Kruskal-Wallis Test Summary**

Total N	1292
Test Statistic	44.568 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	<.001

a. The test statistic is adjusted for ties.

**Pairwise Comparisons of Treatments**

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
NPK+manure-NPK	53.490	25.400	2.106	.035	.106
NPK+manure-No fertilizer	-166.396	25.444	-6.540	<.001	.000

NPK-No fertilizer	-112.906	25.429	-4.440	<.001	.000
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Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

**(A+C)/(B+T) index across Treatments**

**Independent-Samples Kruskal-Wallis Test Summary**

Total N	1187
Test Statistic	17.597 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	<.001

a. The test statistic is adjusted for ties.

**Pairwise Comparisons of Treatments**

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
No fertilizer-NPK	62.000	24.362	2.545	.011	.033
No fertilizer-NPK+manure	101.414	24.377	4.160	<.001	.000
NPK-NPK+manure	-39.414	24.377	-1.617	.106	.318

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

**B-** Mann-Whitney U test for the sampling date effects on DOM composition and DOC concentration in cropland under April 2019 and August 2018 sampling dates (n=216).

**Test Statistics<sup>a</sup>**

	TDN	CN	DOC	SUVA254	BIX	HIX
Mann-Whitney U	4729.000	3244.500	3098.000	1208.000	3347.500	165.500
Wilcoxon W	10615.000	9130.500	8984.000	7094.000	9233.500	6051.500
Z	-2.402	-5.634	-5.953	-10.069	-5.437	-12.397
Asymp. Sig. (2-tailed)	.016	<.001	<.001	<.001	<.001	<.001

**Test Statistics<sup>a</sup>**

	FI	CobleB	CobleT	CobleA	CobleM	CobleC
Mann-Whitney U	5753.000	762.000	3653.500	5537.000	3693.500	2946.000
Wilcoxon W	11639.000	6648.000	9539.500	11423.000	9579.500	8832.000
Z	-.173	-11.048	-4.760	-.642	-4.658	-6.286

Asymp. Sig. (2-tailed)	.863	<.001	<.001	.521	<.001	<.001
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**Test Statistics<sup>a</sup>**

	TA	AC	ACBT
Mann-Whitney U	4005.000	1977.500	2490.000
Wilcoxon W	9891.000	7863.500	8376.000
Z	-4.006	-8.394	-7.277
Asymp. Sig. (2-tailed)	<.001	<.001	<.001

**Section S3:** Mann-Whitney U test for the sampling date effects on DOM composition and DOC concentration in grassland.

A- The tables show the Mann-Whitney test results for comparing the sampling dates effects (August 2018 versus April 2019) on the DOM composition and DOC concentrations in grassland.

**Mann-Whitney Test**

**Test Statistics<sup>a</sup>**

	TDN (mg/kg)	C/N RATIO	DOC (mg/kg)	SUVA254	BIX	HIX
Mann-Whitney U	37.000	30.000	49.000	2.000	38.000	7.500
Wilcoxon W	115.000	108.000	127.000	80.000	116.000	85.500
Z	-2.021	-2.425	-1.328	-4.043	-1.998	-3.811
Asymp. Sig. (2-tailed)	.043	.015	.184	<.001	.046	<.001

**Test Statistics<sup>a</sup>**

	FI	Coble's peak B	Coble's peak T	Coble's peak A	Coble's peak M	Coble's peak C
Mann-Whitney U	54.500	31.000	54.000	43.000	65.500	58.500
Wilcoxon W	132.500	109.000	132.000	121.000	143.500	136.500
Z	-1.015	-2.399	-1.052	-1.677	-.376	-.781
Asymp. Sig. (2-tailed)	.310	.016	.293	.093	.707	.435

**Test Statistics<sup>a</sup>**

	TA	AC	ACBT
Mann-Whitney U	38.000	47.000	62.000
Wilcoxon W	116.000	125.000	140.000
Z	-2.006	-1.446	-.577
Asymp. Sig. (2-tailed)	.045	.148	.564

### Mann-Whitney Test

B- The tables show the Mann-Whitney test results for comparing the sampling dates effects (August 2018 versus June 2018) on the DOM composition and DOC concentrations in grassland.

**Test Statistics<sup>a</sup>**

	TDN (mg/kg)	C/N RATIO	DOC (mg/kg)	SUVA254	BIX	HIX
Mann-Whitney U	11.000	12.000	.000	65.500	.000	.000
Wilcoxon W	89.000	90.000	78.000	143.500	78.000	78.000
Z	-3.523	-3.465	-4.158	-.376	-4.188	-4.269
Asymp. Sig. (2-tailed)	<.001	<.001	<.001	.707	<.001	<.001

**Test Statistics<sup>a</sup>**

	FI	Coble's peak B	Coble's peak T	Coble's peak A	Coble's peak M	Coble's peak C
Mann-Whitney U	60.500	.000	.000	36.000	2.500	44.000
Wilcoxon W	138.500	78.000	78.000	114.000	80.500	122.000
Z	-.670	-4.202	-4.191	-2.086	-4.015	-1.620
Asymp. Sig. (2-tailed)	.503	<.001	<.001	.037	<.001	.105

**Test Statistics<sup>a</sup>**

	TA	AC	ACBT
Mann-Whitney U	.500	54.000	.000
Wilcoxon W	78.500	132.000	78.000
Z	-4.176	-1.041	-4.158
Asymp. Sig. (2-tailed)	<.001	.298	<.001

### Mann-Whitney Test

C- The tables show the Mann-Whitney test results for comparing the sampling dates effects (August 2018 versus October 2018) on the DOM composition and DOC concentrations in grassland.

**Test Statistics<sup>a</sup>**

	TDN (mg/kg)	C/N RATIO	DOC (mg/kg)	SUVA254	BIX	FI
Mann-Whitney U	.000	63.500	.000	22.000	.000	51.000
Wilcoxon W	78.000	141.500	78.000	100.000	78.000	129.000
Z	-4.158	-.491	-4.157	-2.891	-4.188	-1.234
Asymp. Sig. (2-tailed)	<.001	.624	<.001	.004	<.001	.217

**Test Statistics<sup>a</sup>**

	Coble's peak B	Coble's peak A	Coble's peak M	Coble's peak C	AC
Mann-Whitney U	22.500	18.500	5.000	17.500	22.500
Wilcoxon W	100.500	96.500	83.000	95.500	100.500
Z	-2.880	-3.094	-3.871	-3.153	-2.878
Asymp. Sig. (2-tailed)	.004	.002	<.001	.002	.004

D- The tables show the Mann-Whitney test results for comparing the sampling dates effects (August 2018 versus June 2019) on the DOM composition and DOC concentrations in grassland.

**Test Statistics<sup>a</sup>**

	TDN (mg/kg)	C/N RATIO	DOC (mg/kg)	SUVA254	BIX	HIX
Mann-Whitney U	.000	19.500	.000	70.500	21.500	53.000
Wilcoxon W	78.000	97.500	78.000	148.500	99.500	131.000
Z	-4.157	-3.032	-4.157	-.087	-2.970	-1.109
Asymp. Sig. (2-tailed)	<.001	.002	<.001	.931	.003	.267

**Test Statistics<sup>a</sup>**

	FI	Coble's peak B	Coble's peak T	Coble's peak A	Coble's peak M	Coble's peak C
Mann-Whitney U	67.000	24.500	31.500	11.500	.000	18.000
Wilcoxon W	145.000	102.500	109.500	89.500	78.000	96.000
Z	-.292	-2.759	-2.361	-3.497	-4.160	-3.119
Asymp. Sig. (2-tailed)	.770	.006	.018	<.001	<.001	.002

**Test Statistics<sup>a</sup>**

	TA	AC	ACBT
Mann-Whitney U	62.500	30.500	60.000
Wilcoxon W	140.500	108.500	138.000
Z	-.551	-2.399	-.693
Asymp. Sig. (2-tailed)	.581	.016	.488

E- The tables show the Mann-Whitney test results for comparing the sampling dates effects (August 2018 versus July 2019) on the DOM composition and DOC concentrations in grassland.

**Test Statistics<sup>a</sup>**

	TDN (mg/kg)	C/N RATIO	DOC (mg/kg)	SUVA254	BIX	HIX
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Mann-Whitney U	65.000	44.000	1.000	34.000	42.500	45.500
Wilcoxon W	143.000	122.000	79.000	112.000	120.500	123.500
Z	-.404	-1.617	-4.099	-2.196	-1.725	-1.578
Asymp. Sig. (2-tailed)	.686	.106	<.001	.028	.084	.115

**Test Statistics<sup>a</sup>**

	FI	Coble's peak B	Coble's peak T	Coble's peak A	Coble's peak M	Coble's peak C
Mann-Whitney U	27.000	1.000	.000	.000	.000	7.500
Wilcoxon W	105.000	79.000	78.000	78.000	78.000	85.500
Z	-2.630	-4.132	-4.185	-4.164	-4.161	-3.734
Asymp. Sig. (2-tailed)	.009	<.001	<.001	<.001	<.001	<.001

**Test Statistics<sup>a</sup>**

	TA	AC	ACBT
Mann-Whitney U	56.000	69.500	47.000
Wilcoxon W	134.000	147.500	125.000
Z	-.937	-.144	-1.444
Asymp. Sig. (2-tailed)	.349	.885	.149

F- The tables show the Mann-Whitney test results for comparing the sampling dates effects (August 2018 versus September 2019) on the DOM composition and DOC concentrations in grassland.

**Test Statistics<sup>a</sup>**

	TDN (mg/kg)	C/N RATIO	DOC (mg/kg)	SUVA254	BIX	HIX
Mann-Whitney U	.000	.000	.000	8.500	10.500	14.000
Wilcoxon W	78.000	78.000	78.000	86.500	88.500	92.000
Z	-4.157	-4.157	-4.157	-3.669	-3.623	-3.399
Asymp. Sig. (2-tailed)	<.001	<.001	<.001	<.001	<.001	<.001

**Test Statistics<sup>a</sup>**

	FI	Coble's peak B	Coble's peak T	Coble's peak A	Coble's peak M	Coble's peak C
Mann-Whitney U	4.000	.000	.000	.000	.000	.000
Wilcoxon W	82.000	78.000	78.000	78.000	78.000	78.000
Z	-3.943	-4.180	-4.184	-4.161	-4.161	-4.162
Asymp. Sig. (2-tailed)	<.001	<.001	<.001	<.001	<.001	<.001

**Test Statistics<sup>a</sup>**

	TA	AC	ACBT
Mann-Whitney U	.000	41.500	.000
Wilcoxon W	78.000	119.500	78.000
Z	-4.176	-1.769	-4.157
Asymp. Sig. (2-tailed)	<.001	.077	<.001

G- The tables show the Mann-Whitney test results for comparing the sampling dates effects (August 2018 versus October 2019) on the DOM composition and DOC concentrations in grassland.

