

**Soil Properties Controlling Status and
Stabilization of Soil Organic Carbon
in Neutral-to-alkaline Soils
of Indo-Gangetic Plain**

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CHAPTER 1 INTRODUCTION

1.1 General Background

Soil organic carbon (SOC) is a pivotal component of the global carbon cycle, playing a central role in terrestrial ecosystem functioning and agricultural productivity. As the largest terrestrial carbon pool, SOC exceeds the carbon stored in the atmosphere and vegetation, revealing its importance in mitigating climate change (Jobbagy and Jackson, 2000; Schlesinger, 1995). Because the dynamics of SOC are influenced by a complex interplay of climatic conditions, parent materials, soil properties, vegetation, and anthropogenic activities, studies for understanding their relationships are crucial for mitigating global environmental changes and food crises.

In the context of climate change, SOC dynamics are particularly significant due to their influence on atmospheric CO₂ concentrations. Rising temperatures have been shown to accelerate SOC decomposition, enhancing the release of CO₂, a major greenhouse gas, and thus contributing to a positive feedback loop in global warming (Davidson and Janssens, 2006). Land-use changes further accelerate this process, especially the transition from native ecosystems to agriculture, which can significantly reduce SOC stocks, particularly in tropical soils (Lal, 2010). These changes not only impact the global carbon cycle but also affect soil health and fertility, with repercussions for ecosystem services, including water quality and agricultural productivity (Lal, 2018).

The interaction between climatic variables and SOC is complex and multifaceted. For instance, in mountainous regions, SOC concentrations are typically higher in cooler, high-elevation sites, suggesting a negative correlation between SOC stability and mean annual temperature (MAT) (Garten Jr, 2011; Garten Jr and Hanson, 2006). However, the relationship

between SOC components, stability, and temperature is not straightforward, as other factors like net primary production (NPP) and soil geochemistry play critical roles (Djukic et al., 2010) (Tian et al., 2016).

NPP, influenced by climatic factors, directly regulates carbon inputs into the soil, impacting SOC content over shorter timescales. In contrast, soil geochemical properties may exert a more dominant influence on SOC storage over longer timescales (Doetterl et al., 2015). This highlights the need for a nuanced understanding of the different factors influencing SOC storage and distribution, including the interplay of climatic, geochemical, and anthropogenic factors.

Soil pH is a key factor influencing SOC dynamics, affecting microbial community size, composition, and activity, and thereby influencing organic matter mineralization (Cao et al., 2016) (Zhou et al., 2019). Soil pH also relates to the contents of exchangeable cations like Ca^{2+} and Mg^{2+} , which are known to stabilize SOC by bridging negatively charged mineral surfaces with organic matter (Neina, 2019). This bridging action provides a mechanism for the physical and chemical protection of SOC.

Further complicating the SOC dynamics are the roles of fine mineral fractions, such as clay, and active aluminum and iron (Al/Fe). Clay particles preserve organic matter through direct organo-mineral complexation and indirect mechanisms, such as occlusion within soil aggregates (Rodrigues et al., 2022b). Active Al/Fe, namely the acid-oxalate extractable fraction consisting of poorly crystalline minerals and organo-Al/Fe complexes, is proposed as a critical component for SOC preservation, not only in volcanic soils but also across various soil types (Ashida et al., 2021; Rasmussen et al., 2018). Their roles in SOC stabilization, especially in non-volcanic and neutral-

to-alkaline soils, are increasingly recognized, though not fully understood.

The molecular composition of soil organic matter (SOM) is another critical aspect of SOC dynamics. Different organic molecules within SOM exhibit varying decomposition rates and associations with soil properties, which are essential for understanding carbon stabilization and mineralization in soil ecosystems (Angst et al., 2021; Lehmann and Kleber, 2015). For instance, saccharides, considered fragile to degradation, suggest the presence of effective mineral stabilization when abundant in soils (Buurman and Roscoe, 2011; Keiluweit et al., 2015). Moreover, the origins of SOM, whether plant or microbial, significantly influence their stability and stabilization mechanisms (Schmidt et al., 2011).

Despite its significance, many studies have historically focused more on the quantitative aspects of SOC, resulting in notable gaps in current SOC models, often riddled with uncertainties due to a limited understanding of SOM molecular composition, origins, and sequestration mechanisms (Davidson and Janssens, 2006). Specific molecules, like aromatic monomers, are often seen as indicators of independently heightened SOM stability (Dungait et al., 2012). Furthermore, the interactions between organic compounds and soil minerals significantly influence SOC stabilization (Kleber et al., 2015). Therefore, a detailed examination of the molecular-level factors governing SOM stability is imperative, especially in neutral-to-alkaline soils with low SOC.

Analytical pyrolysis emerges as a promising method to assess the overall SOM molecular composition with high resolution, providing insights into the diverse organic constituents of SOC and their interactions with various soil properties. This approach is critical for unraveling the complex dynamics of SOM, particularly in understanding how different molecular forms of

organic matter contribute to the overall stability and turnover of SOC in various soil environments.

Understanding SOC dynamics is particularly essential in the Indo-Gangetic Plain (IGP), a region with diverse climates ranging from hot, humid monsoons to semi-arid conditions. The IGP is characterized by varied soil textures influenced by riverine sediment deposits, creating a gradient of soil properties from coarser textures upstream to finer textures downstream (Pal et al., 2009). This gradient, coupled with the region's climatic diversity, makes the IGP an ideal setting to study the factors controlling SOC stabilization. Meanwhile, the typically low SOC content in these soils (Lal, 2004a) presents challenges for agricultural production, highlighting the need for agricultural management practices that enhance SOC sequestration. Clarifying SOC stabilization mechanisms in the IGP could provide valuable insights for practical agricultural strategies and contribute to a broader understanding of SOC dynamics in similar soil environments.

1.2 Study Objectives

This study aims to elucidate the mechanisms underlying low SOC content in the neutral-to-alkaline topsoil and subsoil of the IGP, from both 1) evaluating the status of SOC and 2) tracing the decomposition and stabilization process of organic matter in soils. Studies that focused on both topsoil and subsoil from upper- to lower-stream in both agriculture and forest zones of IGP were conducted to achieve these objectives. I explore the roles of soil properties and land-use changes from forest to agriculture in shaping the quantity and stability of SOC and molecular composition of SOM, considering the influence of climate and landscape. By conducting SOM fractionation and long-term soil incubation experiments, I partitioned SOC into pools with distinct turnovers. I

also investigate the extent to which agricultural activity influences SOM composition and SOC quantity. Furthermore, by monitoring the decomposition and stabilization of SOC by incubating different soils with ^{13}C labeled plant addition, I explored the possible soil properties regulating SOC stabilization in neutral-to-alkaline soils. Analytical pyrolysis is used to assess the molecular composition of native SOM and trace the stabilization status of added plant materials.

The thesis comprises the following chapters: In Chapter 2, the details of the study sites were described. In Chapter 3, SOC status is evaluated by the sizes and mean residence times of SOC pools. SOM fractions and compositions are also explored, and then the role of active Al/Fe on SOM stability and composition in neutral-to-alkaline soils is discussed. In Chapter 4, the stabilization of SOM is clarified by the isotope tracing method with long-term incubation, and soil factors influencing organic matter decomposition and stabilization are discussed. Chapter 5 provides the general discussion and conclusion, summarizing previous chapters.

CHAPTER 2 GENERAL DESCRIPTION OF THE STUDY SITES

2.1 General of Indo-Gangetic Plain

The Indo-Gangetic Plain (IGP), the world's most extensive fluvial plains, is situated in the northern part of India. It spans a vast area of approximately 43.7 million hectares, stretching from the Himalayan foothills to the Arabian Sea and the Bay of Bengal. The IGP is divided into four subregions, moving from west to east: the Trans-Indo-Gangetic Plains (TGP: Punjab and Haryana) and the Gangetic Plains of Uttar Pradesh, Bihar, and West Bengal. The Upper IGP and a portion of the Middle IGP fall within Uttar Pradesh, while Bihar and West Bengal encompass most of the Middle and the Lower IGP, respectively (Singh et al., 2007). This plain is crucial for livelihoods downstream, heavily relying on the summer monsoon and the seasonal snow melt from glaciers and snowpacks, considered one of the largest plains formed by the huge river systems (Tejwani, 1990). The IGP, shaped by massive river systems like the Ganga and Indus, is known for transporting and depositing large quantities of clay and silt. Historically, the IGP has been a cradle for major South Asian empires and civilizations, such as the Indus Valley, Maurya, Gupta, and Mughal empires, endowing the region with profound cultural, historical, and religious importance (Khan, 2017). Presently, it is one of the most densely populated regions globally, where high population density and intensive agriculture have led to various socio-economic and environmental challenges (Bharati et al., 2011). Economically, the IGP is significant, contributing substantially to the GDPs of the encompassing countries. It serves as a central hub for diverse industries and services essential to the economic dynamics of South Asia. The region's diverse ecosystems range from eastern wetlands to western arid landscapes, though biodiversity faces threats from habitat

loss, pollution, and climate change (Rawat et al., 2015). Environmental issues like air and water pollution, groundwater depletion, and soil degradation are prominent, with unsustainable agricultural practices and resource overexploitation threatening the region's sustainability (Rodell et al., 2009).

The IGP's varied climatic zones and soil properties make it an ideal region for studying factors that influence Soil Organic Carbon (SOC) stabilization. The SOC content in these soils is notably low ($4.2 \pm 0.9 \text{ g kg}^{-1}$) (Lal, 2004b), which may limit agricultural productivity; however, there is potential for agricultural management practices to enhance SOC sequestration. Understanding SOC stabilization is vital for both practical agricultural activities and increasing our mechanistic knowledge of SOC dynamics.

2.2 Climate, Parent Materials and Vegetation

The climate of the IGP exhibits significant variation from west to east. In the western region, specifically Punjab (Pakistan), the climate is predominantly semi-arid, while eastern areas like Bangladesh experience a humid subtropical climate. The Southwest Monsoon is pivotal in shaping the agricultural calendar and water availability in the IGP, bringing substantial rainfall during the summer, which replenishes rivers and supports agriculture (Singh et al., 2016). The IGP experiences a continental climate marked by harsh summers and cold winters. Winter temperatures can drop to freezing due to the Himalayas, which act as a barrier to the cold katabatic winds from Central Asia (Attri et al., 2010).

Geologically, the IGP is an expansive floodplain created by sediment deposition from the

Indus, Ganga, Brahmaputra, and their tributaries. This region extends over parts of Pakistan, northern and eastern India, and Bangladesh, covering about 3,200 km from the mouth of the Indus to the Ganges delta in the Bay of Bengal (Gupta, 2005). Formed approximately 50 million years ago due to epiorogenic movements of the Himalayas, the basin was filled with sediments from northerly and southerly drainage, influenced by climatic changes since the Middle Miocene (Rowley, 1996). The IGP is divided into three major geomorphic units: the piedmont, central alluvial plain, and marginal alluvial plain (Saini, 2008). The region's soils are primarily alluvial, derived from the Indus and Ganges river systems, with textures varying from loamy sand to silty clay loam. While many soils in the IGP are alkaline, acidic soils are also found in mountainous regions and some floodplains (Singh et al., 2002).

The Indo-Gangetic Plains (IGP) are distinguished by tropical deciduous forests, where trees such as Sal (*Shorea robusta*), Teak (*Tectona grandis*), and various species of Bamboo predominate, shedding their leaves in the dry season. These forests, thriving in the monsoonal climate, are home to a myriad of grasses, shrubs, and smaller plants (Hooker, 1885). In the agricultural expanses of the IGP, crops like rice, wheat, and sugarcane are extensively cultivated, leveraging the nutrient-rich soils that are annually replenished by the flooding of the Ganges and its tributaries. This rich vegetation diversity is not only vital for supporting an array of wildlife but also plays an integral role in the local economy and culture. The vegetation of the IGP is essential in maintaining ecological balance, providing habitats for numerous species, and underpinning agricultural activities crucial for the sustenance of millions.

In the Upper IGP, the vegetation is predominantly characterized by xerophytic species and

small deciduous trees, an adaptation to the semi-arid climate of the region. Species such as *Acacia* and *Prosopis* are particularly noteworthy in this context. The region's grasslands are dominated by varieties of grass that are resilient to arid conditions. The original natural vegetation has been significantly altered by extensive agricultural activities. Crops like wheat, maize, and millet are primarily cultivated in drier areas, while rice and sugarcane predominate in regions benefiting from irrigation (Hajra et al., 1993).

Middle IGPs are characterized by a humid subtropical climate that fosters dense forests rich in biodiversity. Dominant tree species in this region include Sal, Sheesham (*Dalbergia sissoo*), and Teak. This lush vegetation is supported by the fertile alluvial soils prevalent in the area, which also contribute to a robust agricultural landscape. Crops such as rice, wheat, and sugarcane are extensively cultivated in these fertile terrains (Singh et al., 1992). In contrast, the Lower IGP is distinguished by a tropical monsoon climate, which supports a wide range of diverse vegetation. This area is particularly renowned for its mangrove forests in the Sundarbans, with key species like the Sundari tree (*Heritiera fomes*) and Golpata palm (*Nypa fruticans*) being notable examples. The agricultural practices in this region are predominantly centered around rice cultivation, taking advantage of the deltaic floodplains conducive to this type of farming (Gopal and Chauhan, 2006).

2.3 Land Management and Agriculture Activities

The IGP ranks among the world's most fertile regions, largely due to the nutrient-rich alluvial soils deposited by its rivers. This region experienced a significant transformation with the advent of the Green Revolution over fifty years ago, leading to a remarkable increase in crop productivity

and a substantial expansion in the area under the rice-wheat system (Pal et al., 2022). As a critical agricultural zone, the IGP plays a vital role in producing a significant portion of the subcontinent's food supply. Rice and wheat, the staple food crops in this region, account for more than 80% of total cereal production. Approximately 13.5 million hectares of the IGP are dedicated to these two crops, often grown in rotation. Other crops, including maize, pigeon pea, sugarcane, and lentil, are also cultivated, sometimes substituting for rice or wheat in certain years (Gupta and Seth, 2007).

Effective land management in the IGP is essential to maintain its agricultural productivity, preserve environmental health, and support the livelihoods of millions. The region is a hub for intensive agriculture, heavily reliant on practices like irrigation, fertilization, and crop rotation. However, it faces challenges such as soil degradation, waterlogging, and declining groundwater levels. The adoption of sustainable practices, including integrated nutrient management and conservation agriculture, is becoming increasingly vital. The principles of conservation agriculture—minimal soil disturbance, retention of crop residue mulch, and rational crop rotations, combined with ensuring farm-level profitability—are now recognized as fundamental for sustainable agriculture in the IGP (Erenstein, 2011).

The Ganges Basin faces a formidable challenge in water resources management due to the marked disparity between seasonal water demand and availability. Over 80% of the annual flow in the Ganges River occurs during the four-month monsoon season, leading to widespread flooding. Conversely, the remaining months of the year are marked by water scarcity, adversely affecting irrigation, navigation, and ecosystems (Khan et al., 2014). To support high cropping intensity, the region employs a variety of irrigation methods, such as canal, tube-well, and tank irrigation.

Despite these efforts, issues like groundwater depletion and inefficient water use pose significant challenges (Dhawan, 1995). In the Indo-Gangetic Plain (IGP), sustainable agriculture is being increasingly emphasized to address concerns such as soil health deterioration, the overuse of chemical fertilizers, and ongoing water scarcity. Practices like conservation agriculture, integrated pest management, and organic farming are becoming more prevalent (Hobbs and Gupta, 2003). Furthermore, the IGP is confronting substantial challenges posed by climate change, including changes in precipitation patterns, rising temperatures, and the occurrence of extreme weather events. These changes have a profound impact on crop productivity and farming practices. As a result, the development and implementation of adaptive strategies and resilience building are crucial for sustaining agriculture in the region (Aggarwal, 2008).

2.4 Sampling Sites and Soil Samples

In this study, sampling sites (Fig. C2-1) were strategically selected across three distinct agro-climatic zones of the Indo-Gangetic Plain (IGP) in India, spanning from west to east (Upper, Middle, and Lower IGP). Within each zone, two locations were chosen: Meerut (I1) and Kanpur (I2) in the Upper IGP; Patna (I3) and Samastipur (I4) in the Middle IGP; and Cooch Behar (I5) and Canning Town (I6) in the Lower IGP. At each site, soil samples were collected from both an agricultural field, reflecting the dominant cropping pattern of the area, and an adjacent non-managed secondary forest (Table C2-1). The forest sites had at least 20 years of continuous tree cover and without cultivation activities, but there might be human influence (considered as best sites in each region as forest sites). Geographically, these sites exhibit a gradient in elevation

(decreasing from 224 to 5 meters) and a corresponding increase in mean annual precipitation (from 840 to 3290 mm) as one moves from west to east (worldclim.org; (Fick and Hijmans, 2017)). Across all sites, the total precipitation in the rainy season from July to September is higher than 640 mm, with the lowest monthly precipitation in Kanpur (I2) in September (140 mm). The soil temperature regime across these locations is hyperthermic, and the soil moisture regime is ustic. Based on the World Reference Base (WRB) classification, the soils are identified as Luvisols, Cambisols, and Gleysols, formed on mixed alluvium (WRB, 2022) (Table C2-1). Clay mineralogy across all samples revealed a mix of mica, vermiculite (with minor hydroxy-Al interlayers), kaolinite, and smectite. Notably, soil I3 showed a higher proportion of smectite, whereas soil I5 was rich in chlorite and kaolinite.

Soil sampling involved collecting well-drained soil profiles up to a depth of approximately 1 meter. While samples were taken from each horizon, the study primarily focused on the topsoil (0–15 cm) and subsoil (40–60 cm) horizons to analyze a gradient in SOC concentrations. The 24 collected samples were categorized into four groups for analysis: forest topsoil (FT), forest subsoils (FS), agricultural topsoil (AT), and agricultural subsoils (AS). Preparation of these samples involved air-drying, gentle grinding, sieving through a 2-mm mesh, and subsequent mixing before analyses.