**Test Statistics<sup>a</sup>**

	TDN (mg/kg)	C/N RATIO	DOC (mg/kg)	SUVA254	BIX	HIX
Mann-Whitney U	71.000	3.000	.000	41.500	4.500	60.000
Wilcoxon W	149.000	81.000	78.000	119.500	82.500	138.000
Z	-.058	-3.984	-4.157	-1.763	-3.951	-.704
Asymp. Sig. (2-tailed)	.954	<.001	<.001	.078	<.001	.482

**Test Statistics<sup>a</sup>**

	FI	Coble's peak B	Coble's peak T	Coble's peak A	Coble's peak M	Coble's peak C
Mann-Whitney U	24.000	36.000	36.000	8.500	.000	13.500
Wilcoxon W	102.000	114.000	114.000	86.500	78.000	91.500
Z	-2.792	-2.089	-2.094	-3.673	-4.160	-3.380
Asymp. Sig. (2-tailed)	.005	.037	.036	<.001	<.001	<.001

**Test Statistics<sup>a</sup>**

	TA	AC	ACBT
Mann-Whitney U	44.500	61.500	45.000
Wilcoxon W	122.500	139.500	123.000
Z	-1.600	-.607	-1.559
Asymp. Sig. (2-tailed)	.110	.544	.119

H- The tables show the Mann-Whitney test results for comparing the sampling dates effects (August 2018 versus July 2020) on the DOM composition and DOC concentrations in grassland.

**Mann-Whitney Test**

<b>Test Statistics<sup>a</sup></b>						
	TDN (mg/kg)	C/N RATIO	DOC (mg/kg)	SUVA254	BIX	HIX
Mann-Whitney U	1.000	.000	.000	33.000	25.500	70.000
Wilcoxon W	79.000	78.000	78.000	111.000	103.500	148.000
Z	-4.099	-4.158	-4.157	-2.254	-2.783	-.119
Asymp. Sig. (2-tailed)	<.001	<.001	<.001	.024	.005	.906

<b>Test Statistics<sup>a</sup></b>						
	FI	Coble's peak B	Coble's peak T	Coble's peak A	Coble's peak M	Coble's peak C
Mann-Whitney U	20.000	38.500	47.000	6.000	.000	7.000
Wilcoxon W	98.000	116.500	125.000	84.000	78.000	85.000
Z	-3.028	-1.953	-1.454	-3.813	-4.160	-3.757
Asymp. Sig. (2-tailed)	.002	.051	.146	<.001	<.001	<.001

<b>Test Statistics<sup>a</sup></b>			
	TA	AC	ACBT
Mann-Whitney U	61.500	37.000	55.000
Wilcoxon W	139.500	115.000	133.000
Z	-.610	-2.024	-.981
Asymp. Sig. (2-tailed)	.542	.043	.326

**Section S4:** Mann-Whitney U test for the comparison results of DOM composition and DOC concentration in grassland compared to control plots in cropland.

**Mann-Whitney Test**

<b>Test Statistics<sup>a</sup></b>					
	TDN (mg/kg)	C/N ratio	DOC (mg/kg)	SUVA254	BIX
Mann-Whitney U	21635.500	17789.000	15551.000	7231.000	17180.500
Wilcoxon W	113870.500	110024.000	107786.000	13117.000	109415.500
Z	-1.062	-3.731	-5.284	-11.013	-4.165
Asymp. Sig. (2-tailed)	.288	<.001	<.001	<.001	<.001

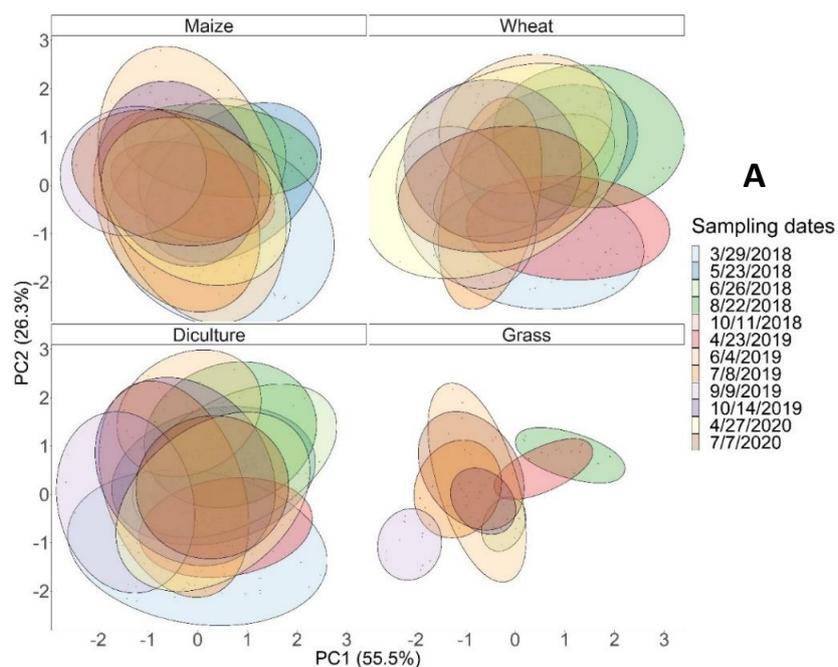
<b>Test Statistics<sup>a</sup></b>						
	HIX	FI	Coble B	Coble T	Coble A	Coble M
Mann-Whitney U	14506.500	9975.500	18005.500	12279.000	19325.000	22148.500

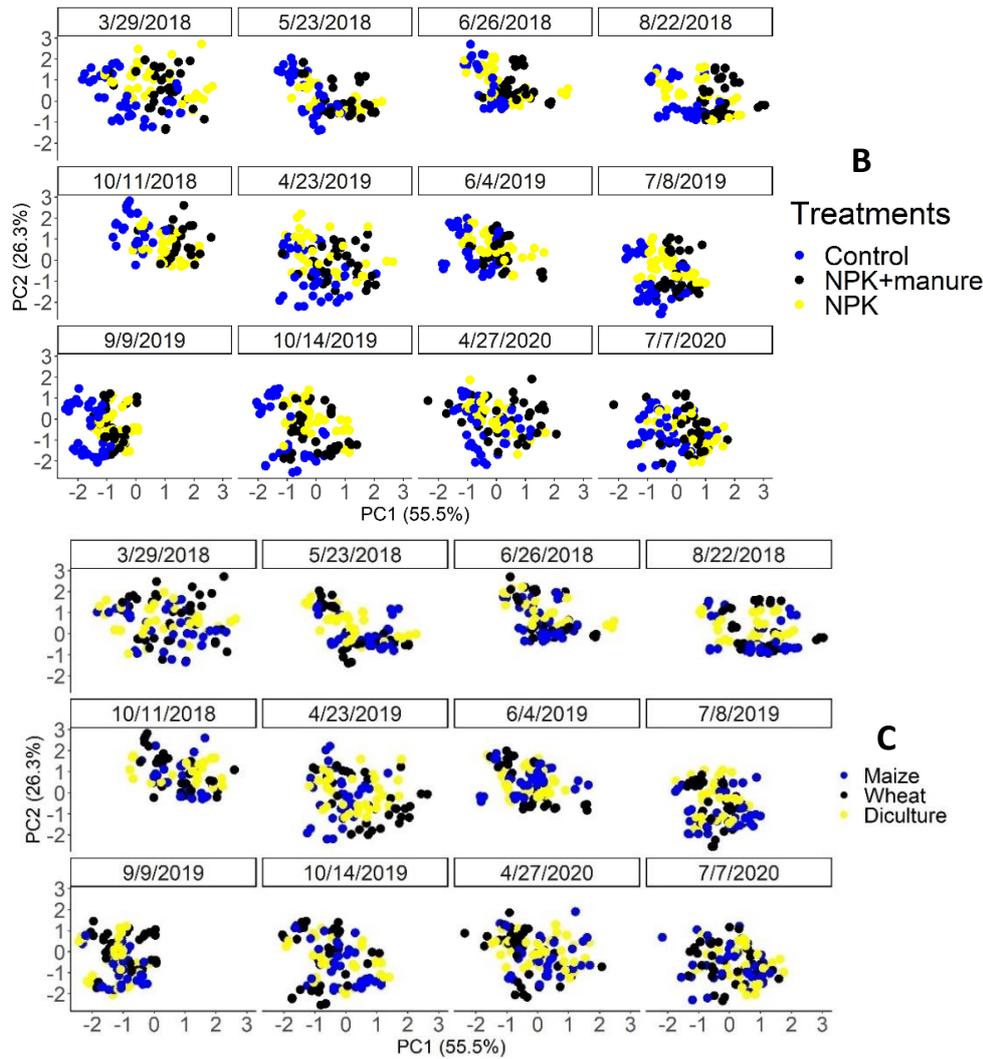
Wilcoxon W	93112.500	15861.500	23891.500	90885.000	25211.000	28034.500
Z	-3.611	-9.168	-3.582	-5.390	-2.665	-.706
Asymp. Sig. (2-tailed)	<.001	<.001	<.001	<.001	.008	.480

**Test Statistics<sup>a</sup>**

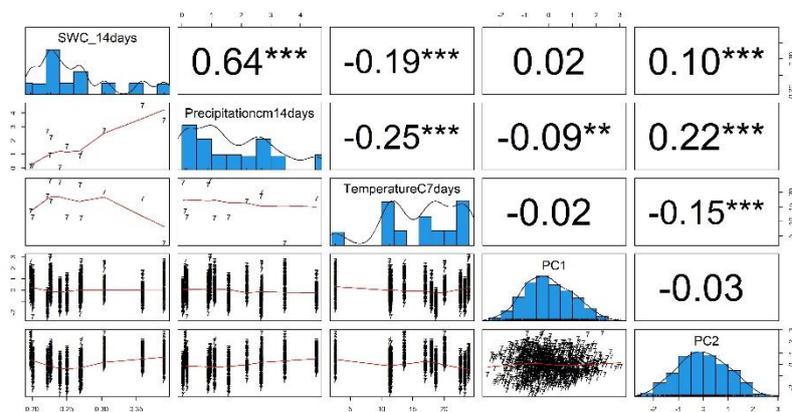
	Coble C	T/A index	A/C index	(A+C)/(B+T) index
Mann-Whitney U	12902.500	11282.000	11378.500	16167.500
Wilcoxon W	18788.500	89888.000	103613.500	20823.500
Z	-7.123	-6.193	-8.180	-2.273
Asymp. Sig. (2-tailed)	<.001	<.001	<.001	.023

**Section S5:** The correlation plot and PCA results figures were shown in this section.



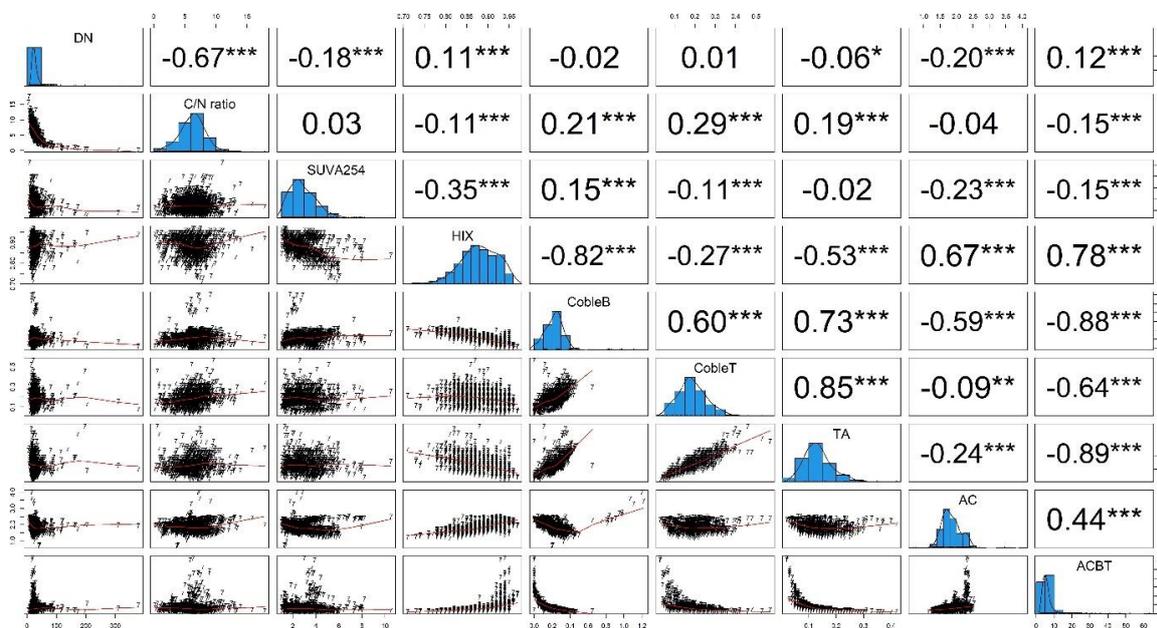


**Figure S12.** PCA results for the measured DOM parameters for twelve sampling dates in grassland and cropland across various crops beside grassland (A), treatment, and plants within cropland (B and C, respectively). The DOM variances were explained in brackets. Oval cloud shapes highlight the seasonal pattern on DOM parameters within grassland and each crop in cropland. NPK: nitrogen, phosphorus, and potassium.

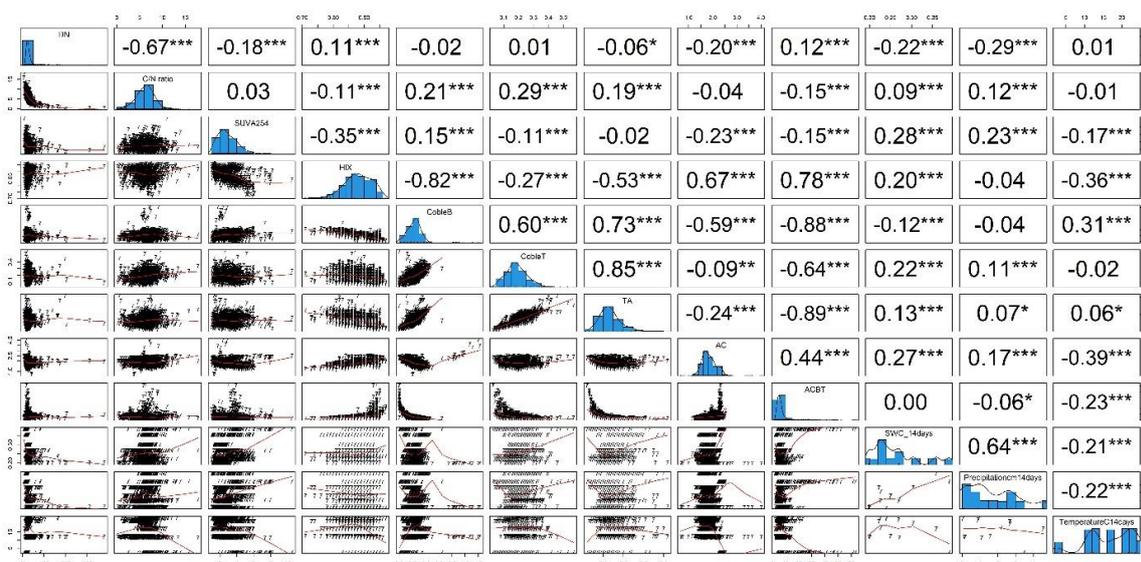


**Figure S13.** The relationship between the measured weather data and each principal component (PC) (n=1404). The significance level was calculated using Spearman's correlation

coefficients ( $\rho$ ). The sign (\*\*\*, \*\*, \*) referred to the test's significance and corresponding to each p-value  $< 0.001$ ,  $< 0.01$ ,  $< 0.05$ , respectively. PC1: The first principal component; PC2: The second principal component; SWC\_14days: Average soil water content before 14 days from the sampling dates, and similar definition keys for average air temperature and the sum of precipitation.



**Figure S14.** The relationship between the measured DOM parameters. The significance level was calculated using Spearman's correlation coefficients ( $\rho$ ). The sign (\*\*\*, \*\*, \*) referred to the test's significance and corresponding to each p-value  $< 0.001$ ,  $< 0.01$ ,  $< 0.05$ , respectively. DOM parameters started from the top: DN (Total dissolved nitrogen), C/N ratio; HIX; Coble peak's B, T, TA (T/A index), AC (A/C index), and ACBT (A+C)/(B+T).



**Figure S15.** The relationship between the measured DOM parameters and weather data. The significance level was calculated using Spearman's correlation coefficients ( $\rho$ ). The sign (\*\*\*, \*\*, \*) referred to the test's significance and corresponding to each p-value  $< 0.001$ ,  $< 0.01$ ,  $< 0.05$ , respectively.

0.05, respectively. DOM parameters started from the top: DN (Total dissolved nitrogen), C/N ratio; SUV<sub>254</sub>; HIX; Coble peak's B, T, TA (T/A index), AC (A/C index), and ACBT (A+C)/(B+T) respectively. SWC\_14days: Average soil water content before 14 days from the sampling dates and similar keys information for sum precipitation and average air temperature.

**Section S6:**

Result of Kruskal-Wallis and Mann-Whitney U test for August 2018. The statistical results include studying treatment's effects on the aggregate stability (A) and crops (Land uses) (B) within cropland. Also, the tillage effect on aggregate stability was studied by comparing grassland data with cropland (control plots) (C).

A- The treatment factor (fertilization) affected aggregate stability within cropland in August 2018.

Null Hypothesis	Test	Sig. <sup>a,b</sup>	Decision
The distribution of AggregateStability is the same across categories of Treatments.	Independent -Samples Kruskal-Wallis Test	0.009	Reject the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

**Independent-Samples Kruskal-Wallis Test**

**Summary**

Total N	36
Test Statistic	9.416 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.009

a. The test statistic is adjusted for ties.

**Pairwise Comparisons of Treatments**

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
NPK-Control	3.583	4.301	.833	.405	1.000
NPK-NPK+manure	-12.792	4.301	-2.974	.003	.009
Control-NPK+manure	-9.208	4.301	-2.141	.032	.097

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

B- The crop factor (land uses) affects aggregate stability within cropland for August 2018.

Null Hypothesis	Test	Sig. <sup>a,b</sup>	Decision
The distribution of AggregateStability is the same across	Independent-Samples Kruskal-Wallis Test	0.996	Retain the null hypothesis.

categories of Landuses.			
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- a. The significance level is .050.
- b. Asymptotic significance is displayed.

### Independent-Samples Kruskal-Wallis Test

#### Summary

Total N	36
Test Statistic	.008 <sup>a,b</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.996

- a. The test statistic is adjusted for ties.
- b. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

C- Tillage factor affects aggregate stability, comparing cropland to grassland for August 2018.

#### Test Statistics<sup>a</sup>

aggregate stability	
Mann-Whitney U	.000
Wilcoxon W	78.000
Z	-2.910
Asymp. Sig. (2-tailed)	.004

a. Grouping Variable: Site\_Groups

### Section S7:

Result of one-way ANOVA for April 2019 data regarding the aggregate stability. It studied the effect of treatments (fertilization) (A) and land uses (crops) (B) on aggregate stability within cropland. Also, the site factor was used to compare grassland to cropland (Control plots) regarding their aggregate stability (C).

A- The treatment factor affected aggregate stability within cropland in April 2019.

#### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	350.471	2	175.235	2.229	.124
Within Groups	2594.576	33	78.624		
Total	2945.047	35			

B- Crops factor affects aggregate stability within cropland in April 2019.

#### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1154.072	2	577.036	10.632	<.001
Within Groups	1790.975	33	54.272		
Total	2945.047	35			

### Multiple Comparisons

(I) crops recode	(J) crops recode	Mean Difference (I-J)	Std. Error	Sig.
Maize	Wheat	3.35833	3.00755	.511
	Diculture	13.33250*	3.00755	<.001
Wheat	Maize	-3.35833	3.00755	.511
	Diculture	9.97417*	3.00755	.006
Diculture	Maize	-13.33250*	3.00755	<.001
	Wheat	-9.97417*	3.00755	.006

C- Tillage factor affects aggregate stability, comparing cropland (control plots) to grassland for April 2019.

### ANOVA

aggregate stability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1138.996	1	1138.996	15.914	.001
Within Groups	1001.985	14	71.570		
Total	2140.981	15			

### Section S8:

Kruskal-Wallis, Mann-Whitney U test, and one-way ANOVA results for August 2018 compared to April 2019. The statistical results include studying treatment's effects on the aggregate stability (A) and crops (Land uses) (B) within cropland. Also, the tillage effect on aggregate stability was studied by comparing grassland data with cropland (control plots) (C). Furthermore, the date (or seasonality) factor affects aggregate stability, including the control plot (D), the NPK+manure (E), and the NPK plot (F) in cropland and grassland data (G).

A- The treatment factor (fertilization) affects aggregate stability within cropland, including sampling dates.

Null Hypothesis	Test	Sig. <sup>a,b</sup>	Decision
The distribution of aggregate stability is the same across categories of Treatments.	Independent-Samples Kruskal-Wallis Test	0.097	Retain the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

### Independent-Samples Kruskal-Wallis Test

#### Summary

Total N	72
Test Statistic	4.673 <sup>a,b</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.097

a. The test statistic is adjusted for ties.

b. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

B- The crop factor (land uses) affects aggregate stability within cropland, including sampling dates.

Null Hypothesis	Test	Sig. <sup>a,b</sup>	Decision
The distribution of aggregate stability is the same across categories of land use.	Independent-Samples Kruskal-Wallis Test	0.045	Reject the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

### Independent-Samples Kruskal-Wallis Test

#### Summary

Total N	72
Test Statistic	6.192 <sup>a</sup>
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.045

a. The test statistic is adjusted for ties.

#### Pairwise Comparisons of Landuses

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
Diculture-Wheat	-11.479	6.041	-1.900	.057	.172
Diculture-Maize	-14.146	6.041	-2.341	.019	.058
Wheat-Maize	2.667	6.041	.441	.659	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

C- Tillage factor affects aggregate stability, comparing cropland (control plots) to grassland, including sampling dates.

**Test Statistics<sup>a</sup>**

aggregate stability	
Mann-Whitney U	5.000
Wilcoxon W	305.000
Z	-3.960
Asymp. Sig. (2-tailed)	<.001

a. Grouping Variable: Site\_group

D- The date (or seasonality) factor affects aggregate stability for control plot data in cropland, including both sampling dates.

**Test Statistics<sup>a</sup>**

aggregate stability	
Mann-Whitney U	66.000
Wilcoxon W	144.000
Z	-.346
Asymp. Sig. (2-tailed)	.729

a. Grouping Variable: Date\_group

E- The date (or seasonality) factor affects aggregate stability for NPK+manure treated plot data in cropland, including both sampling dates.

**ANOVA**

aggregate stability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1062.670	1	1062.670	21.765	<.001
Within Groups	1074.166	22	48.826		
Total	2136.836	23			

F- The date (or seasonality) factor affects aggregate stability for NPK-treated plot data in cropland, including both sampling dates.

**ANOVA**

aggregate stability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	354.202	1	354.202	8.153	.009
Within Groups	955.793	22	43.445		
Total	1309.995	23			

G- The date (or seasonality) factor affects aggregate stability for grassland data, including both sampling dates.