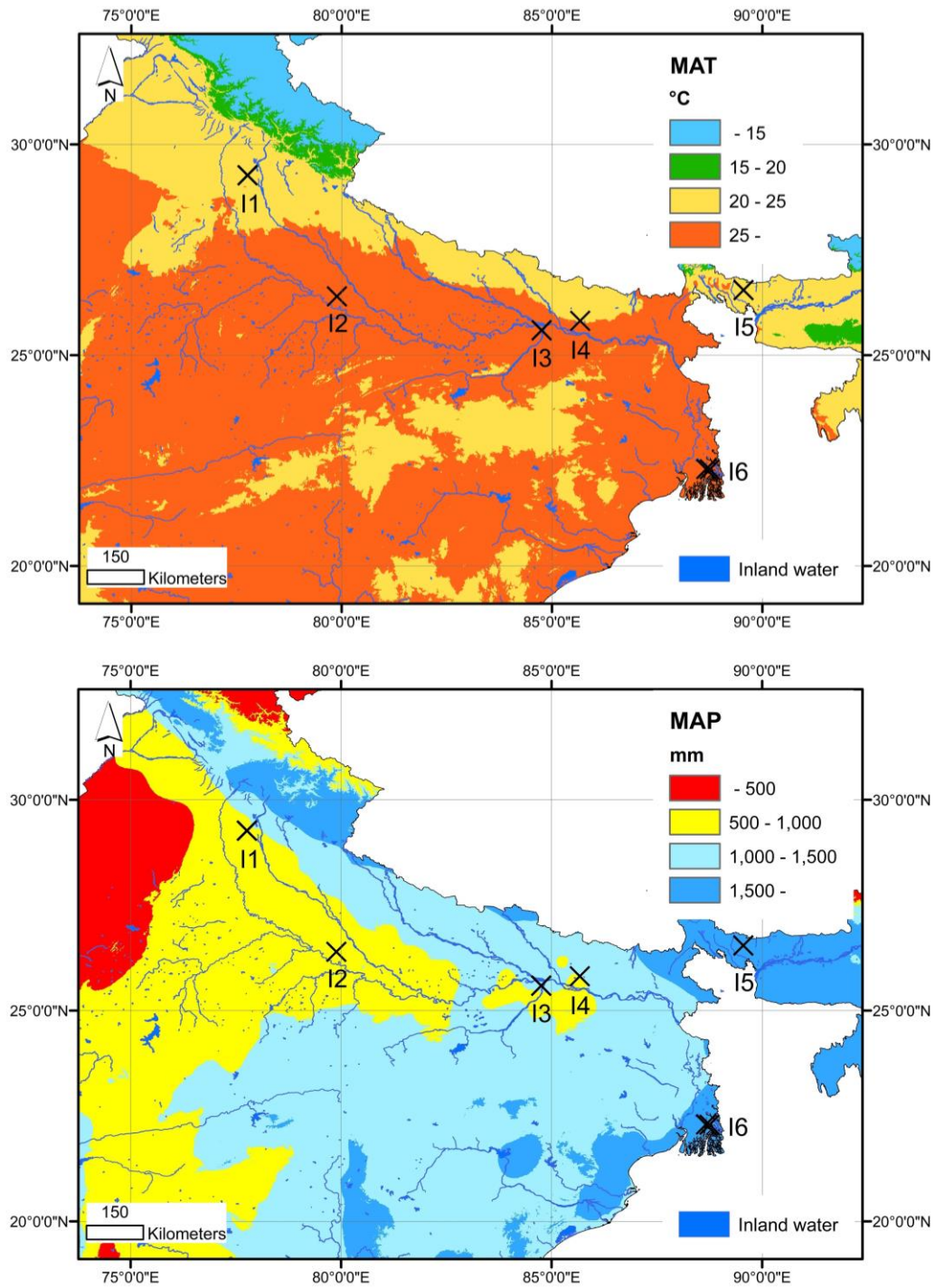


Fig. C2-1 Sampling site locations (black crosses) on the maps of mean annual temperature and precipitation (MAT and MAP). Maps were generated using ArcMap (ESRI).

Table C2-1 Information for soil sampling locations in Indo-Gangetic Plain (IGP)

Region of IGP	Location	Elevation m	Mean annual precipitation mm	Mean annual temperature °C	Soil classification ^a	Cropping pattern in cropland	Forest type
Upper	Meerut (I1)	224	970	24.1	Luvisols	Wheat, sugarcane, vegetables	Secondary forest to bush
	Kanpur (I2)	126	840	25.7	Luvisols	Wheat, sugarcane, vegetables	Secondary forest to bush
	Patna (I3)	55	1050	25.7	Cambisols	Rice, wheat, maize, barley, millet	Secondary forest to bush
Middle	Samastipur (I4)	53	1000	24.9	Cambisols	Rice, wheat, maize, barley, millet	Secondary forest to bush
Lower	Coochbehar (I5)	42	3290	24.9	Cambisols	Rice, vegetables	Secondary forest
	Canning town (I6)	5	1700	26.5	Gleysols	Rice, vegetables	Secondary forest

^a Soil type is classified based on the World Reference Base for Soil Resources (IUSS Working Group WRB, 2022).

CHAPTER 3 ROLES OF ACTIVE Al/Fe AND pH ON SOIL ORGANIC MATTER STABILITY AND COMPOSITION

3.1 General

Reducing soil organic carbon (SOC) diminishes the capability to buffer climate change and vegetative shifts of soils. Notably, a significant portion of neutral-to-alkaline soils exhibits SOC deficiency (Lal, 2004b; Xu et al., 2021), restricting their ability to support natural vegetation and agricultural production (Tiessen et al., 1994). Land-use transitions particularly shift from native to agricultural systems, can lead to substantial reductions in the SOC pool, witnessing decreases of 60% or more (Lal, 2010). Consequently, understanding mechanisms for C stabilization in neutral-to-alkaline soils is crucial to making strategies to respond to climate change and maintain sustainable land use, considering climate, geochemical properties, and land-use patterns.

Climatic and geochemical factors have a synergistic influence on the mineralization and stabilization of SOM. Climatic factors, such as moisture and temperature, regulate microbial decomposition rates (Davidson et al., 2006; Zhang et al., 2020). High excess precipitation can lead to increased leaching, lowering soil pH and retarding the crystallization of active Al and Fe (Lyu et al., 2018; Lyu et al., 2022). This reduced soil pH can alter bacterial and fungal community structures, thereby affecting their activity and reducing organic matter mineralization (Ramirez et al., 2018; Tedersoo et al., 2014). Furthermore, pH changes concurrently with the exchangeable Ca^{2+} and Mg^{2+} levels, which have been recognized to stabilize SOC by bridging negatively charged clay surfaces with organic matter in alkaline soils (Neina, 2019; Rowley et al., 2018). Fine mineral fractions like clay, which can deposit more in rivers downstream, provide additional stability to

organic matter through organo-mineral interactions and aggregation (Rodrigues et al., 2022b). Notably, active Al/Fe, which includes poorly crystalline Al/Fe minerals and organo-Al/Fe complexes, is considered a vital control for SOC stabilization in volcanic or acidic terrains (Rasmussen et al., 2018; Wagai et al., 2015). At the same time, there are suggestions regarding the positive role of active Al/Fe in stabilizing carbon even within non-volcanic and neutral-to-alkaline soils (Ashida et al., 2021; Rasmussen et al., 2018), the precise mechanisms and their confirmation need further exploration. Land use changes from forest to agricultural land may significantly impact SOC dynamics, including priming effects, stock, and stability (John et al., 2005; Lozano-García et al., 2017; Mganga and Kuzyakov, 2018). The intersection of climatic and soil factors with human-induced changes, such as land-use transitions, poses pertinent questions about ecosystem sustainability.

SOC is not a uniform entity but a complex mixture of different components that vary in turnover rates and chemical properties (Trumbore, 2000). Partitioning SOC into different pools based on their stability depending on the density and particle size provides a detailed understanding of specific carbon stabilization mechanisms and the potential carbon release under various disturbances (Paul et al., 2001; von Lützow et al., 2007). By delineating SOC into distinct pools, researchers can identify the more labile fractions susceptible to rapid microbial decomposition and the more stable fractions protected from decomposition due to various physical, chemical, and biological mechanisms (Six and Jastrow, 2002). This partitioning is vital for understanding the factors controlling SOC stocks and stabilities, as well as the resilience of soils to external disturbances, such as land-use change or climatic variations (Doetterl et al., 2015).

Distinct differences exist between topsoil and subsoil horizons regarding their SOC pools and saturation status (Lyu et al., 2021b; Possinger et al., 2021). The saturation degree of SOC, influenced by the balance between soil minerals and SOC, is pivotal in guiding SOC stabilization mechanisms and determining potential SOC sequestration capacity (Rodrigues et al., 2022b). In the subsoil, where saturation status is typically lower, SOC stabilization depends more on soil minerals (Yu et al., 2017). However, studies exploring the differential impacts of SOC conditions in topsoil versus subsoil on SOC dynamics in neutral-to-alkaline soils remain scarce.

Understanding the molecular composition of SOM is crucial for comprehending SOC dynamics. Individual organic molecules in SOM exhibit varied decomposition rates and are associated with climatic and geochemical properties, highlighting the complex mechanisms of SOC stabilization and mineralization (Angst et al., 2021; Lehmann and Kleber, 2015). Specific molecules, like aromatic monomers, are often seen as indicators of independently heightened SOM stability (Dungait et al., 2012). In contrast, though fragile to degradation, saccharides prevail in soils mainly due to stabilization by minerals (Buurman and Roscoe, 2011; Keiluweit et al., 2015). Moreover, whether these SOM originate from plants or microbes reflects their original stability and stabilization mechanisms (Schmidt et al., 2011). While past research often emphasized quantity over quality in SOM studies, modern SOC models grapple with uncertainties due to an incomplete grasp of SOM molecular composition, origins, and sequestration mechanisms (Davidson and Janssens, 2006; Kleber et al., 2021). It is, therefore, imperative to focus on the molecular-level factors that govern SOC stock and stability. In this circumstance, analytical pyrolysis emerges as a promising tool to provide high-resolution insights into the overarching

molecular composition of SOM (Derenne and Quénéa, 2015).

In this chapter, I elucidated the factors underlying the low SOC levels in the neutral-to-alkaline soils by testing topsoil and subsoil of IGP. Specifically, I aimed to determine the influence of climate, geochemistry, and land use shifts from forest to agriculture on the sizes and stabilities of SOC pools, and the molecular composition of SOM in these soils. To achieve this, we conducted SOM fractionation and long-term soil incubation with model fitting for pool partitioning. Also, analytical pyrolysis was conducted to assess SOM molecular composition for both topsoil and subsoil. I hypothesized that the combination of high pH and low active Al/Fe primarily contributes to the low SOC in IGP, by limiting the sizes and stabilities of intermediate and stable SOC pools, organo-mineral associations, and SOM degradation status. Also, I thought that SOM in subsoil is more degraded, and its stability is more dependent on soil minerals than in topsoil. Furthermore, I expected that agricultural activities may change SOM composition as well as its overall quantity and stability.

3.2 Materials and Methods

3.2.1 Climatic and Vegetational Properties

Temperature and precipitation for each site were derived from the WorldClim database (Fick and Hijmans, 2017). Potential evapotranspiration was calculated based on monthly air temperature and solar radiation (Harkin, 1960; Thornthwaite, 1948). I calculated annual excess precipitation (EP) by extracting potential evapotranspiration from precipitation for each month (Lyu et al., 2018).

3.2.2 Physicochemical Properties

Soil pH was measured using a benchtop pH meter (F-70 Series, Horiba, Japan) with a soil-to-deionized water ratio of 1:5. Exchangeable cations (Ca^{2+} , Mg^{2+} , K^{+} , and Na^{+}) were extracted using 1 M NH_4OAc (pH 7.0) and measured: Ca^{2+} and Mg^{2+} via atomic absorption spectroscopy and K^{+} and Na^{+} through flame emission spectroscopy (AA-660, Shimadzu, Japan). Cation exchange capacity (CEC) was assessed by saturating soil with NH_4^{+} , then displacing adsorbed NH_4^{+} with Na^{+} and quantifying NH_4^{+} using the Kjeldahl method (Soil Survey Laboratory Staff, 1996). Total carbon, total nitrogen (TN), and soil organic carbon (SOC) values were obtained using dry combustion on a CN analyzer (EA Isolink, Thermo Fisher Scientific, USA). Dissolved organic carbon (DOC) was extracted using 0.5 M K_2SO_4 at a 1:5 g mL^{-1} (soil to solution) ratio, then measured using the NPOC method (TOC-L, Shimadzu, Japan). Prior to SOC measurement, carbonates were excluded with 0.1 M HCl. Inorganic carbon (IC) was calculated as the difference between total carbon and SOC.

Soil particle size analysis commenced with organic matter removal via H_2O_2 , followed by pH adjustment to 9-10 and a 15-minute ultra-sonication for dispersion. Sand fractions were measured through wet sieving. Meanwhile, silt (2–20 μm) and clay (<2 μm) fractions were determined using the pipette method (Gee and Or, 2002). Active Al and Fe (Al_o and Fe_o) were extracted using ammonium oxalate solution (0.2 M, pH 3.0) for 4 hours in a dark condition (Blakemore, 1987). For free Fe (hydr)oxides (Fe_d) determination, soil was treated with a combined citrate (0.75 M) and sodium dithionite solution (Blakemore, 1987). The extracts were filtered using a 0.45- μm membrane and analyzed with ICP-AES (ICPE-9000, Shimadzu, Japan).

3.2.3 Soil Organic Matter Fractionation

Soil organic matter (SOM) fractionation of original soils using the following procedure (Lyu et al., 2021a): Using density separation (1.6 g cm^{-3}), SOM was divided into light (LF) and heavy (HF) fractions. After sodium hexametaphosphate dispersion and a 15-hour shake, the HF was further separated by particle size into occluded particulate organic matter (oPOM, $\geq 53 \text{ }\mu\text{m}$) and mineral-associated organic matter (MAOM, $< 53 \text{ }\mu\text{m}$). The MAOM underwent a 6 M HCl reflux at $115 \text{ }^{\circ}\text{C}$ for 16 h to identify the non-hydrolyzable SOM in the MAOM fraction. Weight and SOC content of each fraction were subsequently measured.

3.2.4 Long-term Soil Incubation and SOC Pool Partitioning by Model Fitting

To obtain a decomposition curve of SOC, I conducted incubation experiments on both topsoil and subsoil of all sites. Three replications of 20 g soil samples (dry weight based) were placed in sealed 225 mL glass jars and moistened to 55% of field capacity. The CO_2 collection commenced following a 7-day pre-incubation at $25 \text{ }^{\circ}\text{C}$ (Lyu et al., 2021a).

The CO_2 released from the soils was captured using a 10 mL 1 mol L^{-1} NaOH solution inside a plastic bottle within the incubation jar. Another bottle containing 0.01 M HCl maintained soil moisture during the incubation. Over the 196-day incubation period, CO_2 samples of each sample were collected, and CO_2 emissions were determined through titration. The CO_2 -trapping solution was sampled on days 7, 14, 28, 49, 70, 91, 112, 140, 168, and 196 for making cumulative CO_2 release curves.

By fitting cumulative CO₂ release curves to a first-order kinetic model, SOC was segmented into labile, intermediate, and stable SOC pools for each site, as described in the equation following (Paul et al., 2001; Tian et al., 2016):

$$C_r = 1 - C_L\% \times e^{-\left(\frac{1}{MRT_L}\right) \times t} - C_I\% \times e^{-\left(\frac{1}{MRT_I}\right) \times t} - C_S\% \times e^{-\left(\frac{1}{MRT_S}\right) \times t} \quad (1)$$

In this equation, C_r represents the ratio of cumulative CO₂ release to total SOC at day t of incubation. $C_L\%$, $C_I\%$, and $C_S\%$ denote the proportions of the labile, intermediate, and stable pools to total SOC, respectively. MRT_L , MRT_I , and MRT_S (days) are the mean residence time (MRT) for each pool. The value of $C_S\%$ was derived from the ratio of non-hydrolyzable SOM C to total SOC. Relying on prior research and enhancing fitting accuracy (Lyu et al., 2021a; Wattel-Koekkoek et al., 2003; Wiedemeier et al., 2012), I set MRT_S of 1000 years as a constant in Eq (1).

The Universal Global Optimization Algorithm (Auto2Fit 5.5, 7D-Soft High Technology, China) was employed for nonlinear regression of Eq. (1) with specific constraints: $MRT_L = [1, 100]$ and $MRT_I = [1000, 100000]$ (days). The sizes of labile, intermediate, and stable SOC pools (C_L , C_I , and C_S) were calculated using the proportions of each SOC pool relative to the total SOC for each site.

3.2.5 One-shot Py-GC/MS Analysis

The molecular composition of soil organic compounds was examined using pyrolysis–gas chromatography–mass spectrometry (Py-GC/MS). Soil samples were finely ground, homogenized by a ball mill (PM100, Retsch, Germany), and dried at 70 °C for 24 h. Pyrolysis was performed with a multi-functional pyrolyzer (EGA/PY3030D, Frontier Laboratories, Japan) linked to GC/MS

(GCMS-QP2020, Shimadzu, Japan), using samples containing about 100 $\mu\text{g C}$. The samples were pyrolyzed at 550 $^{\circ}\text{C}$ for 1 min. Cryo-trapping at -180°C , with subsequent rapid thermal desorption, was performed using a MicroJet Cryo-Trap (MJT-1035E, Frontier Laboratories, Japan). Pyrolysis products were processed using a stainless-steel capillary column (Ultra ALLOY+-5, Frontier Laboratories, Japan). The GC oven parameters were started at 50 $^{\circ}\text{C}$ for 1 min, raised to 300 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C min}^{-1}$, and then maintained at 300 $^{\circ}\text{C}$ for 5 min. Helium was the carrier gas at a column flow of 1 mL min^{-1} . Mass spectra were scanned across the 29–1000 m/z mass range with an electron ionization. The areas of the top 150 peaks were integrated, and peaks accounting for more than 80% of the total area were identified using the NIST 17 library and personal reference list (Table S1).

Identified compounds were divided into 16 groups (Table S1): undegraded polysaccharides (PS), degraded polysaccharides, long alkanes, short alkanes, n-alkenes, squalene, fatty acids and esters (FAnE), steroids, lignin, phenols, monocyclic aromatic hydrocarbons (MAH), polycyclic aromatic hydrocarbons (PAH), alkylamides, N-containing MAH (N-MAH), other N-containing compounds (N-containing), and other hydrocarbons. For instance, the degraded polysaccharides group includes compounds like furans, while the lignin group comprises derivatives like methoxy phenol. Relative abundance of each group was determined using its chromatogram peak area within the m/z range of 33–500 relative to the total area of identified compounds.

3.2.6 Statistical Analyses

Given non-normal distributions of many parameters even after log transformation, Spearman rank correlations were applied. Differences in overall soil properties and SOM compositions

between forest and agricultural sites or between topsoil and subsoil were assessed using Bray-Curtis distance-based PERMANOVA with Nonmetric multidimensional scaling (NMDS). A pairwise t-test was applied to find the differences in certain soil property and SOM compound group by land use and soil horizon. To assess the influence (both direction and magnitude) of climatic and geochemical properties on SOC pools, I employed partial least squares path modeling (PLS-PM) using the “SEMinR” package (Hair et al., 2021). The significance of path coefficients in the PLS-PM was ascertained through the Bootstrapping method. Redundancy analysis (tb-RDA) was used to clarify relationships between SOM compound groups and climatic and geochemical properties with 999 permutations. For both PLS-PM and tb-RDA, I employed variance inflation factor (VIF) tests and stepwise selection to determine the optimal influencing factors, considering single correlations. NMDS, PERMANOVA, and tb-RDA were carried out with “vegan” and “microeco” packages (Liu et al., 2021; Oksanen et al., 2020). Statistical analyses utilized R 4.3.1. Significance levels were denoted as *, **, and *** for P values < 0.05 , 0.01 , and 0.001 , respectively.

3.3 Results

3.3.1 Climatic and Physicochemical Properties

Table C3-1 summarizes the climatic and geochemical properties of each sampling location. As proximity to the Indian Ocean increases, with a decrease in elevation, MAP and EP also increases ($r_s = -0.83^{***}$ and -0.60^{**} , Fig. C3-1). Forest and agriculture site soils showed minimal physicochemical differences, as shown by PERMANOVA analysis (Fig. C3-2). pH of most soils

was near-neutral to alkaline. As precipitation increased from upper to lower IGP, pH values dropped (Fig. C3-1), with pH being significantly correlated to MAP and EP ($r_s = -0.68^{***}$ and -0.59^{**}). Topsoil and subsoil pH ranged from 6.2 to 9.9 and 5.7 to 9.2, respectively. Inorganic C contents were high for both topsoil and subsoil of I4 (Table C3-1), where apparent carbonate shells were found in the sand fraction. While exchangeable Na^+ ($0.29 \pm 0.25 \text{ cmol}_c \text{ kg}^{-1}$) and K^+ ($0.21 \pm 0.21 \text{ cmol}_c \text{ kg}^{-1}$) were low, exchangeable Ca^{2+} ($11.7 \pm 10.4 \text{ cmol}_c \text{ kg}^{-1}$) and Mg^{2+} ($3.3 \pm 3.0 \text{ cmol}_c \text{ kg}^{-1}$) were notably higher, indicating a low risk of adverse effects from high Na^+ and K^+ contents.