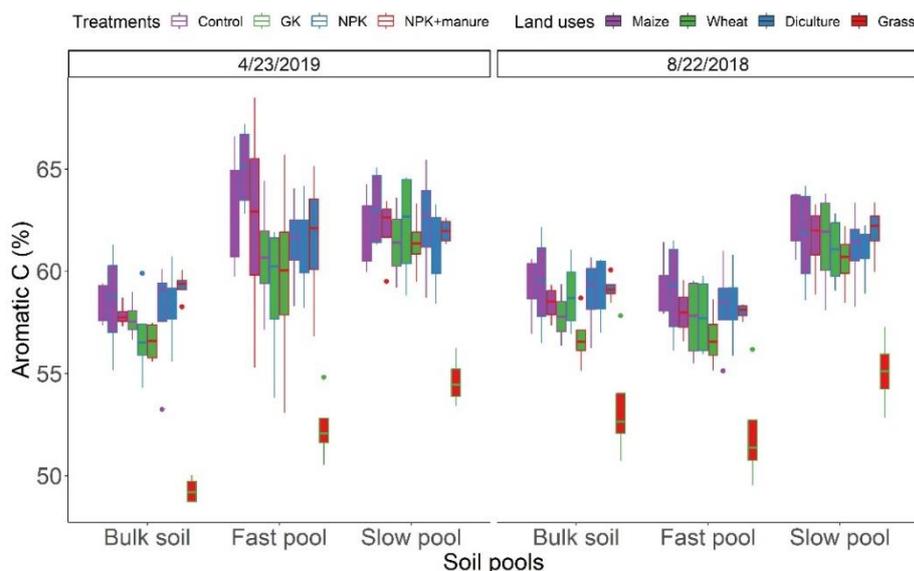
**ANOVA**

aggregate stability

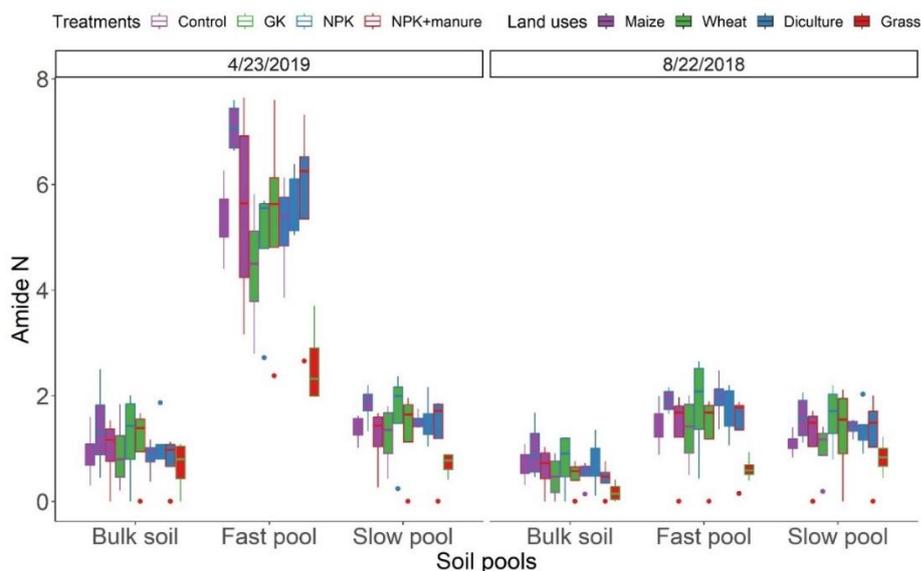
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.682	1	4.682	.497	.507
Within Groups	56.493	6	9.415		
Total	61.174	7			

**Section S9:** This section presents various figures and statistical output results.

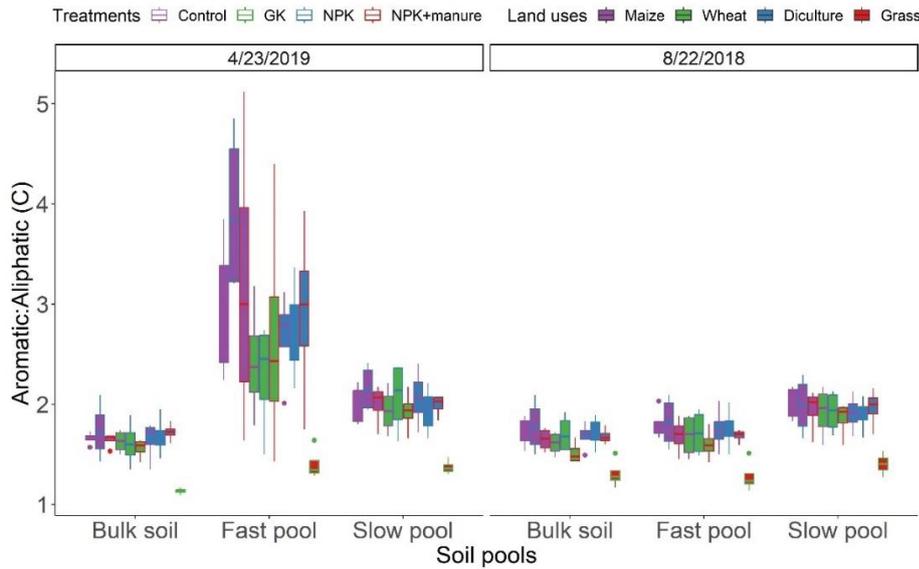
In this section, various figures and output statistical results were displayed.



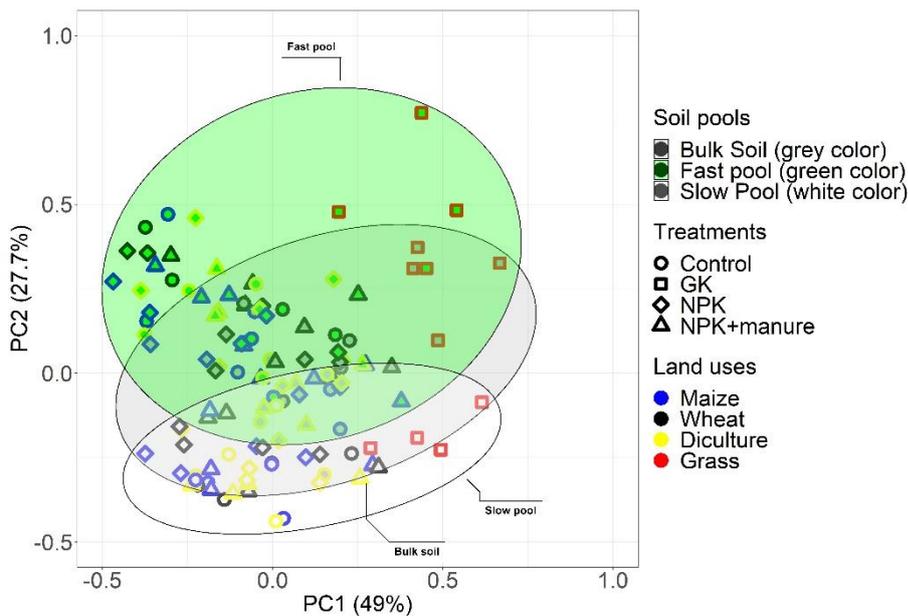
**Figure S16.** The aromatic C component in grassland and cropland sites under different treatments and land uses (crops) across both sampling dates. GK= grass. NPK: nitrogen, phosphorus, potassium.



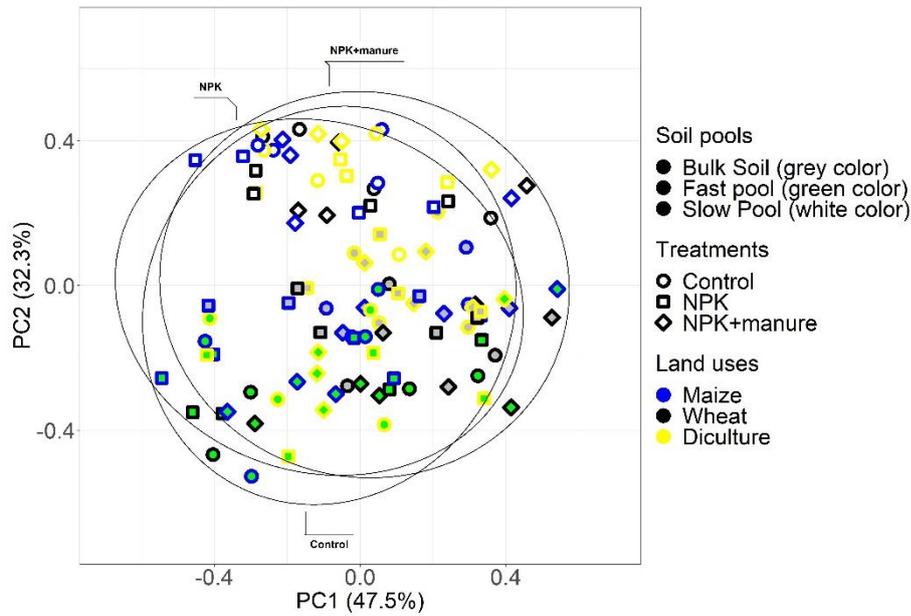
**Figure S17.** The amide N percentage in grassland and cropland sites under different treatments and land uses (crops) across both sampling dates. GK= grass. NPK: nitrogen, phosphorus, potassium.



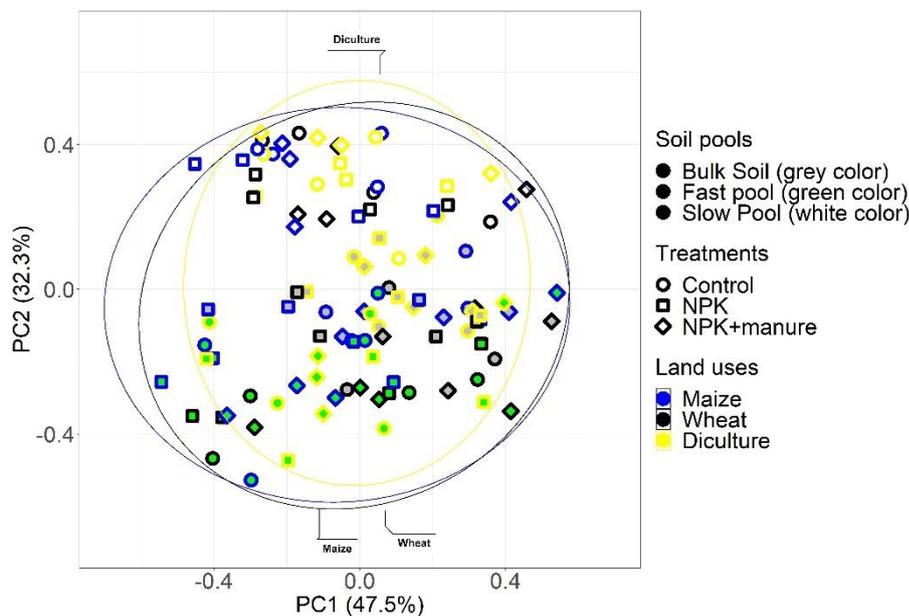
**Figure S18.** The aromatic:aliphatic ratio (aromaticity index) in grassland and cropland sites under different treatments and land uses (crops) across both sampling dates. GK= grass. NPK: nitrogen, phosphorus, potassium.



**Figure S19.** PCA results for the measured SOM variables, including grassland and cropland, in August. It highlighted the soil pool's effects on SOM variables. The SOC variances were explained in brackets. NPK: nitrogen, phosphorus, and potassium. The circled cloud indicated the soil pool's effects on the measured SOM parameters.



**Figure S20.** PCA results for the measured SOM variables within cropland in August. It highlighted the treatment effects (fertilization) on SOM variables. The SOC variances were explained in brackets. The circled cloud was indicated to highlight the treatment effects (fertilization) on the measured SOM parameters.



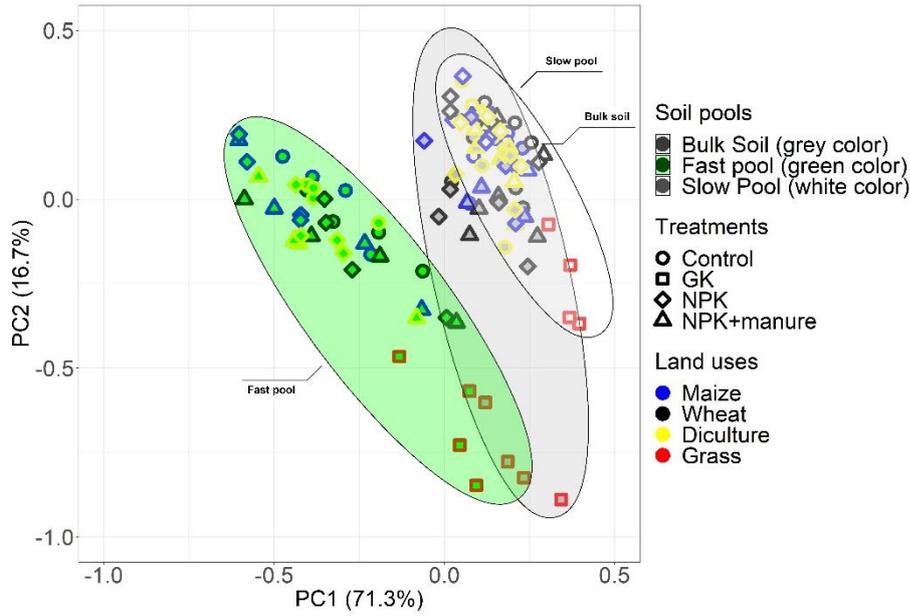
**Figure S21.** PCA results for the measured SOM variables within cropland in August. It highlighted the crop's effects (land uses) on SOM variables in cropland. The SOC variances were explained in brackets. The circled cloud was indicated to highlight the crop's effects (land uses) on the measured SOM parameters.

**Table S2:** The interaction effect results of treatments, fertilization, soil pools, and land uses on all measured SOM variables in August over both study sites (n=120). It showed the output of

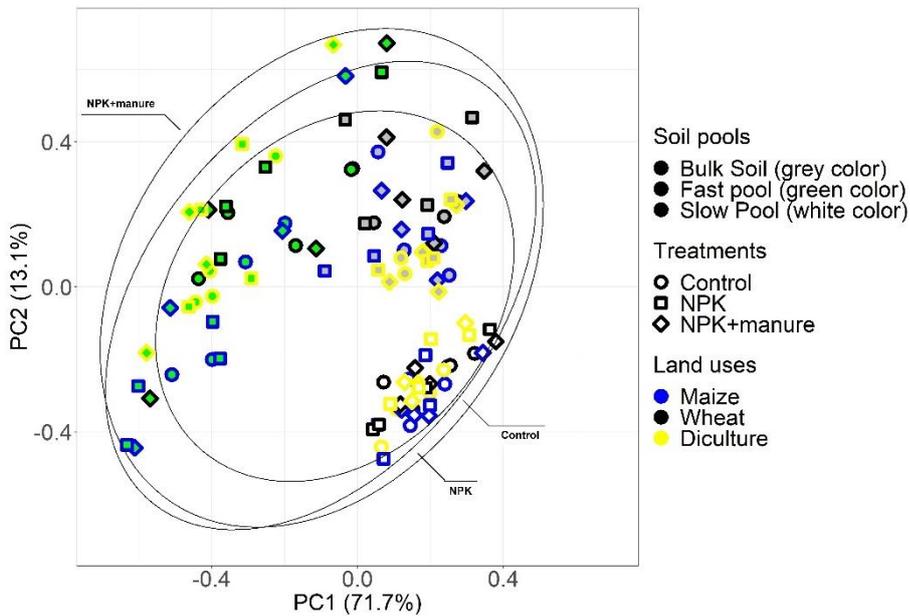
the Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise comparison results. Significance codes: 0 '\*\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1.

<b>Table S2-A-Cropland (n=108)</b>							
Permutation test for adonis under reduced model							
Terms added sequentially (first to last)							
Permutation: free							
Number of permutations: 999							
adonis2(formula = M_SOM3[, 1:10] ~ Soil_pools, data = M_SOM3, permutations = 999, method = "bray")							
	Df	SumOfSqs	R2	F	Pr(>F)		
Soil_pools	2	0.042940	0.33648	26.623	0.001	***	
Residual	105	0.084676	0.66352				
Total	107	0.127616	1.00000				
---							
Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1							
Pairwise comparisons							
pairs		Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted sig
1 Bulk soil vs Fast pool	1	0.01067954	11.94403	0.1457584	0.001	0.003	*
2 Bulk soil vs Slow pool	1	0.02368297	34.65003	0.3311039	0.001	0.003	*
3 Fast pool vs Slow pool	1	0.03004690	35.69800	0.3377358	0.001	0.003	*
<b>Table S2-B-Grassland (n=12)</b>							
Permutation test for adonis under reduced model							
Terms added sequentially (first to last)							
Permutation: free							
Number of permutations: 999							
adonis2(formula = M_SOM5[, 1:10] ~ Soil_pools, data = M_SOM5, permutations = 999, method = "bray")							
	Df	SumOfSqs	R2	F	Pr(>F)		
Soil_pools	2	0.0096343	0.41264	3.1613	0.022	*	
Residual	9	0.0137138	0.58736				
Total	11	0.0233481	1.00000				
---							
Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1							
Pairwise comparisons							
pairs		Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted sig
1 Bulk soil vs Fast pool	1	0.0009981759	0.5425909	0.08293211	0.677	1.000	
2 Bulk soil vs Slow pool	1	0.0050974497	4.4512985	0.42590866	0.037	0.111	
3 Fast pool vs Slow pool	1	0.0083557794	5.2668818	0.46746579	0.027	0.081	
<b>Table S2-C: Grassland versus cropland (n=120)</b>							
Terms added sequentially (first to last)							
Permutation: free							
Number of permutations: 999							

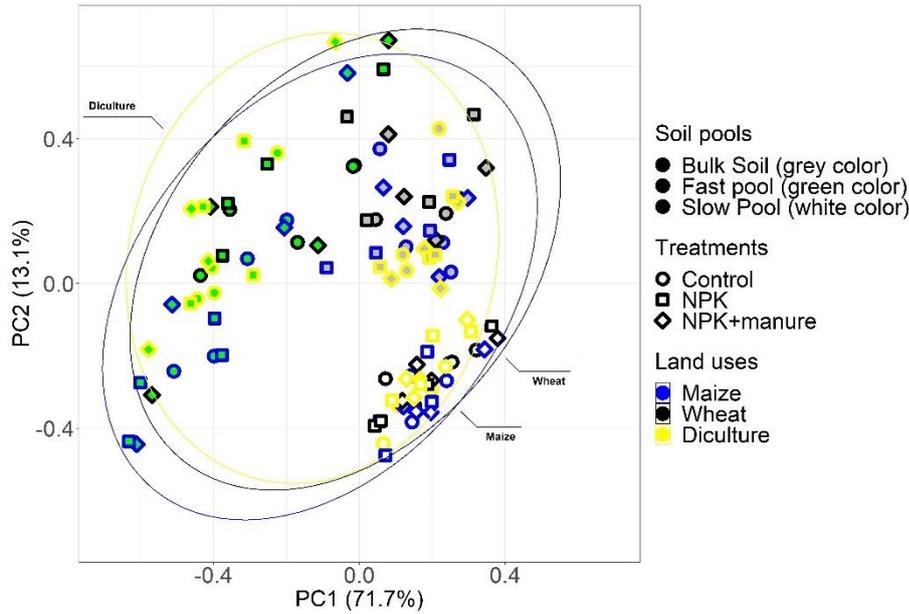
adonis2(formula = M_SOM3[, 1:10] ~ Land_uses * Treatments * Soil_pools, data = M_SOM3, permutations = 999, method = "bray")						
	Df	SumOfSqs	R2	F	Pr(>F)	
Land_uses	3	0.087024	0.37072	30.7840	0.001	***
Treatments	2	0.002750	0.01172	1.4593	0.193	
Soil_pools	2	0.051790	0.22063	27.4803	0.001	***
Land_uses:Treatments	4	0.002771	0.01180	0.7351	0.691	
Land_uses:Soil_pools	6	0.002255	0.00961	0.3989	0.978	
Treatments:Soil_pools	4	0.001944	0.00828	0.5157	0.877	
Land_uses:Treatments:Soil_pools	8	0.001399	0.00596	0.1856	1.000	
Residual	90	0.084808	0.36128			
Total	119	0.234742	1.00000			
Pairwise comparisons						
	pairs	Df	SumsOfSqs	F.Model	R2	p.value p.adjusted sig
1	Control vs NPK+manure	1	0.0013890826	1.1793809	0.016569137	0.328 1.000
2	Control vs NPK	1	0.0007335527	0.6268106	0.008874966	0.627 1.000
3	Control vs GK	1	0.0730085955	53.4353448	0.537387836	0.001 0.006 *
4	NPK+manure vs NPK	1	0.0020028433	1.6423694	0.022924554	0.169 1.000
5	NPK+manure vs GK	1	0.0615312295	42.6958593	0.481373760	0.001 0.006 *
6	NPK vs GK	1	0.0755928274	52.8724660	0.534754195	0.001 0.006 *
Pairwise comparisons						
	Pairs	Df	SumsOfSqs	F.Model	R2	p.value p.adjusted sig
1	Maize vs Wheat	1	0.0030014790	2.4309776	0.033562679	0.073 0.438
2	Maize vs Diculture	1	0.0002072153	0.1801851	0.002567464	0.917 1.000
3	Maize vs Grass	1	0.0766264097	53.4815450	0.537602678	0.001 0.006 *
4	Wheat vs Diculture	1	0.0016606910	1.4209465	0.019895374	0.248 1.000
5	Wheat vs Grass	1	0.0639852514	43.7885462	0.487685212	0.001 0.006 *
6	Diculture vs Grass	1	0.0696449759	52.2706097	0.531904807	0.001 0.006 *
Pairwise comparisons						
	Pairs	Df	SumsOfSqs	F.Model	R2	p.value p.adjusted sig
1	Bulk soil vs Fast pool	1	0.01302162	7.602484	0.08881149	0.002 0.006 *
2	Bulk soil vs Slow pool	1	0.02684791	19.521083	0.20017295	0.001 0.003 *
3	Fast pool vs Slow pool	1	0.03781557	23.591483	0.23221910	0.001 0.003 *



**Figure S22.** PCA results for the measured SOM variables, including grassland and cropland, in April. It highlighted the soil pool's effects on SOM variables. The SOC variances were explained in brackets. The circled cloud indicated the soil pool's effects on the measured SOM parameters.



**Figure S23.** PCA results for the measured SOM variables within cropland in April. It highlighted the treatment effects (fertilization) on SOM variables. The SOC variances were explained in brackets. The circled cloud was indicated to highlight the treatment effects (fertilization) on the measured SOM parameters.



**Figure S24.** PCA results for the measured SOM variables within cropland in April. It highlighted the crop's effects (land uses) on SOM variables in cropland. The SOC variances were explained in brackets. The circled cloud was indicated to highlight the crop's effects (land uses) on the measured SOM parameters.