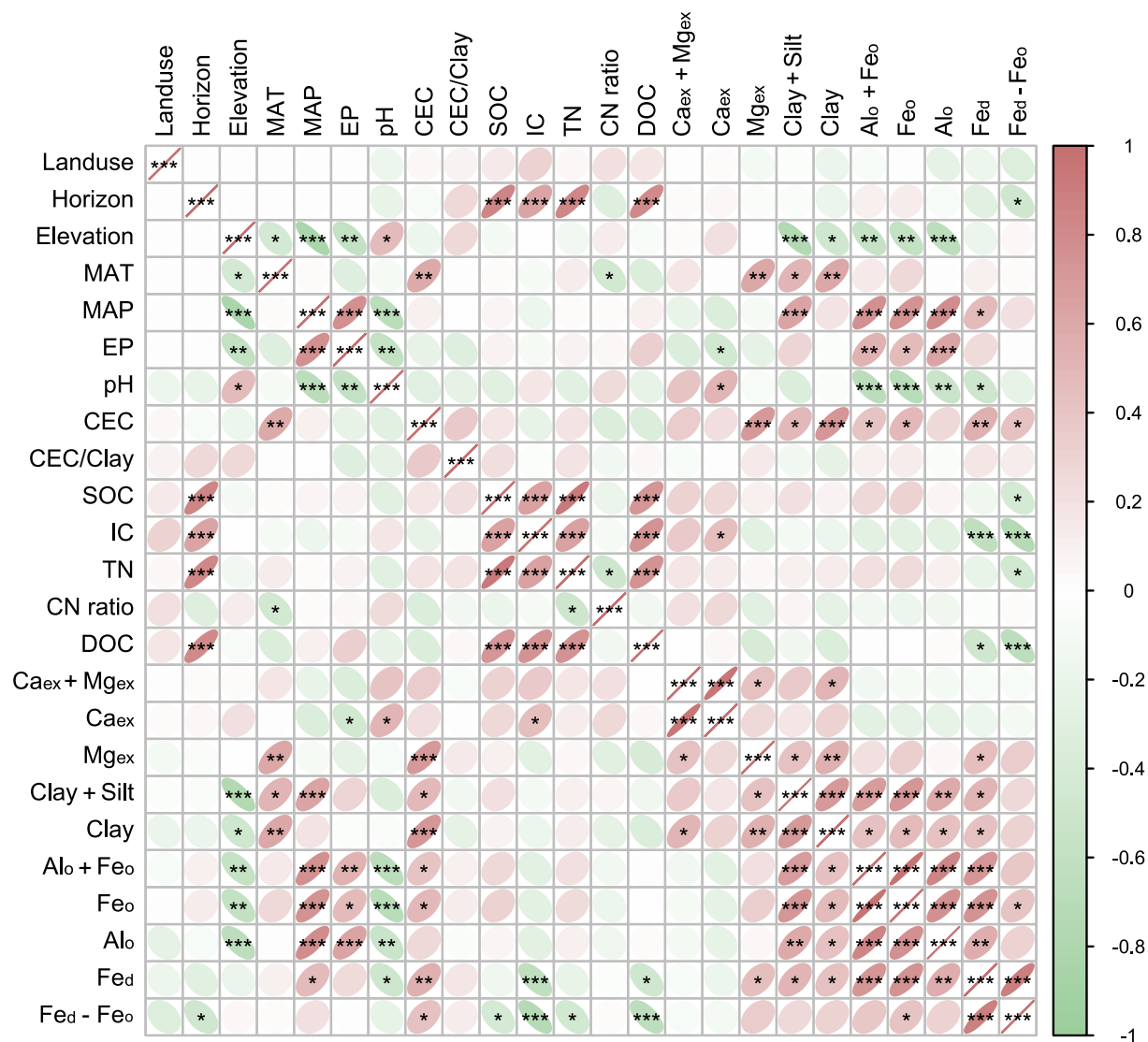


Fig. C3-1 Spearman correlation matrix (r_s) revealing the relationships among climatic and geochemical properties across all soils.

Landuse, forest (1) and agriculture (0); horizon, topsoil (1) and subsoil (0); MAT, mean annual temperature; MAP, mean annual precipitation; EP, excess precipitation; CEC, cation exchange capacity; SOC, soil organic carbon; IC, inorganic carbon; TN, total nitrogen; DOC, dissolved organic carbon; Ca_{ex} and Mg_{ex}, exchangeable Ca²⁺ and Mg²⁺; Fe_o and Al_o, acid oxalate extractable active Fe and Al; Fe_d, dithionite-citrate extractable iron; Fe_d - Fe_o, crystalline Fe (hydr)oxides.

Table C3-1 Climate and geochemical properties of the studied soils

ID	Elevation	MAT	MAP	EP	pH	CEC	SOC	TN	DOC	IC	Clay	Ca _{ex}	Mg _{ex}	Al _o	Fe _o	Fe _d
	m	°C	mm	mm		cmol _c kg ⁻¹			g kg ⁻¹		%	cmol _c kg ⁻¹			cmol kg ⁻¹	
I1FT	224	24.1	965	-671	7.5	9.4	16.4	1.1	0.32	0.4	10	9.8	2.4	1.2	1.3	11.5
I2FT	126	25.7	838	-1165	7.8	8.9	12.0	1.6	0.20	5.1	16	8.5	2.0	1.1	1.6	9.1
I3FT	55	25.7	1046	-975	7.8	10.1	6.7	0.5	0.11	1.9	17	8.3	3.1	1.4	2.9	17.1
I4FT	53	24.9	1003	-745	8.8	8.0	28.9	1.3	0.22	18.4	21	33.6	1.3	1.7	2.2	8.8
I5FT	42	24.9	3288	1860	6.4	9.2	15.9	1.3	0.35	0.3	20	1.5	0.2	5.1	7.0	13.8
I6FT	5	26.5	1704	-153	7.1	21.1	19.3	2.7	0.15	3.2	59	11.4	11.8	3.5	9.9	20.1
I1FS	224	24.1	965	-671	8.5	5.8	3.3	0.2	0.06	0.0	15	6.4	1.6	1.3	2.1	17.9
I2FS	126	25.7	838	-1165	8.3	11.0	3.6	0.3	0.05	0.0	22	7.3	2.2	1.5	1.4	12.1
I3FS	55	25.7	1046	-975	7.7	11.9	3.6	0.2	0.04	0.0	21	9.5	4.2	1.7	3.8	24.4
I4FS	53	24.9	1003	-745	9.2	7.3	3.4	0.2	0.10	3.8	21	37.2	2.3	1.5	0.4	5.7
I5FS	42	24.9	3288	1860	6.0	4.9	3.3	0.2	0.09	0.0	15	0.4	0.1	3.4	4.5	15.4
I6FS	5	26.5	1704	-153	8.0	12.3	4.4	0.3	0.06	0.0	35	8.5	10.3	1.9	4.9	15.1
I1AT	224	24.1	963	-671	8.2	6.6	12.6	0.9	0.22	0.2	15	12.4	3.4	1.7	1.4	11.2
I2AT	126	25.7	838	-1165	9.9	5.7	4.7	0.4	0.08	0.1	13	4.3	3.5	1.0	1.3	8.3
I3AT	55	25.7	1046	-989	7.9	17.1	7.8	0.6	0.08	0.0	30	17.6	6.2	3.0	7.7	28.6
I4AT	53	24.9	1003	-745	8.6	7.1	16.4	0.9	0.19	16.6	26	35.8	0.2	1.6	2	10.0
I5AT	42	24.9	3288	1860	6.4	8.2	11.1	1.0	0.21	0.1	19	1.7	0.1	3.5	4.5	14.8
I6AT	5	26.5	1754	-127	6.2	9.7	10.1	0.8	0.13	0.0	31	4.0	4.2	2.2	5.4	18.6
I1AS	224	24.14	963	-671	8.5	9.3	3.6	0.3	0.06	0.0	26	8.4	3.3	2.0	2.4	24.9
I2AS	126	25.7	838	-1165	8.7	10.0	2.7	0.2	0.05	0.0	22	7.3	3.1	1.6	1.1	11.5
I3AS	55	25.7	1046	-989	8.2	23.3	5.7	0.4	0.04	0.0	33	20.8	6.6	3.1	6.5	40.8
I4AS	53	24.9	1003	-745	9.0	9.2	3.1	0.3	0.08	0.2	25	14.5	0.8	1.8	0.5	11.3
I5AS	42	24.9	3288	1860	8	4.7	2.8	0.1	0.08	0.0	13	4.9	0.3	2.2	3.4	19.2
I6AS	5	26.5	1754	-127	5.7	12.6	5.2	0.8	0.06	0.0	33	6.1	5.4	2.7	5.2	23.5

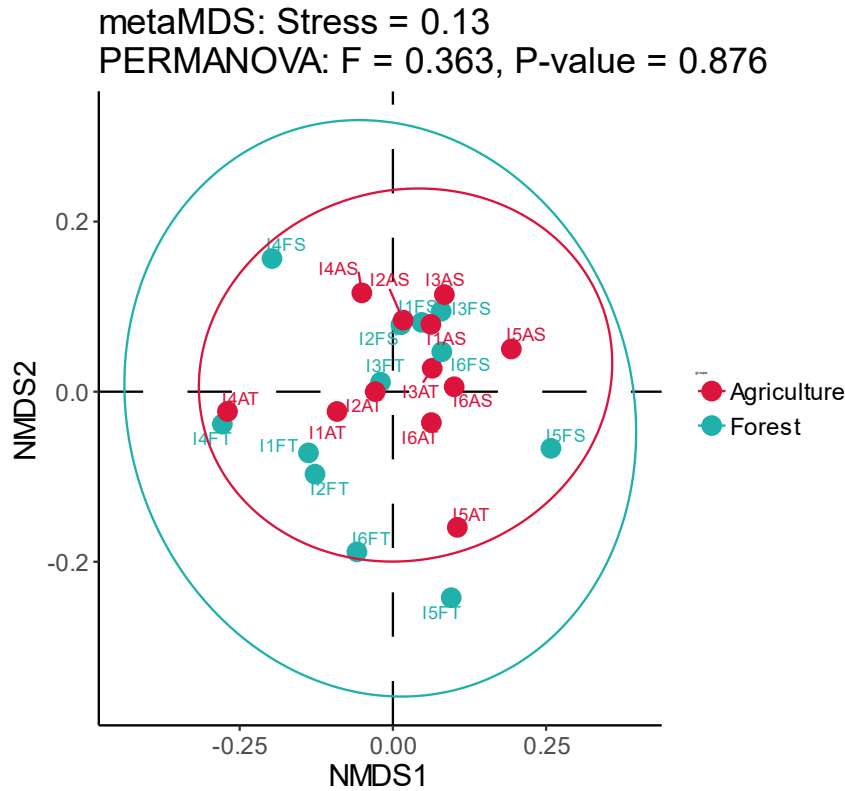


Fig. C3-2 Nonmetric multidimensional scaling (NMDS) diagram with PERMANOVA test revealing difference in soil properties between soils from agriculture and forest land uses.

I1 to I6, sampling location from the upper to lower IGP; FT, FS, AT, AS, forest topsoil, forest subsoil, agricultural topsoil, and agricultural subsoil, respectively.

The following properties were included in this NMDS analysis: EP, pH, CEC, CEC/clay, SOC, IC, TN, CN ratio, DOC, exchangeable Ca^{2+} and Mg^{2+} , clay and silt content, clay content, Fe_o , Al_o , Fe_d , and $\text{Fe}_d - \text{Fe}_o$.

Across all sites, active Al/Fe ($\text{Al}_o + \text{Fe}_o$) was consistently low ($<14 \text{ cmol kg}^{-1}$). Although topsoil displayed similar levels of active Al/Fe, exchangeable $\text{Ca}^{2+}/\text{Mg}^{2+}$, and clay contents as the subsoil (Table C3-2), free Fe (hydr)oxides (Fe_d) and crystalline Fe (hydro)oxides ($\text{Fe}_d - \text{Fe}_o$) were significantly more abundant in the subsoil ($P < 0.05$ and $P < 0.01$, respectively; Table C3-2). Moving from higher to lower elevations (upper to lower IGP), active Al/Fe content increased ($r_s = -0.58^{**}$), correlating positively with MAP and EP ($r_s = 0.78^{***}$ and 0.52^{**}) and negatively with

soil pH ($r_s = -0.67^{***}$) (Fig. C3-1). Clay and clay + silt contents increased from upper to lower IGP, with soil textures primarily being silty loam in upper IGP and silty clay loam to clay in lower IGP (Fig. C3-3). CEC values for topsoil ranged from 5.7 to 21.1, while subsoil fluctuated from 4.7 to 23.3 $\text{cmol}_c \text{kg}^{-1}$. Moreover, only the subsoil CEC correlated with clay content and SOC ($r_s = 0.83^{***}$ and $r_s = 0.81^{**}$).

Table C3-2 Differences of soil properties between topsoil and subsoil

	Unit	Topsoil	Subsoil	<i>P</i> value of paired t-test
pH		7.7 ± 1.1	8 ± 1.1	0.258
CEC	$\text{cmol}_c \text{kg}^{-1}$	10.1 ± 4.5	10.2 ± 5	0.936
SOC	g kg^{-1}	13.5 ± 6.6	3.7 ± 0.9	<0.001
IC	g kg^{-1}	4.7 ± 7.1	0.3 ± 1.1	0.039
TN	g kg^{-1}	1.1 ± 0.6	0.3 ± 0.2	0.001
CN ratio		13 ± 4.2	15.1 ± 4.4	0.261
DOC	g kg^{-1}	0.19 ± 0.09	0.06 ± 0.02	<0.001
$\text{Ca}_{\text{ex}} + \text{Mg}_{\text{ex}}$	$\text{cmol}_c \text{kg}^{-1}$	15.6 ± 11.6	14.3 ± 10.4	0.526
Ca_{ex}	$\text{cmol}_c \text{kg}^{-1}$	12.4 ± 11.4	10.9 ± 9.7	0.473
Mg_{ex}	$\text{cmol}_c \text{kg}^{-1}$	3.2 ± 3.3	3.4 ± 2.9	0.530
Clay + silt	%	81 ± 15.3	82.2 ± 15.5	0.555
Clay	%	23 ± 13	23.3 ± 7.4	0.918
$\text{Al}_o + \text{Fe}_o$	cmol kg^{-1}	6.2 ± 4	5.1 ± 2.6	0.141
Fe_o	cmol kg^{-1}	3.9 ± 2.9	3 ± 2	0.086
Al_o	cmol kg^{-1}	2.2 ± 1.3	2.1 ± 0.7	0.508
Fe_d	cmol kg^{-1}	14.3 ± 6	18.5 ± 9.2	0.023
$\text{Fe}_d - \text{Fe}_o$	cmol kg^{-1}	10.4 ± 4.1	15.4 ± 7.8	0.002

CEC, cation exchange capacity; SOC, soil organic carbon; IC, inorganic carbon; TN, total nitrogen; DOC, dissolved organic carbon; Ca_{ex} and Mg_{ex} , exchangeable Ca^{2+} and Mg^{2+} ; $\text{Al}_o + \text{Fe}_o$, acid oxalate extractable active Al and Fe; Fe_d , dithionite-citrate extractable iron; $\text{Fe}_d - \text{Fe}_o$, crystalline Fe (hydr)oxides.

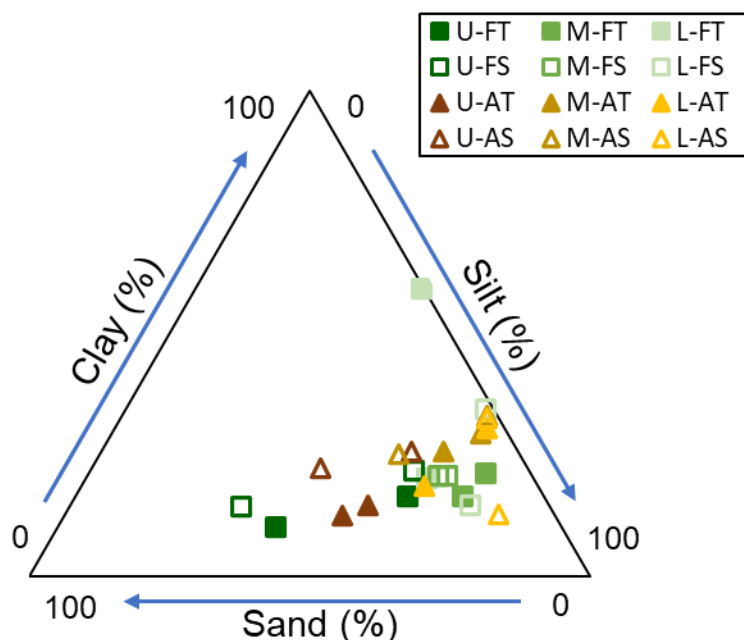
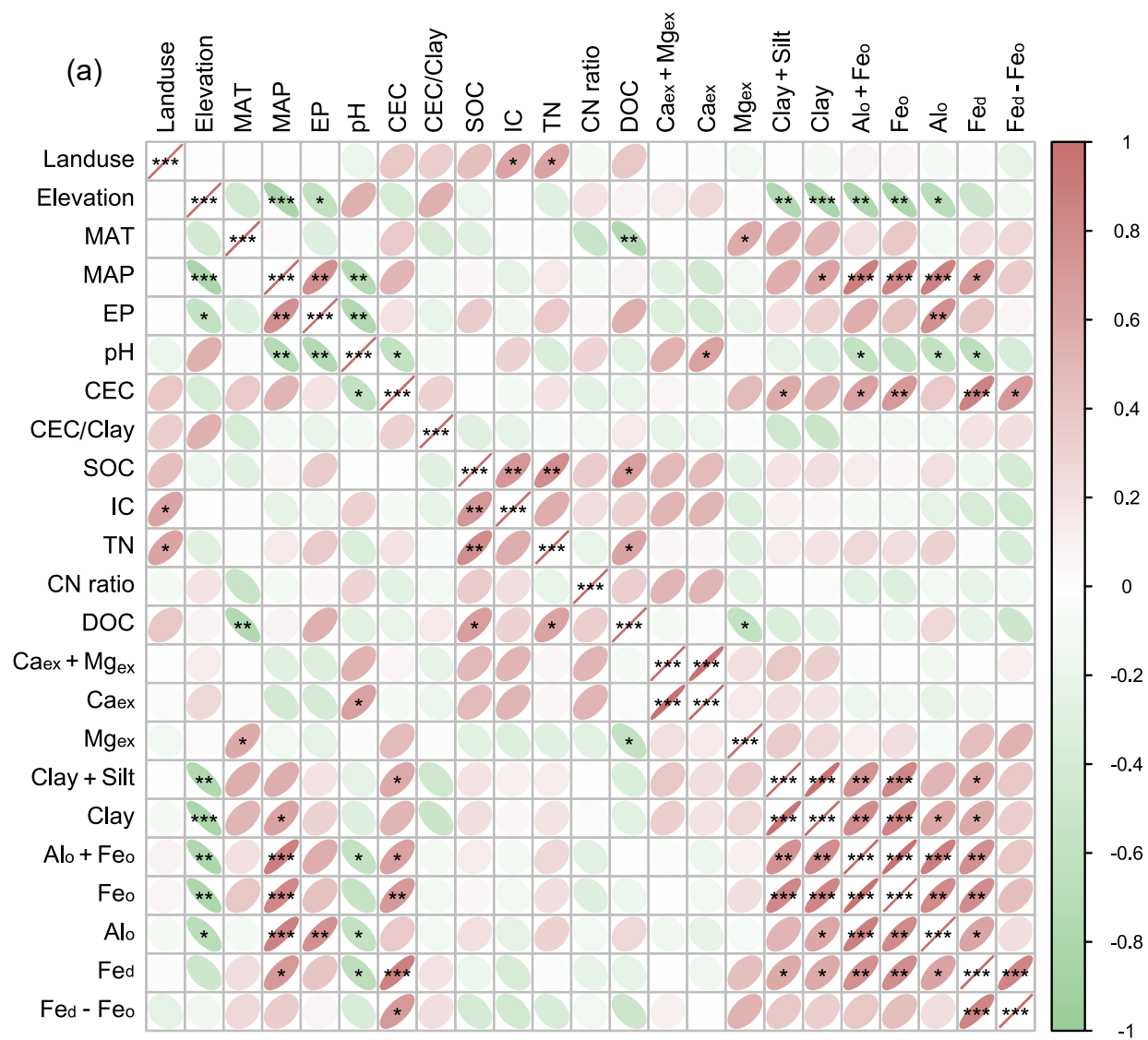


Fig. C3-3 Soil texture distribution of soil samples from Indo-Gangetic Plain (IGP).