**Table S3:** The interaction effect results of treatments, fertilization, soil pools, and land uses on all measured SOM variables in April over both study sites (n=120). It showed the output of the Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise comparison results. Significance codes: 0 '\*\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1.

<b>Table S3-A-Cropland (n=108)</b>							
Permutation test for adonis under reduced model							
Terms added sequentially (first to last)							
Permutation: free							
Number of permutations: 999							
adonis2(formula = M_SOM3[, 1:10] ~ Soil_pools, data = M_SOM3, permutations = 999, method = "bray")							
	Df	SumOfSqs	R2	F	Pr(>F)		
Soil_pools	2	0.37412	0.6659	104.64	0.001	***	
Residual	105	0.18771	0.3341				
Total	107	0.56183	1.0000				
---							
Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1							
Pairwise comparisons							
	pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted sig
1	Bulk soil vs Fast pool	1	0.30318399	131.60523	0.6527868	0.001	0.003 *
2	Bulk soil vs Slow pool	1	0.02821891	33.40648	0.3230598	0.001	0.003 *

3 Fast pool vs Slow pool	1	0.22978061	103.75547	0.5971350	0.001	0.003	*
<b>Table S3-B-Grassland (n=12)</b>							
Permutation test for adonis under reduced model							
Terms added sequentially (first to last)							
Permutation: free							
Number of permutations: 999							
adonis2(formula = M_SOM5[, 1:10] ~ Soil_pools, data = M_SOM5, permutations = 999, method = "bray")							
	Df	SumOfSqs	R2	F	Pr(>F)		
Soil_pools	2	0.026091	0.71423	11.247	0.001	***	
Residual	9	0.010439	0.28577				
Total	11	0.036530	1.00000				
---							
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1							
Pairwise comparisons							
pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted	sig
1 Bulk soil vs Fast pool	1	0.006904899	4.779235	0.4433743	0.026	0.078	
2 Bulk soil vs Slow pool	1	0.015099113	15.496036	0.7208788	0.030	0.090	
3 Fast pool vs Slow pool	1	0.017132228	16.153132	0.7291579	0.017	0.051	
<b>Table S3-C-Grassland versus cropland (n=120)</b>							
Terms added sequentially (first to last)							
Permutation: free							
Number of permutations: 999							
adonis2(formula = M_SOM2[, 1:10] ~ Land_uses * Treatments * Soil_pools, data = M_SOM2, permutations = 999, method = "bray")							
	Df	SumOfSqs	R2	F	Pr(>F)		
Land_uses	3	0.16721	0.22196	31.8600	0.001	***	
Treatments	2	0.00704	0.00934	2.0120	0.101		
Soil_pools	2	0.39109	0.51915	111.7787	0.001	***	
Land_uses:Treatments	4	0.00555	0.00737	0.7932	0.582		
Land_uses:Soil_pools	6	0.01782	0.02365	1.6975	0.098	.	
Treatments:Soil_pools	4	0.00194	0.00258	0.2773	0.973		
Land_uses:Treatments:Soil_pools	8	0.00523	0.00695	0.3739	0.976		
Residual	90	0.15745	0.20900				
Total	119	0.75333	1.00000				
<b>Pairwise comparisons</b>							
Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted	sig
1 Control vs NPK+manure	1	0.003667130	0.7176327	0.010147861	0.451	1.000	
2 Control vs NPK	1	0.003692482	0.7344682	0.010383455	0.423	1.000	
3 Control vs GK	1	0.133081686	31.9905060	0.410184619	0.001	0.006	*
4 NPK+manure vs NPK	1	0.003200095	0.5600739	0.007937548	0.517	1.000	
5 NPK+manure vs GK	1	0.118039139	22.6807610	0.330234562	0.001	0.006	*
6 NPK vs GK	1	0.138064095	27.1851648	0.371457315	0.001	0.006	*
<b>Pairwise comparisons</b>							
pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted	sig

1	Maize vs Wheat	1	0.011392872	2.1141056	0.029316118	0.141	0.846
2	Maize vs Diculture	1	0.002408179	0.4323315	0.006138253	0.586	1.000
3	Maize vs Grass	1	0.148736086	26.9277722	0.369238925	0.001	0.006 *
4	Wheat vs Diculture	1	0.004557017	0.9607012	0.013538497	0.346	1.000
5	Wheat vs Grass	1	0.106433223	24.9528820	0.351682431	0.001	0.006 *
6	Diculture vs Grass	1	0.135315339	29.7975394	0.393120141	0.001	0.006 *
<b>Pairwise comparisons</b>							
	Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted sig
1	Bulk soil vs Fast pool	1	0.30628146	78.85453	0.5027240	0.001	0.003 *
2	Bulk soil vs Slow pool	1	0.03148854	16.40923	0.1738096	0.001	0.003 *
3	Fast pool vs Slow pool	1	0.24887146	71.41171	0.4779526	0.001	0.003 *

**Section S10:** Various statistical outputs and PCA figures were displayed in this section.

**A-** The Mann-Whitney U results include studying sampling date effects on the measured SOM variables in bulk soil in cropland.

**Test Statistics<sup>a</sup>**

	N	SOC	C_Nratio	aliphatic	aromatic	amide_bond
Mann-Whitney U	449.000	533.500	142.500	412.000	525.500	394.000
Wilcoxon W	1115.000	1199.500	808.500	1078.000	1191.500	1060.000
Z	-2.260	-1.290	-5.694	-2.658	-1.380	-2.864
Asymp. Sig. (2-tailed)	.024	.197	<.001	.008	.168	.004

**Test Statistics<sup>a</sup>**

	phenolic_lignin	Polysaccharides	C_Oratio	aromaticity
Mann-Whitney U	468.500	609.500	615.500	609.500
Wilcoxon W	1134.500	1275.500	1281.500	1275.500
Z	-2.022	-.434	-.366	-.434
Asymp. Sig. (2-tailed)	.043	.665	.714	.664

a. Grouping Variable: Date\_Group

**B-** The statistical results include studying sampling date effects on the measured SOM variables in the fast pool in cropland.

**Test Statistics<sup>a</sup>**

	N	SOC	C_Nratio	aliphatic	aromatic	amide_bond
Mann-Whitney U	533.000	463.000	468.000	382.500	243.000	3.000
Wilcoxon W	1199.000	1129.000	1134.000	1048.500	909.000	669.000
Z	-1.303	-2.084	-2.027	-2.990	-4.561	-7.265
Asymp. Sig. (2-tailed)	.193	.037	.043	.003	<.001	<.001

**Test Statistics<sup>a</sup>**

	phenolic_lignin	Polysaccharides	C_Oratio	aromaticity
Mann-Whitney U	22.000	.000	.000	119.500
Wilcoxon W	688.000	666.000	666.000	785.500
Z	-7.050	-7.298	-7.298	-5.953
Asymp. Sig. (2-tailed)	<.001	<.001	<.001	<.001

a. Grouping Variable: Date\_Group

**C-** The statistical results include studying sampling date effects on the measured SOM variables in the slow pool in cropland.

**Test Statistics<sup>a</sup>**

	N	SOC	C_Nratio	aliphatic	aromatic	amide_bond
Mann-Whitney U	519.000	510.000	645.500	540.000	550.500	524.000
Wilcoxon W	1185.000	1176.000	1311.500	1206.000	1216.500	1190.000
Z	-1.471	-1.555	-.028	-1.216	-1.098	-1.397
Asymp. Sig. (2-tailed)	.141	.120	.978	.224	.272	.162

**Test Statistics<sup>a</sup>**

	phenolic_lignin	Polysaccharides	C_Oratio	aromaticity
Mann-Whitney U	535.000	419.000	419.500	531.500
Wilcoxon W	1201.000	1085.000	1085.500	1197.500
Z	-1.273	-2.580	-2.574	-1.313
Asymp. Sig. (2-tailed)	.203	.010	.010	.189

a. Grouping Variable: Date\_Group

**D-** The one-way ANOVA results include studying sampling date effects on the measured SOM variables in bulk soil in grassland.

**ANOVA**

	Sum of Squares	df	Mean Square	F	Sig.
N	Between Groups	.000	1	.000	.141
	Within Groups	.013	6	.002	
	Total	.014	7		
SOC	Between Groups	.344	1	.344	.160
	Within Groups	12.913	6	2.152	
	Total	13.258	7		

C_Nratio	Between Groups	12.202	1	12.202	1.592	.254
	Within Groups	45.982	6	7.664		
	Total	58.184	7			
aliphatic	Between Groups	31.363	1	31.363	9.495	.022
	Within Groups	19.819	6	3.303		
	Total	51.182	7			
aromatic	Between Groups	34.903	1	34.903	7.150	.037
	Within Groups	29.288	6	4.881		
	Total	64.191	7			
amide_bond	Between Groups	.495	1	.495	3.412	.114
	Within Groups	.871	6	.145		
	Total	1.366	7			
phenolic_lignin	Between Groups	4.805	1	4.805	1.456	.273
	Within Groups	19.795	6	3.299		
	Total	24.600	7			
Polysaccharides	Between Groups	1.786	1	1.786	8.888	.025
	Within Groups	1.206	6	.201		
	Total	2.992	7			
C_Oratio	Between Groups	12.375	1	12.375	8.908	.024
	Within Groups	8.336	6	1.389		
	Total	20.711	7			
aromaticity	Between Groups	.061	1	.061	5.624	.055
	Within Groups	.065	6	.011		
	Total	.127	7			

**E-** The one-way ANOVA results include studying sampling date effects on the measured SOM variables in the fast pool in grassland.

#### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.	
N	Between Groups	.001	1	.001	.376	.562
	Within Groups	.020	6	.003		
	Total	.021	7			
SOC	Between Groups	2.773	1	2.773	.929	.372
	Within Groups	17.908	6	2.985		
	Total	20.681	7			
C_Nratio	Between Groups	18.911	1	18.911	1.239	.308

	Within Groups	91.553	6	15.259		
	Total	110.464	7			
aliphatic	Between Groups	.633	1	.633	.398	.552
	Within Groups	9.546	6	1.591		
	Total	10.179	7			
aromatic	Between Groups	.130	1	.130	.023	.884
	Within Groups	33.918	6	5.653		
	Total	34.048	7			
amide_bond	Between Groups	7.644	1	7.644	21.802	.003
	Within Groups	2.104	6	.351		
	Total	9.748	7			
phenolic_lignin	Between Groups	33.497	1	33.497	7.185	.037
	Within Groups	27.971	6	4.662		
	Total	61.468	7			
Polysaccharides	Between Groups	3.538	1	3.538	21.925	.003
	Within Groups	.968	6	.161		
	Total	4.506	7			
C_Oratio	Between Groups	17.761	1	17.761	17.928	.005
	Within Groups	5.944	6	.991		
	Total	23.705	7			
aromaticity	Between Groups	.031	1	.031	1.221	.311
	Within Groups	.153	6	.026		
	Total	.185	7			

**F-** The one-way ANOVA results include studying sampling date effects on the measured SOM variables in the slow pool in grassland.

#### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.	
N	Between Groups	.000	1	.000	.067	.804
	Within Groups	.010	6	.002		
	Total	.010	7			
SOC	Between Groups	.072	1	.072	.047	.836
	Within Groups	9.307	6	1.551		
	Total	9.379	7			
C_Nratio	Between Groups	.852	1	.852	.184	.683

	Within Groups	27.731	6	4.622		
	Total	28.582	7			
aliphatic	Between Groups	.118	1	.118	.055	.822
	Within Groups	12.845	6	2.141		
	Total	12.963	7			
aromatic	Between Groups	.383	1	.383	.155	.707
	Within Groups	14.787	6	2.465		
	Total	15.170	7			
amide_bond	Between Groups	.030	1	.030	.382	.559
	Within Groups	.472	6	.079		
	Total	.502	7			
phenolic_lignin	Between Groups	.300	1	.300	.203	.668
	Within Groups	8.897	6	1.483		
	Total	9.198	7			
Polysaccharides	Between Groups	.010	1	.010	1.342	.291
	Within Groups	.044	6	.007		
	Total	.054	7			
C_Oratio	Between Groups	.252	1	.252	1.639	.248
	Within Groups	.923	6	.154		
	Total	1.175	7			
aromaticity	Between Groups	.002	1	.002	.243	.639
	Within Groups	.052	6	.009		
	Total	.054	7			

**Table S4:** The interaction effect results of sampling date, treatments, and plant effects on all measured SOM variables within the slow pool in cropland (n=72). It showed the output of the Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise comparison results. Significance codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1.

Permutation test for adonis under reduced model					
Terms added sequentially (first to last)					
Permutation: free					
Number of permutations: 999					
adonis2(formula = M_SOM10[, 1:10] ~ Sampling_date * Land_uses * Treatments, data = M_SOM10, permutations = 999, method = "bray")					
	Df	SumOfSqs	R2	F	Pr(>F)
Sampling_date	1	0.001585	0.03162	2.0334	0.128
Land_uses	2	0.001022	0.02040	0.6558	0.569
Treatments	2	0.001546	0.03085	0.9920	0.378
Sampling_date:Land_uses	2	0.000529	0.01056	0.3394	0.847

Sampling_date:Treatments	2	0.000732	0.01460	0.4695	0.732
Land_uses:Treatments	4	0.001909	0.03809	0.6124	0.738
Sampling_date:Land_uses:Treatments	4	0.000713	0.01423	0.2288	0.979
Residual	54	0.042082	0.83965		
Total	71	0.050118	1.00000		
> pairwise.adonis(M_SOM10[ 1:10],M_SOM10\$Sampling_date)					
	pairs	Df	SumsOfSqs	F.Model	R2 p.value p.adjusted sig
1	April_2019 vs August_2018	1	0.001584593	2.285469	0.03161727 0.126 0.126
> pairwise.adonis(M_SOM10[ 1:10],M_SOM10\$Land_uses)					
	pairs	Df	SumsOfSqs	F.Model	R2 p.value p.adjusted sig
1	Maize vs Wheat	1	1.128774e-03	1.4757016	0.031083303 0.232 0.696
2	Maize vs Diculture	1	3.267822e-04	0.5069578	0.010900687 0.640 1.000
3	Wheat vs Diculture	1	7.768375e-05	0.1071355	0.002323621 0.908 1.000
> pairwise.adonis(M_SOM10[ 1:10],M_SOM10\$Treatments)					
	pairs	Df	SumsOfSqs	F.Model	R2 p.value p.adjusted sig
1	Control vs NPK+manure	1	0.0008944396	1.4195895	0.029936773 0.242 0.726
2	Control vs NPK	1	0.0002865549	0.3841738	0.008282433 0.648 1.000
3	NPK+manure vs NPK	1	0.0011381916	1.5467707	0.032531561 0.203 0.609

**Table S5:** The interaction effect results of sampling date, treatments, and plant effects on all measured SOM variables within the fast pool in cropland (n=72). It showed the output of the Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise comparison results. Significance codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1.

Permutation test for adonis under reduced model					
Terms added sequentially (first to last)					
Permutation: free					
Number of permutations: 999					
adonis2(formula = M_SOM10[, 1:10] ~ Sampling_date * Land_uses * Treatments, data = M_SOM10, permutations = 999, method = "bray")					
	Df	SumOfSqs	R2	F	Pr(>F)
Sampling_date	1	0.24324	0.59523	102.0774	0.001 ***
Land_uses	2	0.01453	0.03556	3.0490	0.041 *
Treatments	2	0.00529	0.01294	1.1097	0.320
Sampling_date:Land_uses	2	0.00542	0.01327	1.1383	0.325
Sampling_date:Treatments	2	0.00221	0.00541	0.4637	0.666
Land_uses:Treatments	4	0.00546	0.01336	0.5729	0.711
Sampling_date:Land_uses:Treatments	4	0.00382	0.00935	0.4007	0.863
Residual	54	0.12868	0.31488		
Total	71	0.40865	1.00000		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					
> pairwise.adonis(M_SOM10[ 1:10],M_SOM10\$Sampling_date)					
	pairs	Df	SumsOfSqs	F.Model	R2 p.value p.adjusted sig
1	April_2019 vs August_2018	1	0.2432382	102.9362	0.5952265 0.001 0.001 **
> pairwise.adonis(M_SOM10[ 1:10],M_SOM10\$Land_uses)					
	pairs	Df	SumsOfSqs	F.Model	R2 p.value p.adjusted sig

1	Maize vs Wheat	1	0.013334108	2.3151145	0.04791698	0.123	0.369
2	Maize vs Diculture	1	0.003327081	0.5341507	0.01147868	0.503	1.000
3	Wheat vs Diculture	1	0.005135041	0.9976343	0.02122733	0.339	1.000
> pairwise.adonis(M_SOM10[ 1:10],M_SOM10\$Treatments)							
		pairs	Df	SumsOfSqs	F.Model	R2	p.value p.adjusted sig
1	Control vs NPK+manure	1	0.003139494	0.5504933	0.011825723	0.490	1
2	Control vs NPK	1	0.001989005	0.3790536	0.008172948	0.578	1
3	NPK+manure vs NPK	1	0.002804481	0.4257586	0.009170740	0.569	1

**Table S6:** The interaction effect results of sampling date, treatments, and plant effects on all measured SOM variables within bulk soil in cropland (n=72). It showed the output of Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise comparison results. Significance codes: 0 ‘\*\*\*\*’ 0.001 ‘\*\*\*’ 0.01 ‘\*\*’ 0.05 ‘.’ 0.1 ‘ ’ 1.

Permutation test for adonis under reduced model							
Terms added sequentially (first to last)							
Permutation: free							
Number of permutations: 999							
adonis2(formula = M_SOM10[, 1:10] ~ Sampling_date * Land_uses * Treatments, data = M_SOM10, permutations = 999, method = "bray")							
		Df	SumOfSqs	R2	F	Pr(>F)	
Sampling_date		1	0.005601	0.08746	6.3887	0.001	***
Land_uses		2	0.003832	0.05983	2.1852	0.051	.
Treatments		2	0.002873	0.04485	1.6382	0.139	
Sampling_date:Land_uses		2	0.000317	0.00494	0.1805	0.981	
Sampling_date:Treatments		2	0.001025	0.01601	0.5847	0.741	
Land_uses:Treatments		4	0.002155	0.03364	0.6144	0.827	
Sampling_date:Land_uses:Treatments		4	0.000895	0.01398	0.2553	0.994	
Residual		54	0.047345	0.73927			
Total		71	0.064042	1.00000			
Signif. codes: 0 ‘****’ 0.001 ‘***’ 0.01 ‘**’ 0.05 ‘.’ 0.1 ‘ ’ 1							
> pairwise.adonis(M_SOM10[ 1:10],M_SOM10\$Sampling_date)							
		pairs	Df	SumsOfSqs	F.Model	R2	p.value p.adjusted sig
1	April_2019 vs August_2018	1	0.005601312	6.709197	0.08746274	0.003	0.003 *
> pairwise.adonis(M_SOM10[ 1:10],M_SOM10\$Land_uses)							
		pairs	Df	SumsOfSqs	F.Model	R2	p.value p.adjusted sig
1	Maize vs Wheat	1	0.002518527	2.5528469	0.052578728	0.069	0.207
2	Maize vs Diculture	1	0.000269181	0.3275522	0.007070355	0.833	1.000
3	Wheat vs Diculture	1	0.002959891	3.6564568	0.073635073	0.010	0.030 .
> pairwise.adonis(M_SOM10[ 1:10],M_SOM10\$Treatments)							
		pairs	Df	SumsOfSqs	F.Model	R2	p.value p.adjusted sig
1	Control vs NPK+manure	1	0.0004712246	0.6320246	0.01355345	0.652	1.000
2	Control vs NPK	1	0.0017553809	1.8112245	0.03788283	0.152	0.456

3	NPK+manure vs NPK	1	0.0020822325	2.2038759	0.04571989	0.110	0.330
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**Table S7:** The interaction effect results of sampling date within the slow pool in grassland (n=8). It showed the output of Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise comparison results. Significance codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1.

Permutation test for adonis under reduced model					
Terms added sequentially (first to last)					
Permutation: free					
Number of permutations: 999					
adonis2(formula = M_SOM10[, 1:10] ~ Sampling_date, data = M_SOM10, permutations = 999, method = "bray")					
	Df	SumOfSqs	R2	F	Pr(>F)
Sampling_date	1	0.0001819	0.0393	0.2454	0.942
Residual	6	0.0044466	0.9607		
Total	7	0.0046285	1.0000		

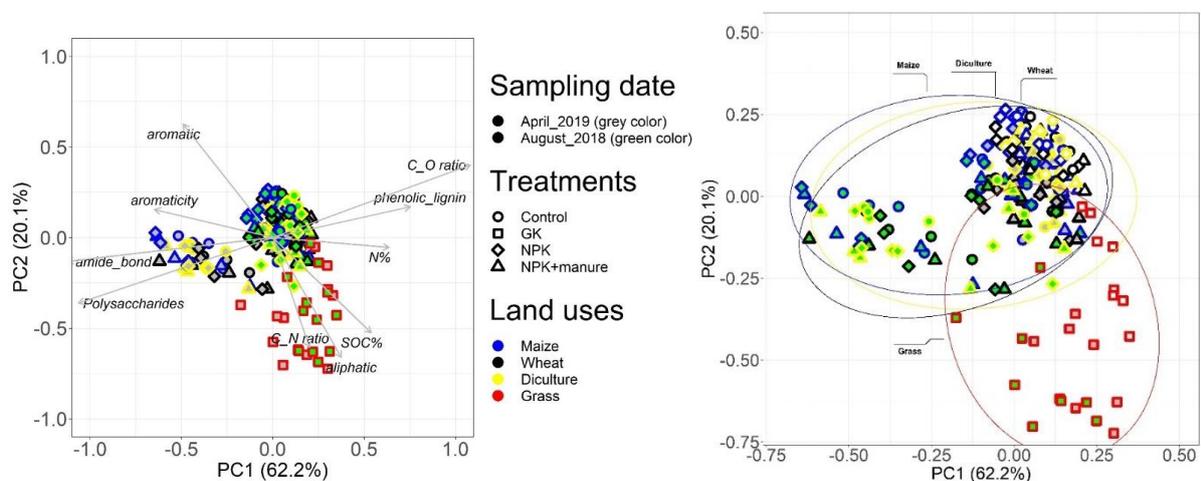
**Table S8:** The interaction effect results of sampling date on all measured SOM variables within the fast pool in grassland (n=8). It showed the output of Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise comparison results. Significance codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1.