U, M, and L, upper, middle, and lower IGP, respectively; FT, FS, AT, and AS, forest topsoil, forest subsoil, agricultural topsoil, and agricultural subsoil, respectively.

Comparing the two layers, topsoil showed a significantly higher SOC ($13.5 \pm 6.6 \text{ g kg}^{-1}$) relative to subsoil ($3.7 \pm 0.9 \text{ g kg}^{-1}$) across all sites (Table C3-2). Correspondingly, TN and DOC levels in topsoil surpassed those in subsoil. SOC positively correlated with TN for both topsoil and subsoil and positively correlated with DOC for topsoil (Figs. C3-4ab). In the subsoil, SOC positively aligned with Mg_{ex} , clay + silt, clay, and Fe_o ($r_s = 0.80^{**}$, 0.61^* , 0.66^* , 0.67^* , respectively), with a near-significant association with active Al/Fe ($r_s = 0.53$, $P = 0.08$). Importantly, topsoil SOC content only significantly correlated to IC, TN, and DOC, without any evident ties to other soil physiochemical properties (Fig. C3-4a). Across all soils, no significant correlation emerged between SOC and variables such as land use, elevation, or climate (Figs. C3-1 and C3-4ab).



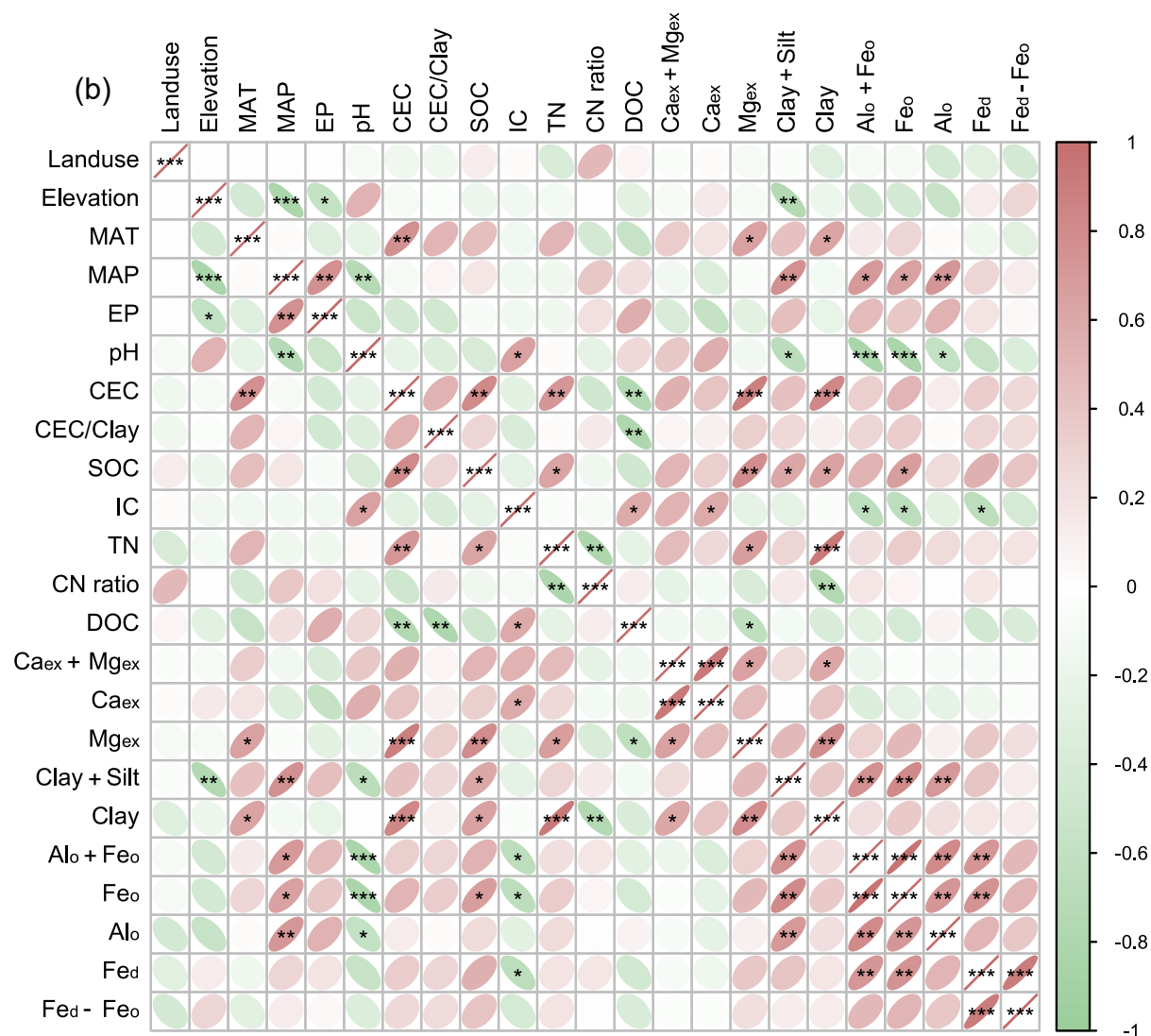


Fig. C3-4 Spearman correlation matrix (r_s) reveal the relationships among climatic and geochemical properties for topsoil (a) and subsoil (b).

3.3.2 SOM Fractionation and SOC Pools

Figure C3-5 presents the organic carbon content and proportion of each SOM fraction. Notably, no significant differences in organic carbon factors (i.e., SOC, DOC, SOM fractions, and SOC pools) between agricultural and forest soils were observed in both topsoil and subsoil, as indicated by NMDS diagrams with the PERMANOVA test (Figs. C3-6ab). Thus, for subsequent

analysis, agricultural and forest soils were consolidated. Consequently, MAOM ($9.5 \pm 6.1 \text{ g kg}^{-1}$), LF ($1.8 \pm 1.1 \text{ g kg}^{-1}$), and oPOM ($0.6 \pm 0.4 \text{ g kg}^{-1}$) content were significantly higher in topsoil than subsoil (2.6 ± 0.9 , 0.3 ± 0.3 , $0.1 \pm 0.04 \text{ g kg}^{-1}$, respectively, with MAOM representing the majority ($68 \pm 15\%$) of all fractions. Non-hydrolyzable MAOM contents were similar for topsoil and subsoil, yet it constituted a larger proportion in subsoil (Fig. C3-5).

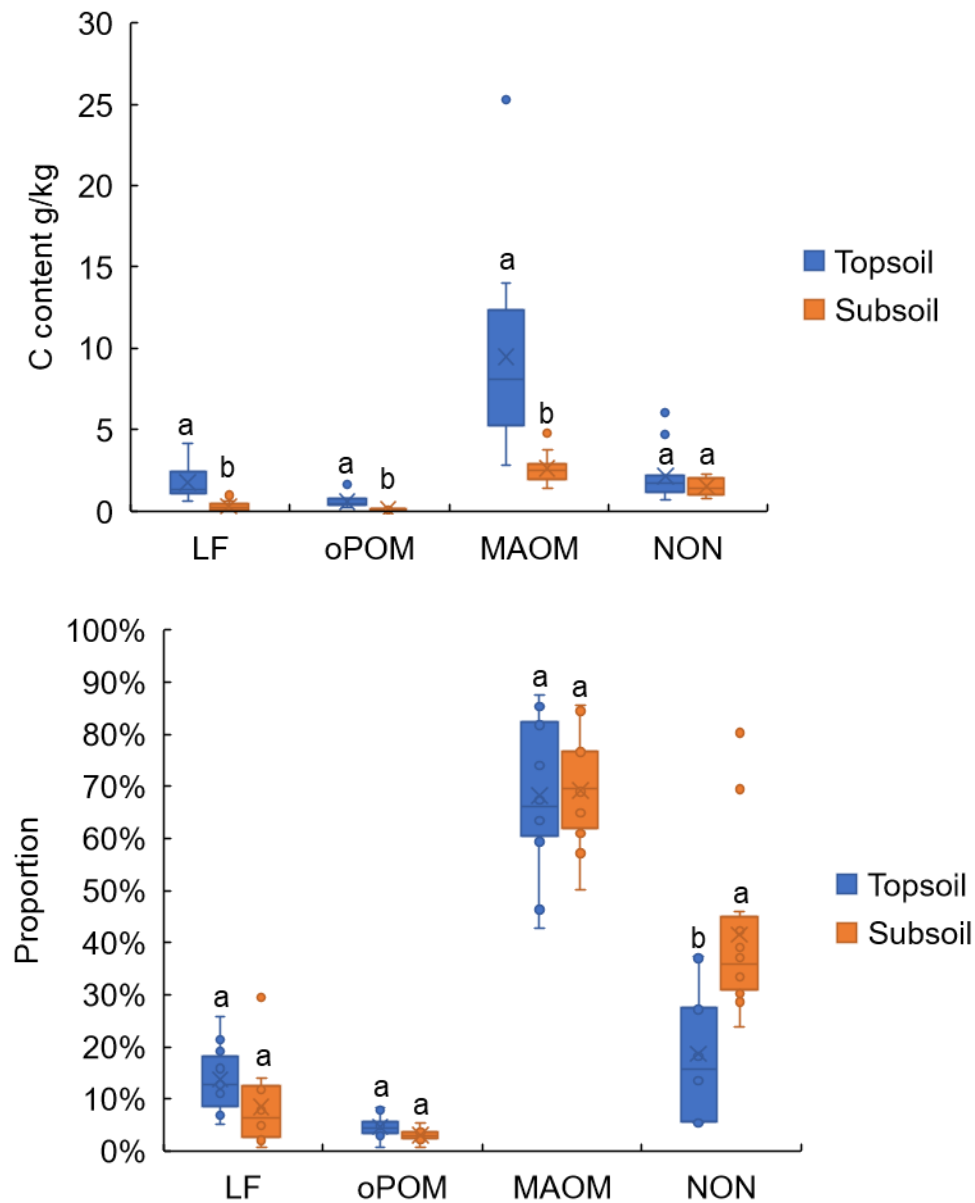


Fig. C3-5 Box diagrams of sizes (a) and proportions (b) for SOM fractions in topsoil and subsoil across all sites

Small letters show the significance of differences between topsoil and subsoil ($P < 0.05$).

LF, light fraction; oPOM, occluded particulate organic matter in aggregates; MAOM, maize-derived C as mineral-associated organic matter.

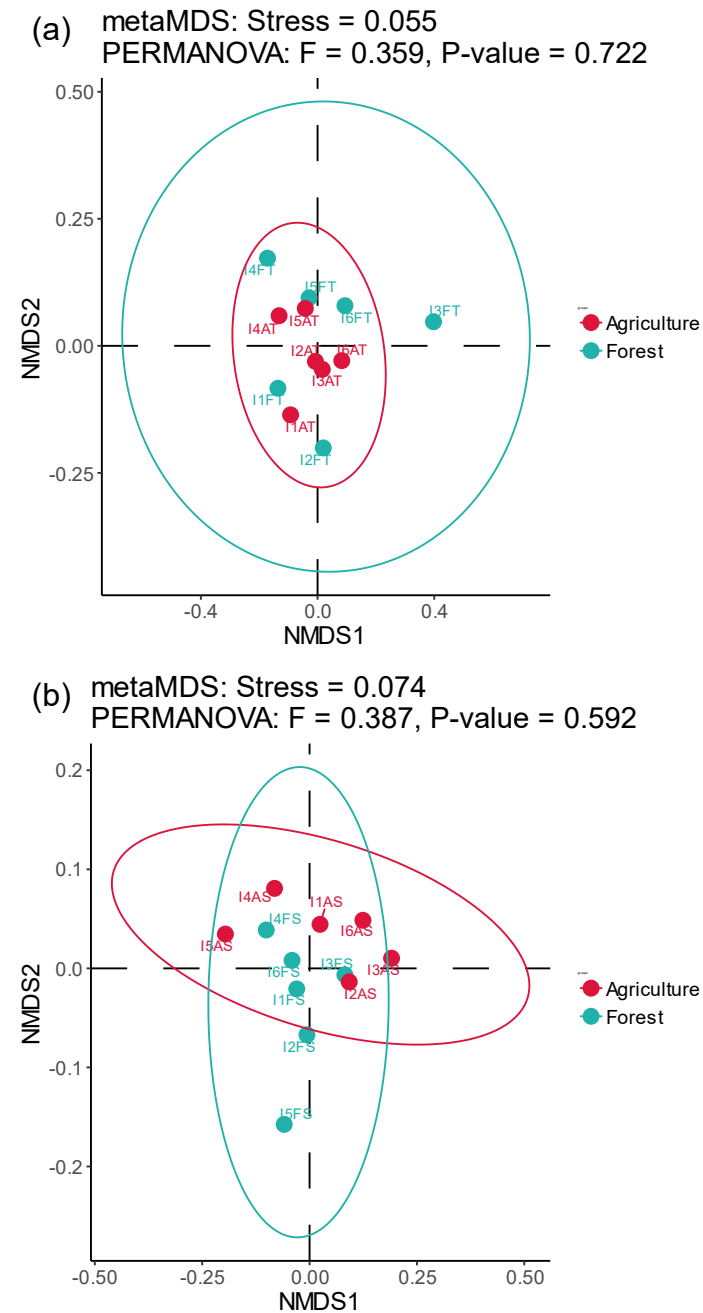


Fig. C3-6 Nonmetric multidimensional scaling (NMDS) diagram with PERMANOVA test

revealing difference in SOC-related properties (SOC, DOC, SOM fractions, and SOC pools) between soils from agriculture and forest land uses in topsoil (a) and subsoil (b).

Curves of cumulative CO₂ releases across all sites are shown in Fig. C3-7. Early respiration was intensive, primarily within the initial two months for topsoil and the first month for subsoil. Then, cumulative CO₂ releases increased steadily in the later stages of incubation for most soils. A total of $4.1 \pm 2.6\%$ of SOC was respired for topsoils, significantly higher than that of subsoil $1.8 \pm 1.2\%$. Cumulative CO₂ release curves (Fig. C3-7) adeptly fitted to Eq. (1), as evidenced by mean square error roots lower than 0.001 and sum squared residuals lower than 10^{-5} ($r > 0.995$; $R^2 > 0.995$).

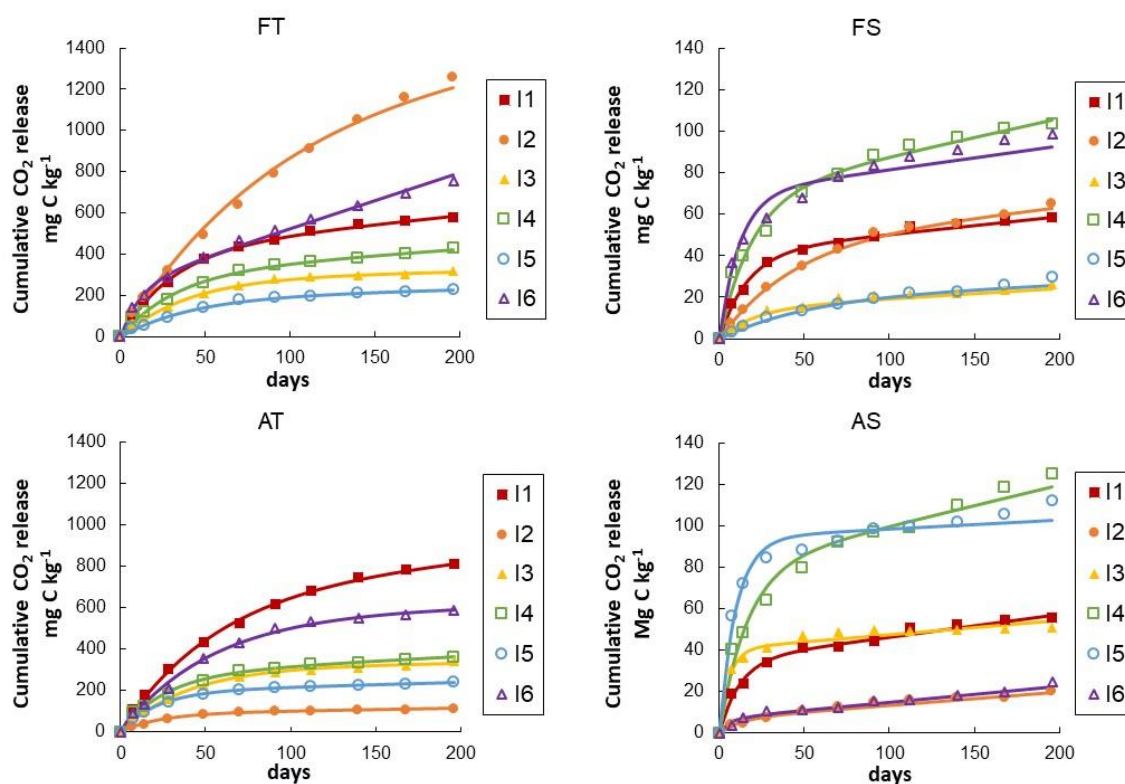


Fig. C3-7 Cumulative CO₂ release with fitting curve across all soils.