Permutation test for adonis under reduced model					
Terms added sequentially (first to last)					
Permutation: free					
Number of permutations: 999					
adonis2(formula = M_SOM10[, 1:10] ~ Sampling_date, data = M_SOM10, permutations = 999, method = "bray")					
	Df	SumOfSqs	R2	F	Pr(>F)
Sampling_date	1	0.0077997	0.40548	4.0922	0.056 .
Residual	6	0.0114359	0.59452		
Total	7	0.0192356	1.00000		
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Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

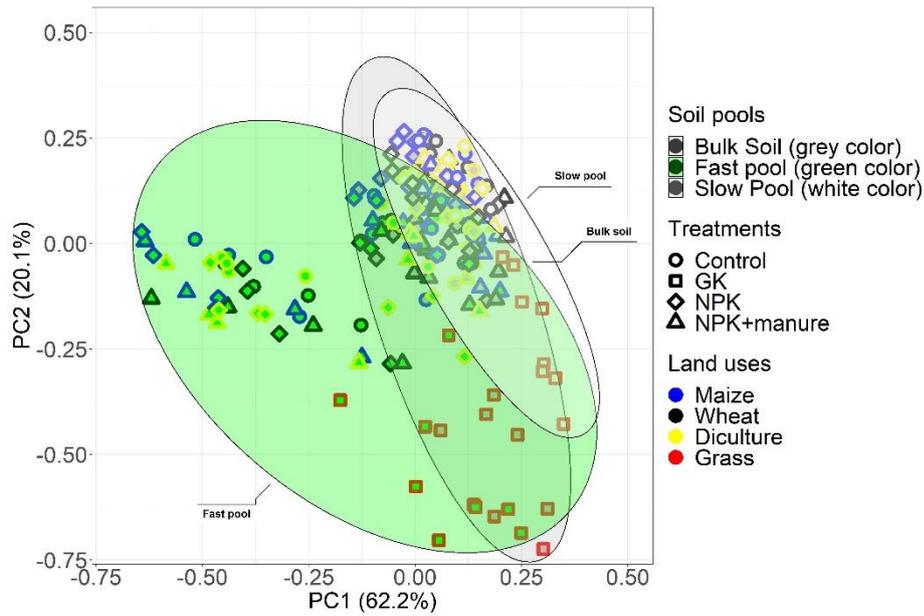
**Table S9:** The interaction effect results of sampling date on all measured SOM variables within the bulk soil in grassland (n=8). It showed the output of Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise comparison results. Significance codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1.

Permutation test for adonis under reduced model					
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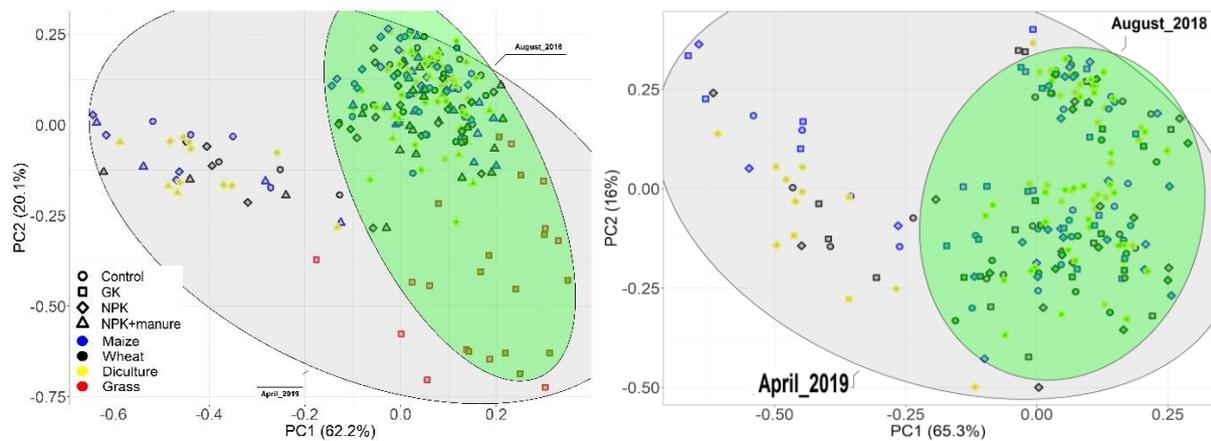
Terms added sequentially (first to last)				
Permutation: free				
Number of permutations: 999				
adonis2(formula = M_SOM10[, 1:10] ~ Sampling_date, data = M_SOM10, permutations = 999, method = "bray")				
	Df	SumOfSqs	R2	F Pr(>F)
Sampling_date	1	0.0072788	0.46811	5.2805 0.068 .
Residual	6	0.0082706	0.53189	
Total	7	0.0155494	1.00000	
---				
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1				



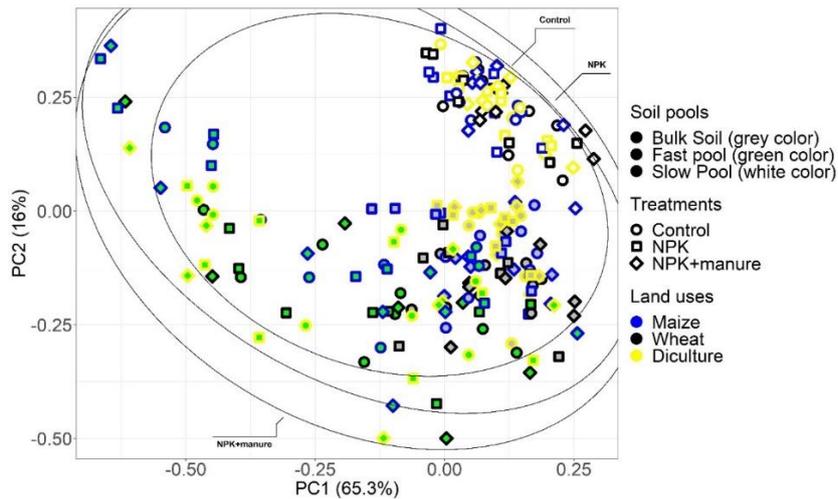
**Figure S25.** Biplot of the first two components showing the loading values in PC 1 and 2 (left side), and PCA results for the measured SOM variables (right side) for both sampling dates within both study sites. The measured SOM parameter variances were explained in brackets. NPK: nitrogen, phosphorus, and potassium. The following variables shortcut referred to: (aromatic\_aliphatic\_C (aromaticity index), C\_O (C/O ratio), amide\_bond (amide N), SOC (soil organic carbon), N (total N), C\_N (C/N ratio)).



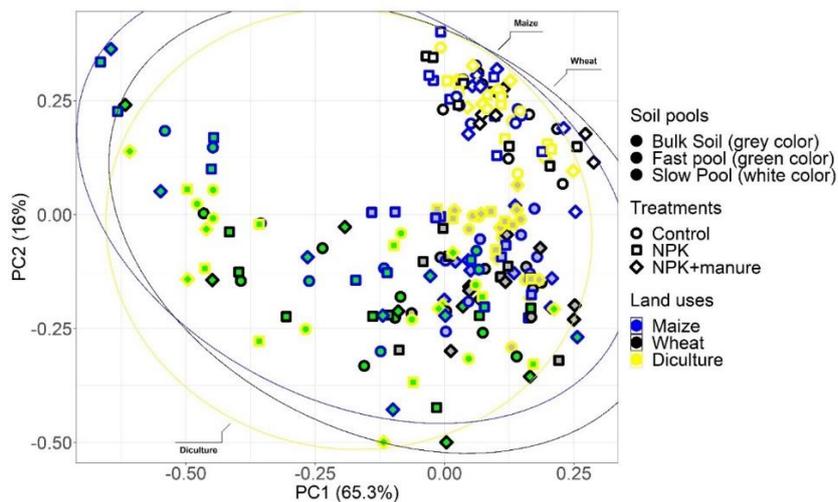
**Figure S26.** PCA results for the measured SOM variables, including grassland and cropland, for both sampling dates. It highlighted the soil pool's effects on SOM variables. The SOC variances were explained in brackets. The circled cloud indicated the soil pool's effects on the measured SOM parameters.



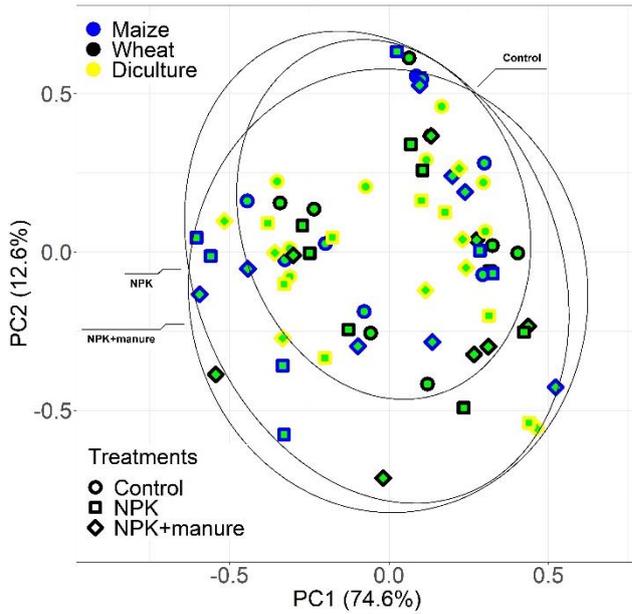
**Figure S27.** PCA results for the measured SOM variables for both sampling dates, including grassland and cropland (left side) and only cropland (right side). It highlighted sampling date effects on SOM variables. The SOC variances were explained in brackets. The circled cloud was indicated to highlight the sampling date's effects on the measured SOM parameters.



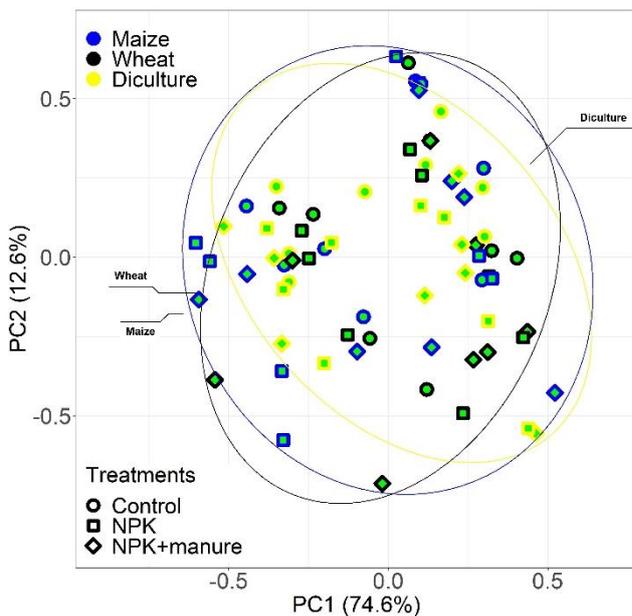
**Figure S28:** PCA results for the measured SOM variables for both sampling dates within cropland. It highlighted the treatment effects (fertilization) on SOM variables. The SOC variances were explained in brackets. The circled cloud was indicated to highlight the treatment effects (fertilization) on the measured SOM parameters.



**Figure S29:** PCA results for the measured SOM variables for both sampling dates within cropland. It highlighted the crop's effects (land uses) on SOM variables in cropland. The SOC variances were explained in brackets. The circled cloud was indicated to highlight the crop's effects (land uses) on the measured SOM parameters.



**Figure S30.** PCA results for the measured SOM variables for both sampling dates within cropland in the fast pool. It highlighted the treatment effects (fertilization) on SOM variables. The SOC variances were explained in brackets. The circled cloud was indicated to highlight the treatment effects (fertilization) on the measured SOM parameters.



**Figure S31.** PCA results for the measured SOM variables for both sampling dates within cropland in the fast pool. It highlighted the crop's effects (land uses) on SOM variables in cropland. The SOC variances were explained in brackets. The circled cloud was indicated to highlight the crop's effects (land uses) on the measured SOM parameters.