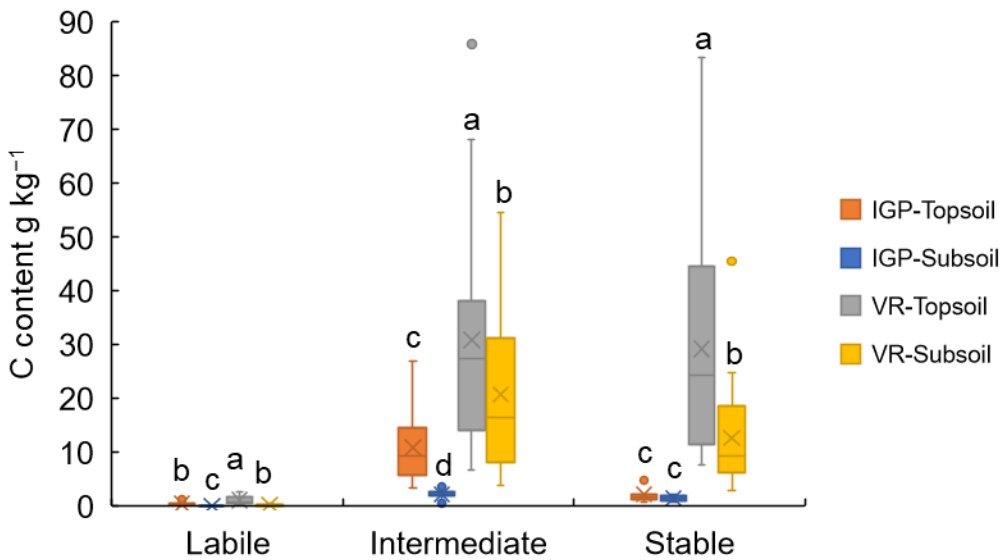
The size, proportion, and MRT of each SOC pool are listed in Table C3-3. The intermediate SOC pool was the largest within three pools for both topsoil and subsoil ($78 \pm 13\%$ and $57 \pm 17\%$ of total SOC, respectively), with sizes ranging from 3.3 to 27.0 g C kg^{-1} and 0.5 to 3.7 g C kg^{-1} , while the labile pool size was smallest (0.1 to 1.2 g C kg^{-1} and 0.01 to 0.09 g C kg^{-1} for topsoil and subsoil, respectively). MRTs of intermediate SOC pools were decades to tens of decades with no significant difference between topsoil and subsoil (10,000 to 88,600 days for topsoil and 9,800 to 57,800 days for subsoil; Table C3-3). Notably, subsoil had significantly shorter MRTs for the labile pool (6 to 52 days) than topsoil (22 to 94 days), with a significance of $P < 0.01$.

Table C3-3 Differences of soil organic carbon pools between topsoil and subsoil

Topsoil		I1FT	I2FT	I3FT	I4FT	I5FT	I6FT	I1AT	I2AT	I3AT	I4AT	I5AT	I6AT
C _L	g kg ⁻¹	0.42	1.21	0.28	0.31	0.19	0.25	0.69	0.09	0.31	0.28	0.19	0.57
C _I	g kg ⁻¹	9.93	8.60	4.55	27.02	14.81	17.99	7.21	3.31	5.33	13.94	10.21	8.16
C _S	g kg ⁻¹	6.08	2.17	0.16	1.58	0.85	1.09	4.73	1.29	2.11	2.21	0.66	1.35
C _L %		2.5	10.1	4.3	1.1	1.2	1.3	5.5	1.9	4.0	1.7	1.8	5.6
C _I %		60.5	71.8	68.3	93.5	93.5	93.1	57.1	70.6	68.8	84.9	92.2	80.9
C _S %		37.0	18.1	27.4	5.5	5.4	5.6	37.4	27.5	27.2	13.4	6.0	13.4
MRT _L	days	31	94	39	33	42	66	56	23	40	26	22	53
MRT _I	days	11700	11300	29900	46300	75200	88600	10000	24600	54600	31700	44800	46100
Subsoil		I1FS	I2FS	I3FS	I4FS	I5FS	I6FS	I1AS	I2AS	I3AS	I4AS	I5AS	I6AS
C _L	g kg ⁻¹	0.04	0.05	0.02	0.07	0.02	0.07	0.04	0.01	0.04	0.08	0.09	0.01
C _I	g kg ⁻¹	1.99	2.68	2.07	2.28	0.98	2.29	2.22	1.90	3.67	1.96	0.47	3.46
C _S	g kg ⁻¹	1.31	0.85	1.53	1.02	2.26	2.02	1.33	0.77	1.98	1.04	2.28	1.74
C _L %		1.2	1.3	0.4	2.1	0.6	1.6	1.0	0.3	0.7	2.6	3.3	0.1
C _I %		59.6	74.9	57.3	67.6	29.9	52.3	61.8	71.2	64.5	63.6	16.4	66.5
C _S %		39.2	23.8	42.3	30.3	69.5	46.1	37.2	28.8	34.7	33.9	80.3	33.5
MRT _L	days	17	44	20	20	52	15	12	13	6	17	10	9
MRT _I	days	24200	31400	46200	12500	35100	21300	20800	30500	57800	9800	12300	47600

C_L, C_I, and C_S, sizes of labile, intermediate, and stable SOC pools, respectively; C_L%, C_I%, and C_S%, proportions of labile, intermediate, and stable SOC pools, respectively; MRT_L and MRT_I, mean residence times of labile and intermediate SOC pools.

In terms of sizes and proportions, topsoil outpaced subsoil for labile and intermediate SOC pools (Fig. C3-8). Conversely, subsoil had a higher stable SOC pool proportion (Fig. C3-3). All pool sizes in both topsoil and subsoil were significantly lower in IGP than in tropical volcanic regions (VR) (Lyu et al., 2021b), especially the stable pool. Furthermore, the proportion of the stable SOC pool in IGP topsoil was significantly lower than in VR. Contrarily, IGP topsoil had higher proportions for labile and intermediate pools compared to VR.



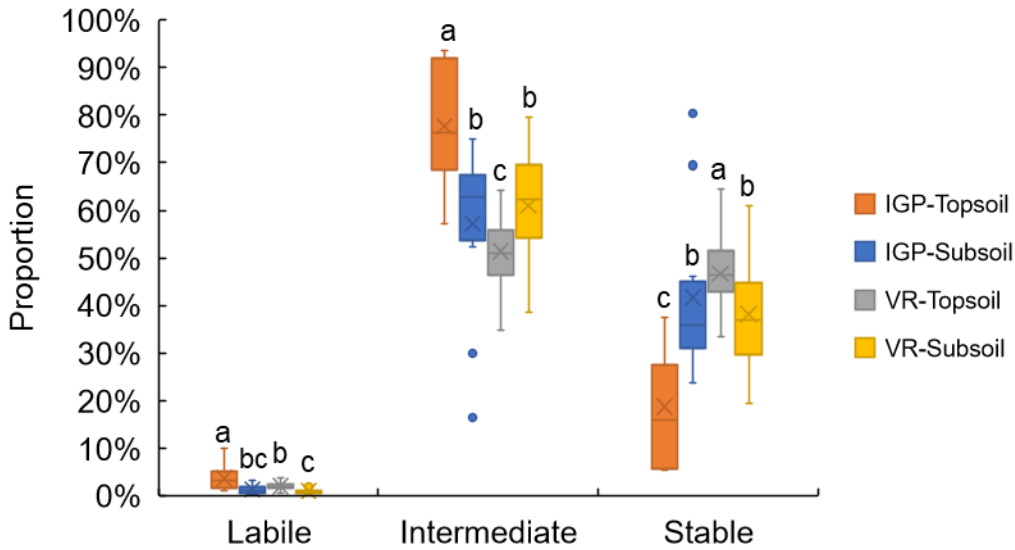


Fig. C3-8 Box diagrams of sizes (a) and proportions (b) for SOC pools in topsoil and subsoil of sites from Indo-Gangetic plain (IGP) and tropical volcanic regions (VR). Small letters show the significance of differences in each pool among the soils ($P < 0.05$). The data of VR soils from Lyu et al., 2021b.

Figure C3-9 shows correlation matrices to elucidate the relationship between SOM fractions and SOC pools. The most potent correlation emerged between MAOM and C_1 for both topsoil and subsoil ($r_s = 0.80^{**}$ and 0.87^{***}). Additionally, the topsoil featured significant negative correlations between MAOM and the labile pool proportion and between LF and the stable pool proportion (Fig. C3-9a). In subsoil, MAOM positively correlated with MRT_1 (Fig. C3-9b).

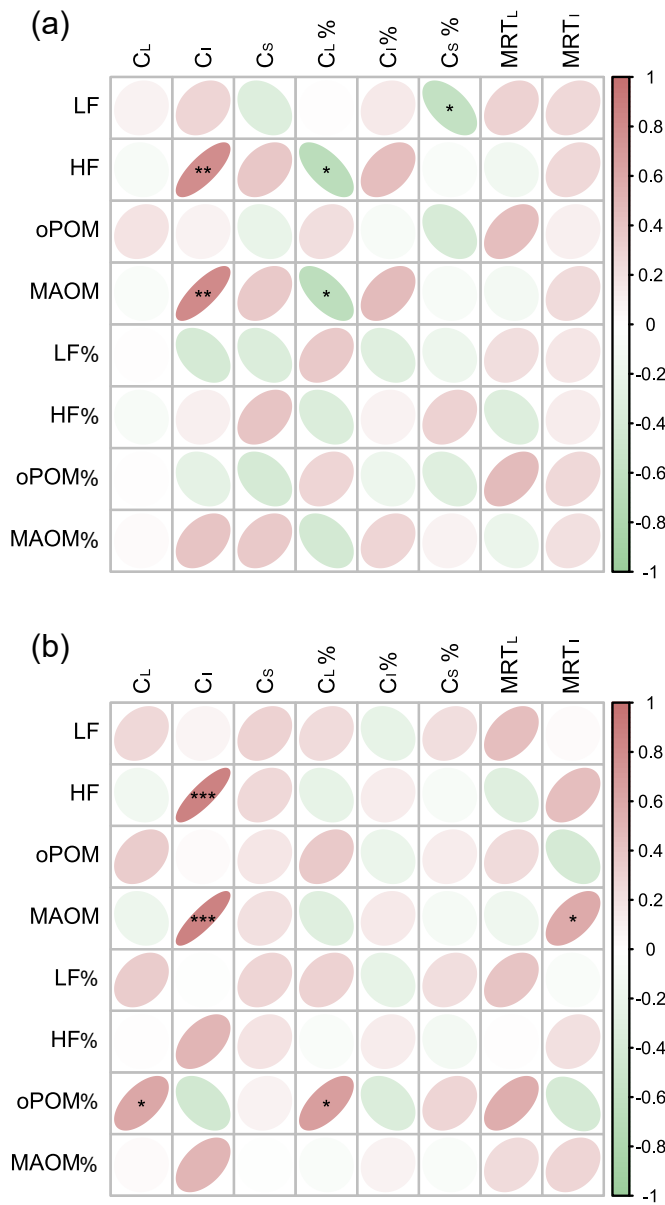


Fig. C3-9 Spearman correlation matrix (r_s) revealing the relationships between SOM fractions and SOC pools in topsoil (a) and subsoil (b).

C_L , C_I , and C_S , sizes of labile, intermediate, and stable SOC pools, respectively; $C_L\%$, $C_I\%$, and $C_S\%$, proportions of labile, intermediate, and stable SOC pools, respectively; MRT_L and MRT_I , mean residence times of labile and intermediate SOC pools. LF and HF, light and heavy fractions; oPOM, occluded particulate organic matter in aggregates; MAOM, mineral-associated organic matter.

3.3.3 Contributions of Climatic and Geochemical Properties to SOM Fractions and SOC Pools

The potential determinants of SOM fractions and SOC pools for IGP topsoil are shown in Figs. C3-10a and C3-11a. Land use demonstrated a positive correlation solely with LF, indicating a higher LF content in forest soils (Fig. C3-10). Soil pH negatively correlated with LF and oPOM ($r_s = -0.63^*$ and -0.66^*) in topsoil. A traditional index showing the SOM quality, C to N ratio was positively correlated with both content and proportions of MAOM ($r_s = 0.59^*$ and 0.84^{***}). Additionally, CEC exhibited positive correlations with LF and oPOM in topsoil (Fig. C3-10a). Unlike LF or oPOM in topsoil, MAOM positively correlated with CEC in subsoil (Fig. C3-10b). In subsoil, the correlation between land use and LF was positive ($r_s = 0.68^*$), as was the association between land use and MRT_L (Fig. C3-10b). There, exchangeable Ca/Mg, clay, and active Fe showed positive correlations with MAOM, with patterns resembling C_i in subsoil (Fig. C3-10b).

Notably, MRT_I , indicative of the stability of the intermediate SOC pool, positively correlated with active Al/Fe, clay + silt, and clay ($r_s = 0.87^{***}$, 0.78^{**} , and 0.81^{**}). Also, MRT_I was higher in lower IGP (i.e., area of lower elevation) (Fig. C3-10a). Similar to topsoil, MRT_I increased with the increase of active Al/Fe in subsoil ($r_s = 0.62^*$, Fig. 4b). Moreover, active Al/Fe strongly positively correlated with C_S ($r_s = 0.84^{***}$), succeeded by correlations with clay + silt and EP ($r_s = 0.78^{**}$ and 0.76^{**}) in subsoil. In subsoil, clay and exchangeable Ca/Mg showed positive correlations with C_I ($r_s = 0.73^{**}$ and 0.59^*), while pH negatively correlated with C_S and MRT_I (Fig. C3-10b).

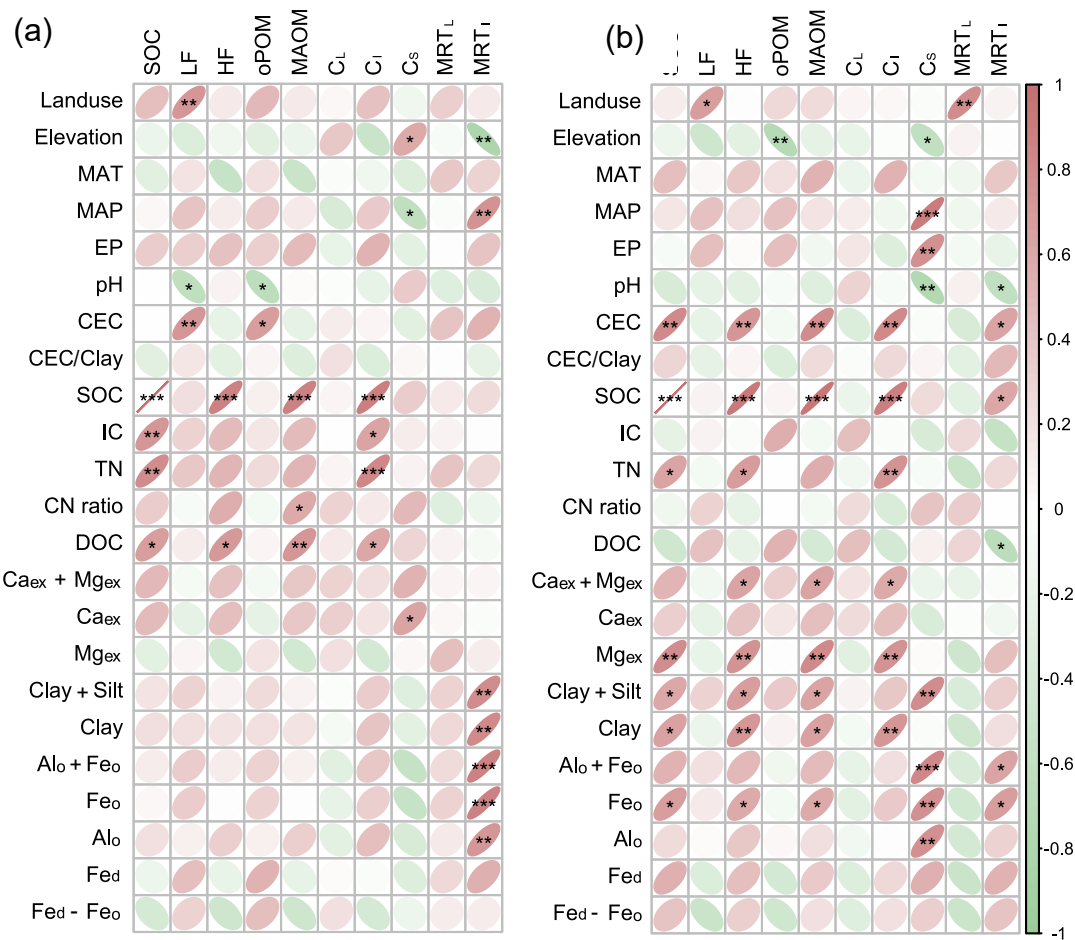


Fig. C3-10 Spearman correlation matrix (r_s) between carbon properties and climatic and geochemical properties for topsoil (a) and subsoil (b).

To assess the influence of climate, soil properties, and land use on the sizes and residence times of SOC pools, Partial Least Squares Path Modeling (PLS-PM) was employed (Fig. C3-11). The mean residence time of the intermediate SOC pool (i.e., MRT_I) was well explained ($r^2 = 0.89$ and 0.49), primarily due to the direct contribution from active Al/Fe in both topsoil and subsoil ($\beta = 0.75^{***}$ and 0.64^*). Topsoil showed that exchangeable Ca/Mg and active Al/Fe potentially contribute positively to C_I ($\beta = 0.64$ and 0.40), whereas this relationship might not be consistent, as suggested by the insignificance from the Bootstrapping test (Fig. C3-11a).

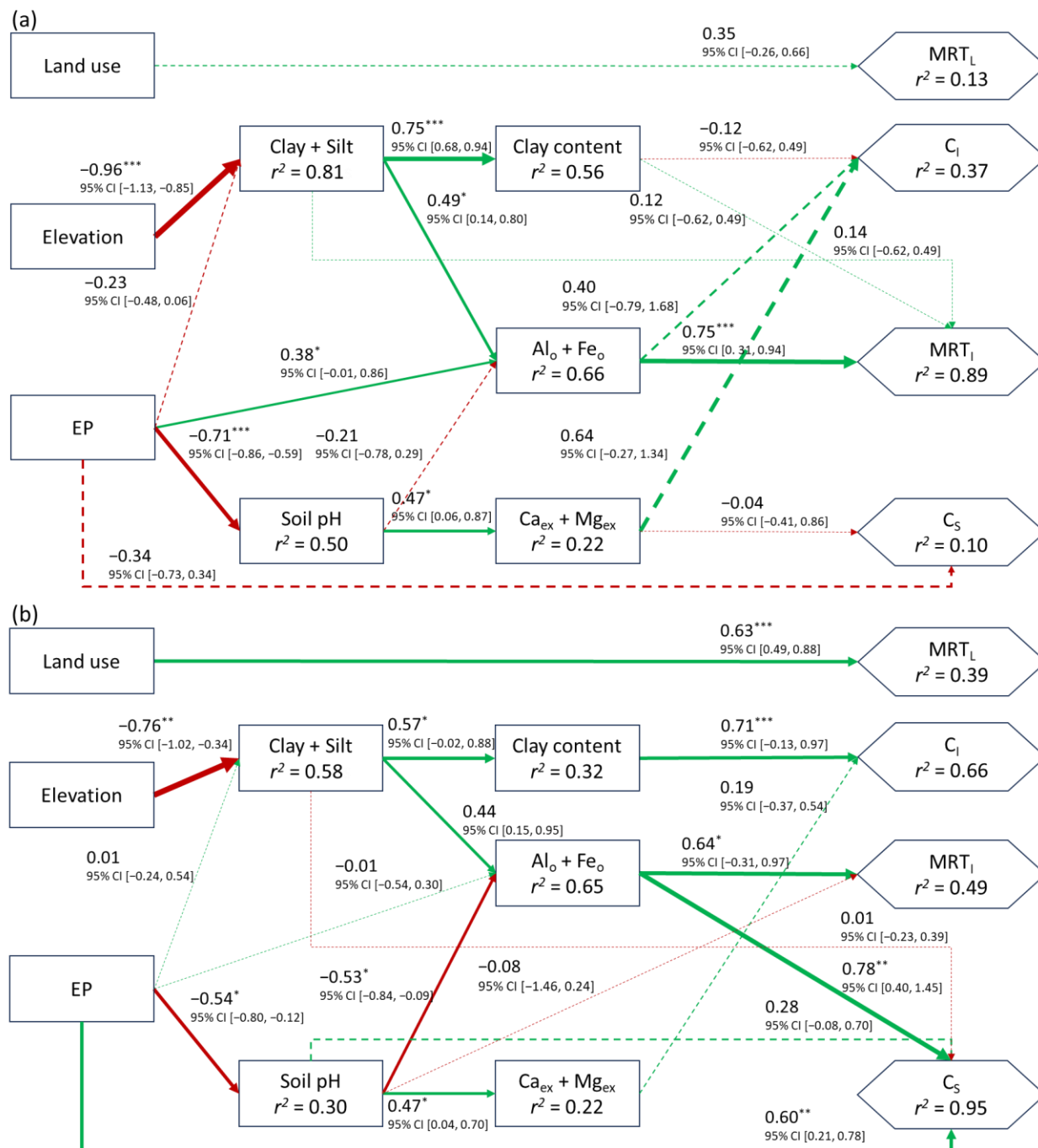


Fig. C3-11 Partial Least Squares Path Modeling (PLS-PM) revealing the contribution of climatic and geochemical properties on sizes and mean residence times (MRT) of SOC pools in topsoil (a) and subsoil (b).

Solid and dotted arrows represent the significant and non-significant contributions, respectively; green and red colors represent the positive and negative contributions, respectively; the number above the line represents the normalized path coefficient (β). The significance and the confidence interval (CI) of the path coefficient were provided by the Bootstrapping method.

Differing from topsoil, MRT_L , which also reflects the carbon quality of litter input, revealed a positive contribution from land use in subsoil ($\beta = 0.63^{***}$). The intermediate SOC pool sizes in subsoil drew contributions exclusively from clay content ($\beta = 0.71^{***}$), which was inversely affected by elevation (i.e., river flow; Fig. 5b). C_s in subsoil was well explained ($r^2 = 0.95$) and primarily contributed by active Al/Fe ($\beta = 0.78^{**}$), which was negatively affected by soil pH (Fig. C3-11b). EP also manifested a positive contribution to C_s in subsoil ($\beta = 0.60^{**}$).

3.3.4 SOM Molecular Composition Evaluated by Py-GC/MS Analyses

The comprehensive SOM molecular compositions exhibited no significant distinctions between agriculture and forest soils in both topsoil and subsoil, as shown in Fig. C3-12. Consequently, I combined these datasets to probe into the determinants of SOM composition. Delving into the specifics, forest soils revealed a higher proportion of degraded PS, long alkanes and N-MAH, whereas agricultural soils showed a higher proportion of squalene (Table C3-4). N-containing group was the predominant SOM component, followed by MAH and degraded PS (Fig. C3-13 and Table C3-5). Among aromatic hydrocarbons, MAH proportion was significantly higher than PAH in both topsoil (MAH: $12.5 \pm 2.6\%$, PAH: $1.5 \pm 0.7\%$) and subsoil (MAH: $16.9 \pm 3.8\%$, PAH: $0.8 \pm 1.1\%$). N-containing compounds primarily comprised non-aromatic nitriles ($44.4 \pm 19.4\%$ for all samples). Compounds in the degraded PS group were mainly heterocyclic compounds like furans ($5.5 \pm 2.5\%$ for all samples). Remarkably, no undegraded PS, a biomarker for cellulose and starch, was detected (Fig. C3-13 and Table C3-5). Additionally, lignin proportions were minimal in topsoil ($1.2 \pm 0.6\%$) and were not detected in subsoil.

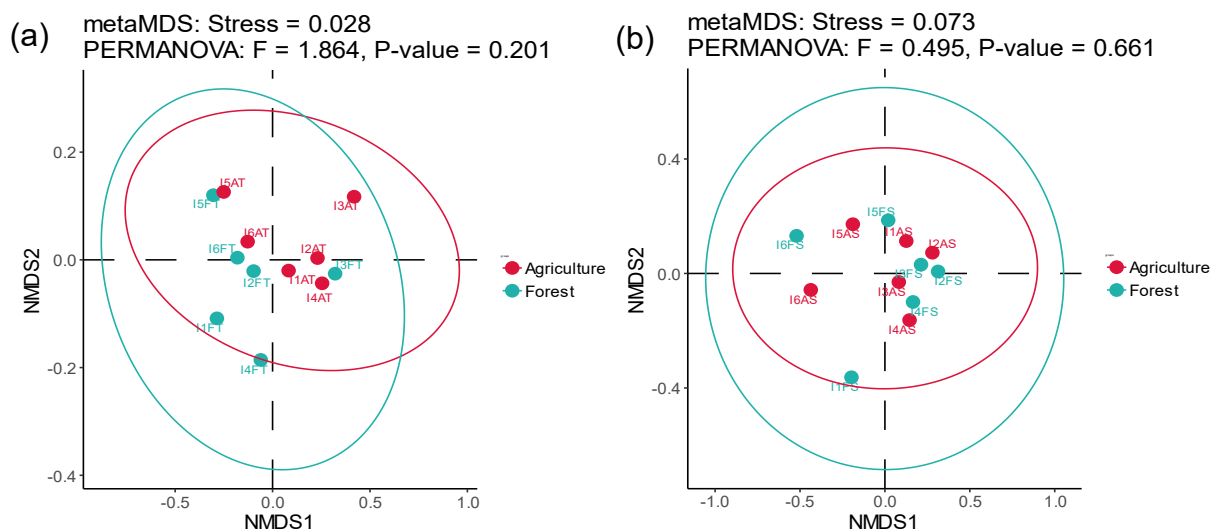


Fig. C3-12 Nonmetric multidimensional scaling (NMDS) diagram with PERMANOVA test revealing difference in SOM molecular composition between soils from agriculture and forest land uses for topsoil (a) and subsoil (b).

Table C3-4 Differences in the relative abundance of SOM compounds between forest and agricultural

Organic compounds	Forest (%)	Agriculture (%)	P value of paired t-test
Undegraded PS	n.d.	n.d.	-
Degraded PS	15.8 ± 10.4	11 ± 9.5	0.002
Long alkanes	1.6 ± 1.1	1.1 ± 0.8	0.018
Short alkanes	2.1 ± 1.0	1.9 ± 0.7	0.578
n-alkenes	3.1 ± 2.0	3.4 ± 2.1	0.649
Squalene	3.0 ± 4.2	5.1 ± 5.2	0.036
FAnE	2.2 ± 5.6	2.2 ± 3.7	0.957
Steroids	0.1 ± 0.2	0.1 ± 0.2	0.948
Lignin	0.7 ± 0.9	0.5 ± 0.5	0.120
Phenols	2.7 ± 3.0	1.8 ± 2.4	0.075
MAH	14.5 ± 4.4	14.9 ± 3.5	0.798
PAH	1.1 ± 1.0	1.2 ± 1.0	0.825
N-MAH	1.6 ± 0.7	1.3 ± 0.6	0.046
Alkylamides	1.0 ± 2.2	0.5 ± 1.6	0.616
N-containing	48.5 ± 15.7	54.1 ± 12.7	0.122
Other hydrocarbons	1.9 ± 2.3	0.9 ± 0.9	0.085

PS, polysaccharides; FAnE, fatty acids, and their esters; MAH, monocyclic aromatic hydrocarbons; PAH, polycyclic aromatic hydrocarbons; N-MAH, N-containing MAH; N-containing, other N-containing compounds.

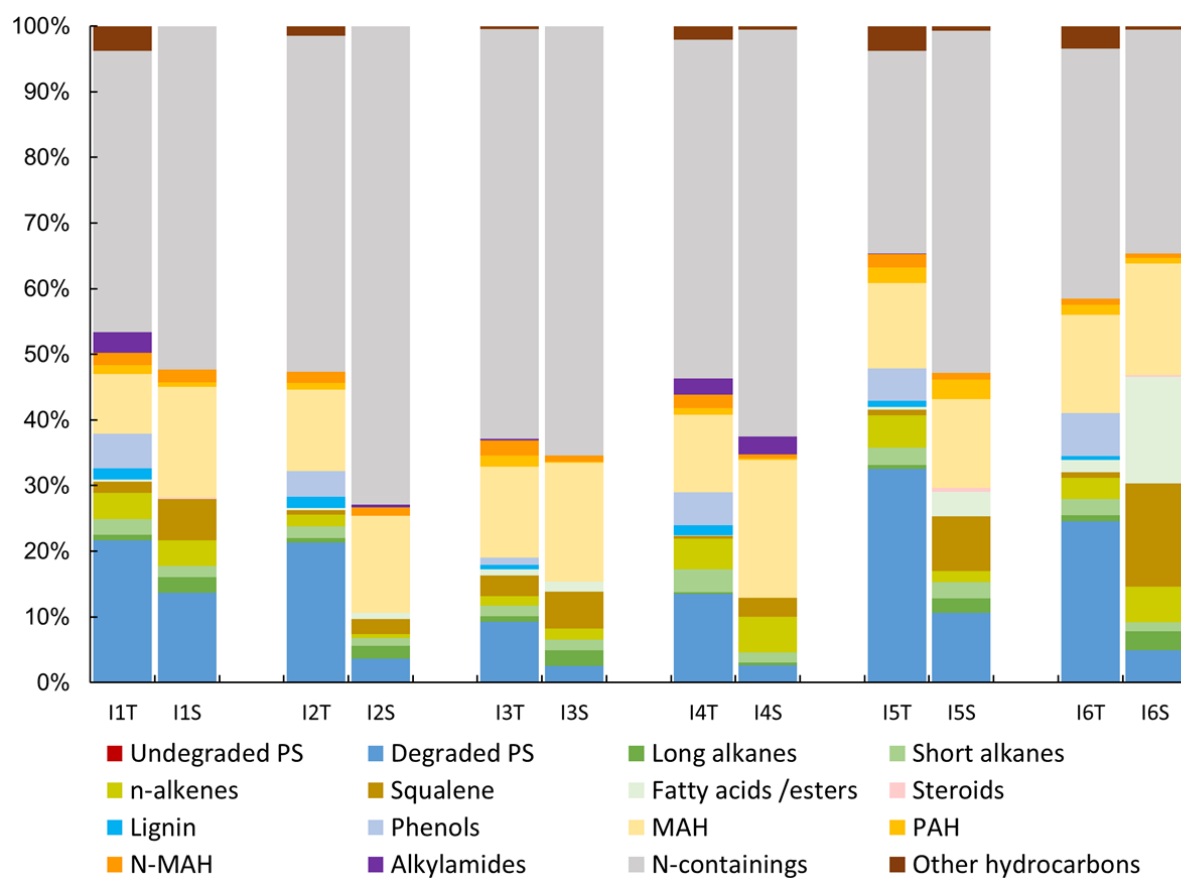


Fig. C3-13 Distribution of SOM molecular composition across all sites.

I1 to I6, sampling location from the upper to lower IGP; T and S, topsoil and subsoil. PS, polysaccharides; MAH, monocyclic aromatic hydrocarbons; PAH, polycyclic aromatic hydrocarbons, N-MAH, N-containing MAH; N-containing, other N-containing compounds.

Table C3-5 Differences in the relative abundance of SOM compounds between topsoil and subsoil, as determined by pyrolysis–gas chromatography–mass spectrometry.

Organic compounds	Topsoil (%)	Subsoil (%)	P value of paired t-test
Undegraded PS	n.d.	n.d.	-
Degraded PS	20.5 ± 8.5	6.3 ± 5.5	0.045
Long alkanes	0.7 ± 0.3	2 ± 0.9	0.009
Short alkanes	2.4 ± 0.8	1.7 ± 0.8	0.008
n-alkenes	3.3 ± 1.7	3.1 ± 2.4	0.024
Squalene	1.3 ± 1	6.9 ± 5.4	0.054
FAnE	0.7 ± 0.8	3.7 ± 6.3	0.063
Steroids	n.d.	0.2 ± 0.3	0.003
Lignin	1.2 ± 0.6	n.d.	<0.001
Phenols	4.5 ± 2.2	n.d.	<0.001
MAH	12.5 ± 2.6	16.9 ± 3.8	0.038
PAH	1.5 ± 0.7	0.8 ± 1.1	0.011
N-MAH	1.8 ± 0.5	1.1 ± 0.6	0.006
Alkylamides	1 ± 2.2	0.5 ± 1.6	0.016
N-containing	46.2 ± 12.4	56.4 ± 14.6	0.146
Other hydrocarbons	2.5 ± 1.9	0.3 ± 0.6	0.006

Results of PERMANOVA show a significant difference in SOM composition between topsoil and subsoil (Fig. C3-14). As illustrated in Table C3-5, subsoil demonstrated significantly lower degraded PS, lignin, and phenols but higher MAH and N-containing compounds compared to topsoil. Interestingly, lignin and phenol groups were absent in subsoil.

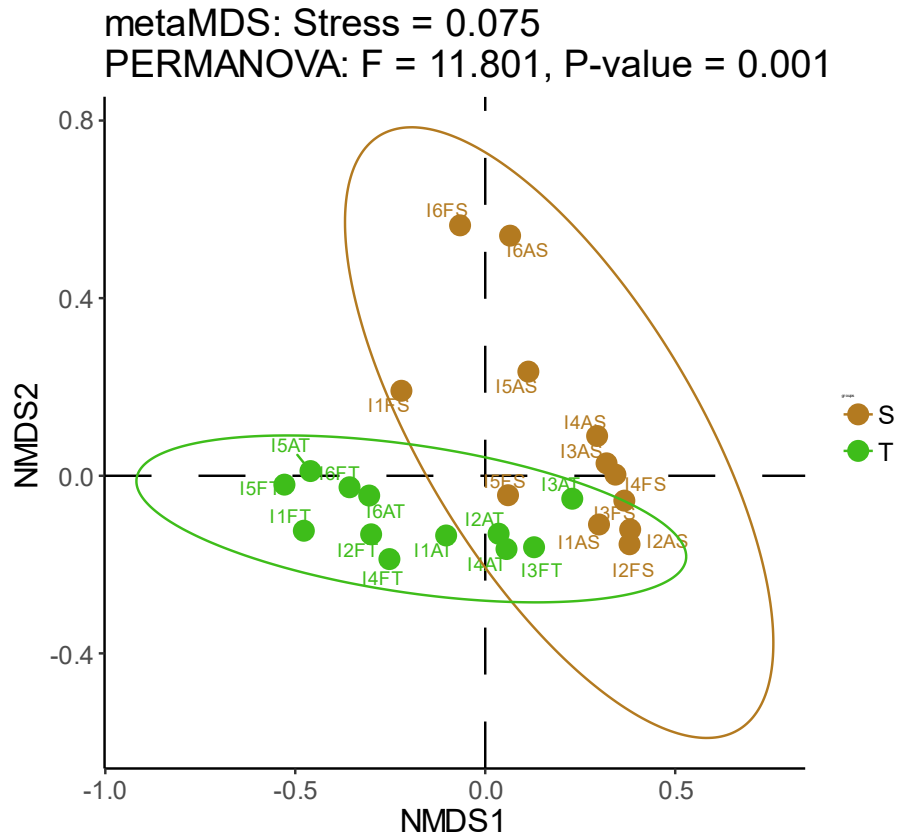


Fig. C3-14 Nonmetric multidimensional scaling (NMDS) diagram with PERMANOVA test revealing difference in SOM composition between topsoil (T) and subsoil (S).

RDA diagram (Fig. C3-15a) elucidated that climate, horizon, along with soil physicochemical and mineralogical properties explained 74.6% of SOM composition variations ($F = 2.89$, $P < 0.001$). The dominant RDA1 axis, encapsulating 58.1% of the variance, was primarily shaped by SOC and differences between topsoil and subsoil, accompanied by a minor influence of moisture-associated factors like EP, pH, and active Al/Fe. Meanwhile, RDA2 (16.5%) drew significant contributions from EP ($P < 0.001$), pH ($P < 0.01$), and active Al/Fe ($P < 0.05$). However, land use, clay, clay + silt, and exchangeable $\text{Ca}^{2+}/\text{Mg}^{2+}$ did not significantly impact SOM composition ($P > 0.05$; Fig. C3-15a).

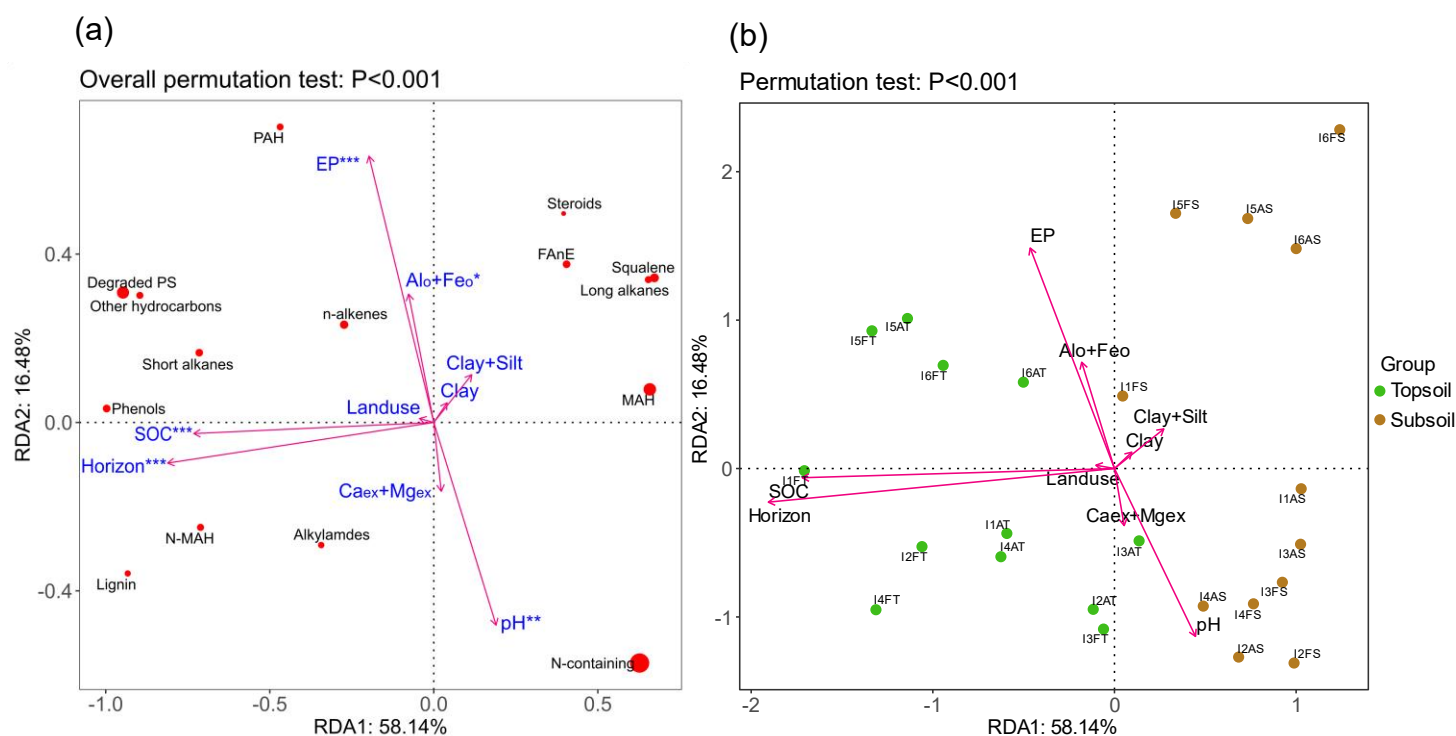
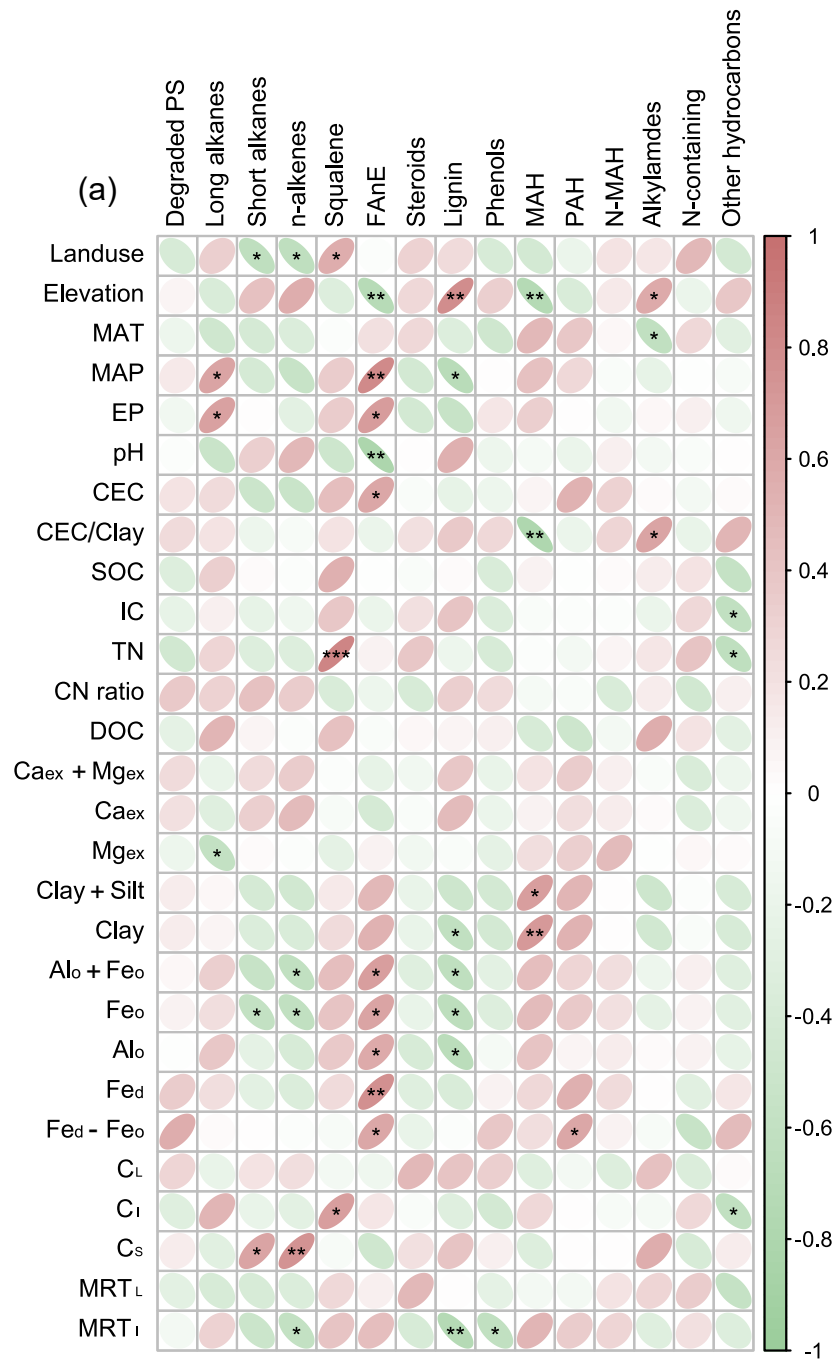


Fig. C3-15 Redundancy analysis (RDA) diagram of SOM compound groups and their controls (a) and plots diagram showing the SOM composition distribution across all soils (b) with significance test.

EP, excess precipitation; Horizon, topsoil (1) and subsoil (0); Landuse, forest (1) and agriculture (0).

Topsoil exhibited lower RDA1 scores, indicating a higher abundance of degraded PS, lignin, and phenols, but lower levels of MAH and N-containing groups (Figs. C3-15ab). The SOC content effectively distinguished topsoil from subsoil along the RDA1 axis. Factors like EP, pH, and active Al/Fe accentuated disparities in FAnE, long alkanes, n-alkanes, and N-containing compounds along the RDA2 axis. Congruent with RDA, FAnE positively correlated with EP and active Al/Fe in topsoil, but negatively correlated with pH (Fig. C3-16a). FAnE was also closely related to active Al and EP in subsoil ($r_s = 0.58$, $P = 0.05$; $r_s = 0.53$, $P = 0.07$). Long alkanes, another faction

representing less decomposed lipids, correlated positively with EP in topsoil (Fig. C3-16a) and EP and active Al/Fe in subsoil (Fig. C3-16b). In contrast, as the decomposition residue, MAH was higher in topsoils from lower IGP (lower elevation) with higher clay content.



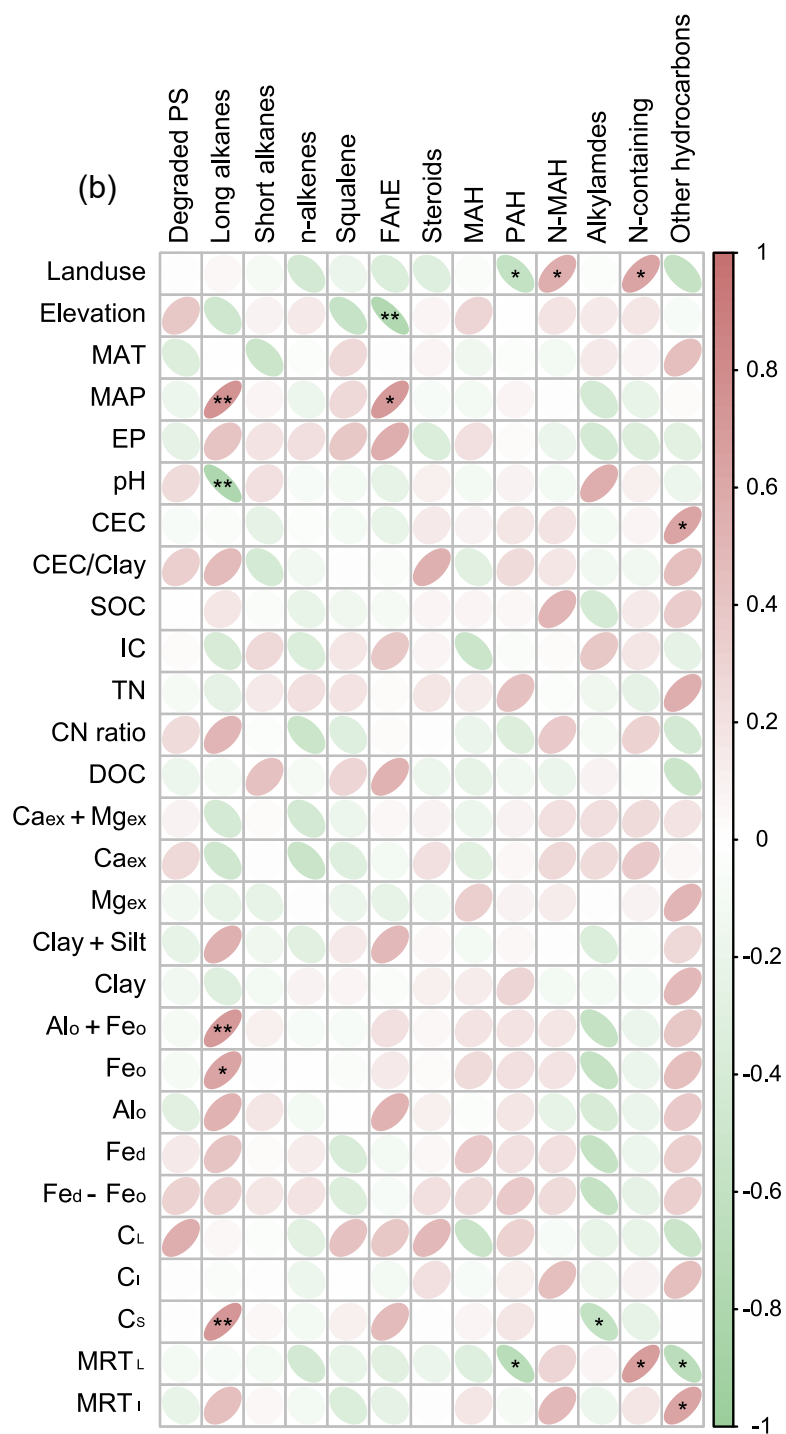


Fig. C3-16 Spearman correlation matrix (r_s) reveal the correlations between climatic and geochemical properties and SOM composition in topsoil (a) and subsoil (b).

3.4 Discussion

3.4.1 Image of overall SOC, SOM fractions and SOC pools in IGP soils

SOC content in IGP soils is low but lacks a significant controlling factor (Fig. C3-1). Given the similarity in soil properties between forest and agriculture soils (Fig. C3-2), data from both land-use types have been combined for further discussion. SOC content changes simultaneously with TN (Fig. C3-4), showing its importance in the soil ecosystem of studied regions. Subsoil SOC content appears to be influenced by active Al/Fe, clay, and exchangeable Ca/Mg (Fig. C3-4b). However, in the IGP topsoil, no conspicuous influence of climatic and geochemical attributes on total SOC was observed (Fig. C3-4a). To decipher the underlying cause of low SOC in IGP, particularly within topsoil, a deeper exploration into the nuances of SOM, including its fractions, SOC pools, and molecular composition, was applied.

The predominant proportion of MAOM in both topsoil and subsoil (Fig. C3-5b) underscores its significance in overall SOC storage. The significantly higher SOC ($13.3 \pm 6.7 \text{ g kg}^{-1}$) in topsoil (subsoil SOC $3.7 \pm 0.9 \text{ g kg}^{-1}$) arises from its greater MAOM content in topsoil ($9.5 \pm 6.1 \text{ g kg}^{-1}$) than subsoil ($2.6 \pm 0.9 \text{ g kg}^{-1}$). Compared to topsoil, the higher proportion of non-hydrolyzable MAOM in subsoil (Fig. C3-5b) points to a more abundant intensively degraded and/or directly stabilized OM (von Lützow and Kögel-Knabner, 2009; Yan et al., 2017). Because the drying-wetting process promotes the dissociation of soil aggregates (Utomo and Dexter, 2006; Xu et al., 2017), the limited oPOM in both topsoil and subsoil (Fig. C3-5a) might be influenced by clear drying-wetting cycles in IGP having a moderate MAP but predominant negative EP value at most sites (Table C3-1).

Central to my discussion, the intermediate SOC pool emerges as the predominant pool of total SOC ($78 \pm 13\%$ and $57 \pm 17\%$ of topsoil and subsoil SOC), controlling SOC decomposition over durations spanning years to decades, as its MRT suggests (Table C3-3). The stable SOC pool is resistant to decomposition and had limited contribution to cumulative CO₂ release ($1.0 \pm 1.1\%$, calculated using Eq (S1)) during the entire incubation, working as a persistent part of SOC. Thus, intermediate SOC pools controlled mid to long-term SOC turnover. Delving deeper, the larger sizes and proportions of the intermediate SOC pool in topsoil than subsoil (Fig. C3-8) indicate that topsoil SOC storage has a higher dependency on the intermediate pool. Conversely, the higher proportion of stable SOC pool in subsoil SOC stability leans more towards its stable SOC fraction than topsoil. Labile SOC pool regulates the short-term stability at a scale of days to dozen days (i.e., MRT_L, Table C3-3), contributing to $72.9 \pm 16.2\%$ of cumulative CO₂ release (Eq (S1)) and closely reflecting the characteristics of inputted organic matter (Lyu et al., 2021a; Lyu et al., 2024). The marked contrast in MRT_L between the topsoil (44 ± 21 days) and subsoil (20 ± 14 days) indicates diminished labile pool stability in subsoil, potentially driven by an influx of more bioavailable DOC from topsoil and root exudates (Sokol and Bradford, 2019).

Consistent with findings from tropical volcanic regions (Lyu et al., 2021b), the size and MRT of intermediate SOC pool show SOC storage and stabilization. Compared to volcanic regions, the significantly lower intermediate and stable SOC pool in IGP (Fig. C3-8a) might be attributed to its significantly lower active Al/Fe (Table C3-1), which controls intermediate and stable SOC pool sizes in volcanic regions (Lyu et al., 2021a; Lyu et al., 2021b). Also, a significantly lower proportion of stable pool and a higher proportion of labile pool in IGP topsoil (Fig. C3-8b) indicate

its lower stability than volcanic topsoils.

To improve soil carbon accumulation and stability, as indicated by SOC pool sizes and MRTs, the mineral association is essential in neutral to alkaline soils. The robust correlation between MAOM content and CI, which was a major part of SOM, across both horizons (Fig. C3-9) infers the pivotal role of MAOM in dictating SOC storage. In topsoil with increased MAOM levels, there was a diminished proportion of labile SOC pool but an increased size of the intermediate pool (Fig. C3-9a), underscoring the crucial role of mineral association in SOC stabilization. In subsoil, MAOM also positively contributes to mid to long-term SOC stability, as revealed by the positive correlation between MAOM and MRT_I (Fig. C3-9b). The correlation between soil mineral-associated properties, such as exchangeable Ca/Mg, clay, and active Fe, with MAOM in subsoil further underscores the significance of mineral associations there (Fig. C3-10b).

3.4.2 Controlling factors and their effects on SOC

Despite its minimal levels in neutral to alkaline soils compared to acidic ones, active Al/Fe is essential for stabilizing SOC. Increased moisture (MAP and EP) tends to decrease soil pH, promoting the formation of active Al/Fe (Figs. C3-1 and C3-4) (Lyu et al., 2018; Lyu et al., 2022). These active Al/Fe can contribute to the preservation of SOC, which is evident from the positive relationship between active Al/Fe and SOC in subsoil (Fig. C3-4b). Rather than Ca/Mg or clay, active Al/Fe cooperates with fine mineral particles to preserve SOM and enhance stability, revealed by predominant contributions of active Al/Fe to MRT_I in both topsoil and subsoil (Fig. C3-11), supporting my first hypothesis. More active Al/Fe, showing a positive correlation with fine mineral

particles, were found in soils from downstream of the river (lower IGP) (Figs. C3-1 and C3-4). The higher EP and lower pH promote the formation of active Al/Fe (Figs. C3-4 and C3-11) (Lyu et al., 2022). These active Al/Fe tend to preserve organic matter and prolong the mean residence time of SOC, possibly by making covalent bonds with organic matter on mineral surfaces, forming organic-metal complexes, and promoting aggregation (Churchman et al., 2020; Kleber et al., 2021). Among these mechanisms, acting as bonding agents and facilitating aggregation appear to be the most promising in natural to alkaline soils, as this approach would remain efficient even when the absolute value of active Al/Fe is low (Wagai et al., 2020; Zhong et al., 2023). Furthermore, the stabilizing effect of active Al/Fe on SOM was particularly pronounced in subsoil (Fig. C3-11), possibly attributed to subsoil minerals' less carbon-saturated nature and propensity for inner-sphere interactions (Kleber et al., 2007; McNally et al., 2018). The higher reactivity of soil minerals in subsoil is also revealed by the enlarged MAOM as the relatively stable SOM fraction (von Lützow et al., 2007) in soils with more active Al/Fe, clay, and exchangeable Ca/Mg in subsoil but not in topsoil horizon.

Exchangeable Ca/Mg may amplify the stability of SOC, especially in alkaline soils (Carmes Filho et al., 2017). However, due to the interference of shell carbonates in I4 and secondary carbonates in the upper IGP (Table C3-1) in gauging the accuracy of exchangeable Ca on mineral surfaces, only exchangeable Mg exhibited a positive association with SOC in subsoil. Exchangeable Ca^{2+} and Mg^{2+} potentially act as a bridge, linking the negatively charged mineral surfaces (i.e., clays) and organic matter (Fig. C3-4b), thus enhancing the retention of organic matter (Rowley et al., 2018).

Though clay and exchangeable Ca/Mg can enlarge the intermediate SOC pool, the influence of the former is more conspicuous in the subsoil (Fig. C3-11). In topsoil, exchangeable Ca/Mg can potentially increase intermediate SOC pool, but the positive contribution was not significant across all sites (Fig. C3-11a). In subsoil, given the reduced SOC-to-clay ratio (Table C3-1), more SOM appears to stabilize directly on clay surfaces, forming an intermediate SOC pool (Fig. C3-11b). In neutral and alkaline soils, Ca and Mg ions can serve as connectors, linking charged clay surfaces and OM (Kleber et al., 2021). However, the intensity of Ca/Mg bridging would be weaker than active Al/Fe, as revealed by the significant correlation between C_s and active Al/Fe, rather than exchangeable Ca/Mg (Figs. C3-10 and C3-11b). Additionally, high EP would promote carbon leaching and clay movement from topsoil to subsoil, resulting in a strong positive contribution of EP on the size of the stable pool in subsoil (Fig. C3-11b).

Meanwhile, high pH enhances the enzyme activities (Fujii et al., 2020; Puissant et al., 2019) to decompose litter residues and also reduces Al/Fe activities that stabilize OM (Candra et al., 2019; Parfitt and Kimble, 1989). As a result, the stability of intermediate SOC pool (i.e., MRT_I) was reduced in high pH soils of subsoil horizon (Figs. C3-10 and C3-11b) and less LF and oPOM remained in high pH soils of topsoil horizon (Fig. C3-10). Interestingly, these less decomposed fractions (LF and oPOM) seem to enlarge soil CEC more potently than its stable counterpart in topsoil (Figs. C3-4 and C3-10). Contrarily, subsoil soil minerals (e.g., clay) are more reactive, enlarging MAOM and controlling soil CEC (Figs. C3-4 and C3-10).

Regarding agricultural soils, a reduced quantity of input, coupled with disturbances, curtail the less decomposed residues of LF but not the more stable fractions in both layers (Fig. C3-10).

The residues from woody plants (i.e., forest) have a higher resistance to microbial utilization than those from herbs (e.g., crops) (Mack et al., 2001). The agriculture activity may reduce the stability of the labile SOC pool by changing the residue input and increasing disturbance, but it has limited influence on more stable SOC pools, as no effects were found on CI and CS (Figs. C3-10 and C3-11), partially refuting my third hypothesis. Alternatively, these minimal differences in SOC between agricultural and forest soils could also suggest a significant human influence on the forest sites.

3.4.3 SOM Molecular Composition

SOM in both agriculture and forest sites in IGP are highly degraded, evident from a predominant proportion of N-containing compounds and limited detection of less degraded compounds like undegraded PS (Fig. C3-13 and Table C3-4). N-containing group was the predominant SOM component, followed by MAH and degraded PS, indicating the advanced degradation of SOM (Buurman and Roscoe, 2011; Lu et al., 2018; Vancampenhout et al., 2009). The significantly higher proportion of N-containing in IGP, especially when compared to tropical volcanic regions (Lyu et al., 2024), known for much higher SOM degradation than subtropical or temperature regions (Girona-García et al., 2019; Thomas et al., 2021), infers the extreme SOM degradation in IGP. This SOM degradation can be attributed to the elevated pH in IGP, which promotes SOM decomposition and limits the presence of active Al/Fe, a known SOM stabilizer, in neutral-to-alkaline soils.

Moreover, the absence of undegraded PS (e.g., cellulose) reveals the intensive microbial

degradation of SOM in the study sites. While lignin is generally considered resilient to decomposition (Marschner et al., 2008), its intensive degradation is observed in tropical soils (Vancampenhout et al., 2009) and is evidenced by its limited proportion in studied IGP soils (Fig. C3-13 and Table C3-5). Moreover, possibly due to drier conditions in IGP, lignin degradation appears weaker than in tropical volcanic regions, as suggested by a higher lignin proportion in the IGP ($1.2 \pm 0.6\%$) than in tropical volcanic topsoil ($0.7 \pm 0.7\%$; Lyu et al., 2024). In these soils with high SOM degradation, PAH was significantly lower than MAH in both topsoil and subsoil (Table C3-5). Consistent with prior studies in tropical regions (Lyu et al., 2024), the scarce presence of PAHs infers limited aromatic polymerization in tropical regions, challenging certain aspects of conventional humification concepts (Lehmann and Kleber, 2015).

In terms of influencing SOM composition, active Al/Fe stands out, while clay and exchangeable Ca/Mg play a minor role. This impact of active Al/Fe can be observed in stabilizing less-degraded and less-recalcitrant OM, such as fatty acids and saccharides, and reducing N-containing compounds (Figs. C3-15 and C3-16). The importance of clay content in enlarging weakly degraded OM seems marginal (not significant, Fig. C3-15a), with only a positive correlation with MAH in topsoil (Fig. C3-16). Similarly, exchangeable Ca/Mg does not substantially influence the overall SOM composition (Fig. C3-15a). On the contrary, the impact of active Al/Fe on SOM composition is significant (Fig. C3-15a). The creation of organo-Al/Fe complexes is attributed to the abundance of less-resistant OM, like long-chain fatty acids (FAnE and long alkane, Figs. C3-15a and C3-16). These OM compounds are probably stabilized as the intermediate decomposable SOC pool (see 4.2). This stabilization arises from organo-mineral

interactions and aggregation, primarily regulated by active Al/Fe (Rasmussen et al., 2018; Takahashi and Dahlgren, 2016; Wagai et al., 2015). The biochemical stability of fatty acids and saccharides, which are easily decomposed, may be further strengthened via hydrogen bonding with active Al/Fe (Fig. C3-15a) (Kleber et al., 2021). Thus, a higher moisture environment with a reduced pH that supports the formation of active Al/Fe facilitates the stabilization of less-recalcitrant OM. In contrast, in conditions with reduced active Al/Fe, as seen in higher IGP regions with higher pH, more degraded compounds persist (e.g., N-containing compounds, Fig. C3-15a). Additionally, high pH (Table C3-1) promotes activities of extracellular enzymes, like acetylsterase, leading to enhanced SOM degradation (Puissant et al., 2019), resulting in a surge of N-containing compounds as degradation residues (Fig. C3-15a), also supporting my first hypothesis.

Subsoil SOM in IGP is more degraded than topsoil. The SOM composition of subsoil was notably different from that of topsoil (Figs. C3-14 and C3-15, Table C3-5), characterized by elevated levels of N-containing compounds and MAH, which are results of microbial activity and reduced less degraded compounds like lignin (Buurman and Roscoe, 2011; Carr et al., 2013; Vancampenhout et al., 2009). Furthermore, RDA1 indicates a higher proportion of less-degraded compounds in SOC-rich topsoil (Fig. C3-15, Table C3-5). These results indicate a more intensive degradation in subsoil, consisting of the second hypothesis. High soil pH and active microbial processing in IGP increase OM solubility and mobility, driving the downward movement of SOM from topsoil to subsoil (Curtin et al., 2016). Consequently, subsoil SOM is more degraded, containing a larger proportion of microbial derivatives, a trend also influenced by reduced fresh

litter input than topsoil. The absence of phenols, primary degradation products of lignin and tannin (Leinweber and Schulten, 1999), as well as the significantly lower degraded PS than tropical volcanic subsoil further attest to the degraded nature of IGP subsoil (Table C3-5). The limited lignin and phenol in subsoil might also result from the prevalence of microbes like Actinomycetes, Chloroflexi, and lignin-digesting fungi such as Ascomycetes, which survive under carbon-scarce conditions and breakdown recalcitrant compounds (Abdel-Hamid et al., 2013; Fierer et al., 2007).

Although agricultural activities can accelerate SOM degradation (John et al., 2005; Mganga and Kuzyakov, 2018), their effect seems minimal in the IGP, as indicated by similar SOM compositions in agricultural and forest soils. Indicators of weakly degraded saccharides and lipids, such as degraded PS and long alkanes (Carr et al., 2013; Vancampenhout et al., 2012; Vancampenhout et al., 2009), were significantly lower in agriculture than in forest soils (Table C3-4), indicating that agricultural activities promote the degradation of weakly degraded plant-derived materials. Nevertheless, this influence was not substantial as the overall SOM composition and SOC-related properties remain largely consistent between agriculture and forest soils (Figs. C3-6 and C3-12), with land use imparting an insignificant effect on SOM composition (Fig. C3-15a). Conversely, the similarity of SOM composition between agricultural and forest soils may also imply substantial human impact on forest soils.

3.5 Summary

In neutral-to-alkaline IGP soils, the intermediate SOC pool, which has high affinity with MAOM, was the majority of SOC rather than the stable SOC pool and controls SOC stability at a

scale of decades. Active Al/Fe, rather than clay content and exchangeable Ca/Mg, predominantly controls the stability of this intermediate SOC pool in both topsoil and subsoil. In the less carbon-saturated subsoil, this carbon preservation effect of active Al/Fe was stronger, as active Al/Fe mainly determines the size of the stable SOC pool, with clay content influencing the size of intermediate SOC pool in subsoil.

The degradation of SOM in these soils was very intensive, which may stem from enhanced microbial activity and the notably low active Al/Fe levels, partially due to high soil pH. As it moves from topsoil to subsoil, both the proportion of the stable SOC pool and SOM degradation increase. Higher precipitation lowers soil pH, enhancing active Al/Fe, slowing SOM decomposition, and potentially promoting OM translocation from topsoil to subsoil. Active Al/Fe helps retain long-chain fatty acids and weakly degraded polysaccharides, which are highly bioactive. In contrast, while exchangeable Ca/Mg and clay content positively affect the size of the intermediate SOC pool in topsoil and subsoil, they do not significantly influence overall SOM composition. Agricultural activity diminishes LF and accelerates SOM degradation, yet its effects on the majority of SOC (C_1 and C_s) and general SOM composition can be minimal. Nonetheless, the similarity between agricultural and forest soils could also suggest a concealed, significant human influence extending even to forest soils. Across all sites, the total precipitation in the rainy season from July to September is higher than 640 mm, with the lowest monthly precipitation in Kanpur (I2) in September (140 mm).

CHAPTER 4 CONTRIBUTIONS OF FINE MINERAL PARTICLES AND ACTIVE Al/Fe TO STABILISATION OF PLANT MATERIAL

4.1 General

Soil organic carbon (SOC) is the largest carbon pool in terrestrial ecosystems (Jobbagy and Jackson, 2000) and plays a key role in controlling carbon cycling, soil health, and agricultural production. Severe depletion of the SOC pool degrades several ecosystem services, such as climate regulation, food/fiber/forage production, and water quality (Lal, 2018). SOC depletion may be further exacerbated by global warming, thereby creating a positive feedback loop of diminishing SOC. Moreover, land-use change from native to agricultural ecosystems can lead to a pronounced decrease in the SOC pool by up to 60% in temperate soils and 75% or more in tropical soils (Lal, 2010). Many tropical soils are deficient in inorganic nutrients, hence maintaining soil fertility relies critically on the recycling of nutrients through the soil organic matter (SOM) (Lu et al., 2021; Tiessen et al., 1994). Consequently, soil organic carbon conservation and restoration are especially crucial to sustainable land use in the tropics.

The magnitude of the SOC pool depends on C inputs to the soil by primary production and C outputs by decomposition (Zhang et al., 2018). Stabilization of SOC links the C input and output, thereby regulating SOC storage. Various soil properties are recognized as controlling factors for organic matter mineralization and stabilization. For example, soil pH influences the size, composition, and activity of the microbial community, thereby affecting organic matter mineralization (Cao et al., 2016; Zhou et al., 2019). The pH is also directly associated with the composition of exchangeable cations, especially Ca^{2+} and Mg^{2+} , which stabilize SOC (Lyu et al.,

2022; Neina, 2019) via bridging negatively charged mineral surfaces and organic matter (Rowley et al., 2018). The fine-mineral fraction, such as clay, further preserves organic matter by direct (organo-mineral complexes) and indirect (occlusion by aggregation) mechanisms (Rodrigues et al., 2022a). Active Al/Fe, defined here as the acid-oxalate extractable fraction and composed of poorly crystalline Al/Fe minerals and organo-Al/Fe complexes, was proposed as the most important soil component for SOC preservation in various soil types, especially acidic soils (Ashida et al., 2021; Rasmussen et al., 2018). The effect of active Al/Fe on carbon stabilization in non-volcanic and neutral-to-alkaline soils is suggested (Rasmussen et al. 2018; Ashida et al. 2021), but its roles are still unclear as their content is generally low (Ashida et al., 2021).

Topsoil and subsoil horizons have large differences in SOC pool size, as well as SOC saturation status (Dai et al., 2022; Lyu et al., 2021b; Possinger et al., 2021). The SOC saturation status, controlled by the relative abundance of soil minerals versus SOC, may regulate SOC stabilization processes and the potential SOC sequestration capacity (Rodrigues et al., 2022b; Yu et al., 2017). Soils with a low level of SOC saturation could store a considerable amount of additional SOC, particularly in subsoil horizons of cropland soils (Vågen et al., 2005). However, there is limited research on the influence of contrasting SOC saturation conditions in topsoil and subsoil on the mineralization and stabilization of exogenous organic matter. Thus, clarifying the effects of SOC saturation conditions will enhance our ability to manage SOC pools in topsoil and subsoil.

Most studies investigating the influence of exogenous organic matter on SOC pools have either examined organic matter mineralization alone (Rakhsh et al., 2017; Shahbaz et al., 2017;

Zhao et al., 2017) or the remaining C in SOM fractions (Rodrigues et al., 2022a; Rodrigues et al., 2022b; Tokarski et al., 2020). Tracing the C in plant residue using ^{13}C -labeled material is a powerful approach for assessing the mineralization of exogenous organic matter (Chen et al., 2019; Zhang et al., 2018). Moreover, SOM fractionation after the incubation of ^{13}C plant material added to soils helps clarify the SOC stabilization mechanisms. Furthermore, the transformation of organic matter (OM) molecular composition is rarely examined in soil incubation studies, which is essential in assessing SOC mineralization and stabilization mechanisms. Analytical pyrolysis is a valuable technique for characterizing the molecular composition of bulk SOC, including the identification of biomarkers (Derenne and Quénée, 2015; Picó and Barceló, 2020). A comparative study at the molecular level using analytical pyrolysis in incubated soils with and without plant-residue addition is warranted to gain a comprehensive understanding of the stabilization mechanisms of exogenous OM in soils.

This chapter aims to clarify the roles of soil physicochemical properties (e.g., pH, clay, active Al/Fe and C saturation status) that control the mineralization and stabilization of newly added plant material to neutral-to-alkaline soils of the IGP; and evaluate the influence of plant material additions on SOM molecular composition by comparing incubated soils with and without maize-addition. I conducted a 1-year soil incubation with and without addition of ^{13}C -labeled maize using topsoil and subsoil from the upper-to-lower IGP. The objectives were achieved by tracing the maize-derived ^{13}C in respired CO_2 and SOM fractions of the incubated soils. Analytical pyrolysis was conducted at the completion of the incubation to evaluate the influence of maize addition at the molecular level. I anticipated that the wide range of soil properties and SOC saturation

conditions in the IGP soils would allow us to elucidate the determinant factors affecting the mineralization and stabilization of soil organic matter.

4.2 Materials and Methods

4.2.1 Indices for Estimating SOC Saturation Degree

Fine mineral particles (clay + silt, <20 µm) and active Al_o and Fe_o are posited to contribute to SOC stabilization. As an index of SOC saturation, the soil C deficit can be calculated based on SOC and clay + silt contents (Rodrigues et al., 2022a; Stewart et al., 2008), with the protective capacity of clay and silt based on the formula developed by (Stewart et al., 2008):

$$C\ deficit = 1 - (SOC/protective\ capacity) \quad (1)$$

$$Protective\ capacity_{clay+silt} (g\ kg^{-1}) = 0.29 \times (clay + silt\ content) + 6.9 \quad (2)$$

Similarly, the SOC to active Al_o and Fe_o ratio has a reported maximum value of ~30 mol C mol⁻¹ Al_o + Fe_o for non-volcanic soils (Ashida et al., 2021). Thus, the protective capacity of active Al_o and Fe_o was calculated as:

$$Protective\ capacity_{Al_o + Fe_o} (cmol) = 30 \times (Al_o + Fe_o) \quad (3)$$

Furthermore, we used the SOC/(clay + silt) (g g⁻¹) and SOC/(Al_o + Fe_o) (mol mol⁻¹) ratios as simple metrics to estimate the degree of SOC saturation.

4.2.2 Long-term Soil Incubation with Maize Addition

I incubated soils with and without the addition of ¹³C-labeled plant material (12 sites, 2 horizons, 3 replicates, and 2 treatments) to investigate the mineralization and stabilization of

organic matter. Firstly, ^{13}C -labeled maize (*Zea mays L.*) (35% ^{13}C) was grown in a $^{13}\text{CO}_2$ -rich atmosphere, harvested about one month after germination, and then dried at 70 °C. Maize leaf and stem tissues were ball-milled at 450 rpm for 5 min (PM100, Retsch) (<1 mm) for the incubation experiment. The ground maize (TC: $340 \pm 18 \text{ mg C kg}^{-1}$, TN: $85 \pm 14 \text{ mg N kg}^{-1}$) was uniformly mixed into soil samples at a rate of 420 mg C kg^{-1} soil, which is roughly equal to 420 kg C ha^{-1} (assuming a 10-cm depth and bulk density of 1 g cm^{-3}). This amendment amount is similar to the input of post-harvest residues in croplands within the study area (Sukhoveeva, 2022). Twenty grams of each soil sample were prepared with three replications and placed in a sealed glass jar (225 mL, 7-cm diameter). The soil moisture content was adjusted to 55% of field capacity by adding deionized H_2O . Soil samples were pre-incubated at 25 °C for 7 days prior to the addition of ^{13}C -labeled maize and the start of CO_2 collection (Lyu et al., 2021a; Ma et al., 2021).

The CO_2 released from soils was trapped in a plastic bottle with 10 mL 1 mol L^{-1} NaOH trapping solution placed within the incubation jar. Another glass bottle with 0.01 M HCl solution was also placed in the incubation jar to maintain the soil moisture content. Soils were incubated for 364 days and the CO_2 -trapping solution was collected at 7, 14, 28, 49, 70, 91, 112, 140, 168, 196, 224, 280, 336 and 364 days for CO_2 quantification. Half (5 mL) of the CO_2 -trapping solution was used for determining total CO_2 emission via titration with 0.1 M HCl. The remaining 5 mL of CO_2 -trapping solution was used for determining the ^{13}C ratio of the respired CO_2 after treating with SrCl_2 to precipitate CO_3^{2-} as SrCO_3 (Ma et al., 2021). The ^{13}C ratio of the added maize, the soil sample after incubation, and precipitated SrCO_3 were measured with EA-IRMS (EA-IsoLink and DELTA V Advantage, Thermo Fisher Scientific). Replicated standard samples (glucose) were

determined at 12-sample intervals to ensure data quality, and the analytical precision for $\delta^{13}\text{C}$ was $\pm 0.06\text{‰}$.

The contribution of ^{13}C -labeled maize to total respiration ($\text{Pres}_{\text{M-derived}}$) was estimated as follows:

$$\text{Pres}_{\text{M-derived}} = (\text{atom}\%^{13}\text{C}_{\text{Mafter}} - \text{atom}\%^{13}\text{C}_{\text{Mbefore}}) / (\text{atom}\%^{13}\text{C}_{\text{M}} - \text{atom}\%^{13}\text{C}_{\text{Mbefore}}) \quad (4)$$

where $\text{atom}\%^{13}\text{C}_{\text{Mafter}}$, $\text{atom}\%^{13}\text{C}_{\text{Mbefore}}$ and $\text{atom}\%^{13}\text{C}_{\text{M}}$ are the ^{13}C atom% of the respired C after maize addition, respired C before maize addition (assumed as ^{13}C atom% of the original soil) and the C of the maize, respectively.

To determine the mineralization rate at an arbitrary date, the mineralization of added maize was fitted to a modified two-pool, first-order kinetic model:

$$C_r = 1 - (a \times e^{-k_a \times t} + b \times e^{-k_b \times t}) \quad (5)$$

where C_r is the ratio of cumulative mineralized carbon to total added carbon amount at time t (days), a and b are the portion of decomposable C pools ($a + b = 1$) in maize, and k_a and k_b are the rate constants of the C pools. The mineralization rate at each sampling date was calculated by the derivative: $C_r' = a \times k_a \times e^{-k_a \times t} + b \times k_b \times e^{-k_b \times t}$.

4.2.3 Soil Organic Matter Fractionation

Soil organic matter fractionation for soils after incubation was conducted based on previous protocols (Kadono et al., 2008; Lyu et al., 2021a; Paul et al., 2001). Briefly, SOM was fractionated into light (LF) and heavy (HF) fractions using NaI (1.6 g cm^{-3}) density separation. The HF was

subsequently dispersed by adding sodium hexametaphosphate and shaking for 15 h for particle size separation: $\geq 53 \mu\text{m}$ (occluded particulate organic matter, oPOM) and $< 53 \mu\text{m}$ (mineral-associated organic matter, MAOM). Furthermore, the MAOM was collected to determine the non-hydrolyzable SOM fraction by refluxing with 6 M HCl at 115 °C for 16 h. After measuring the weight of each fraction, total C content was determined by dry combustion.

4.2.4 One-shot Py-GC/MS Analysis

After incubation, pyrolysis–gas chromatography–mass spectrometry (Py-GC/MS) was applied to all control and maize-treated soils to evaluate the residual status of added maize after incubation and assess how soil organic compounds were affected by maize addition. Soil samples and maize plant material without ^{13}C labeling were separately ground to a fine powder and homogenized by a ball mill at 450 rpm for 5 min (PM100, Retsch), then dried at 70 °C for 24 h. Analytical pyrolysis was conducted using a multi-functional pyrolyzer (EGA/PY3030D, Frontier Laboratories) coupled with GC/MS (GCMS-QP2020, Shimazu). Same pyrolysis conditions were applied as 3.2.5 in Chapter 3. The 100 highest peaks for each sample were manually adjusted to reduce baseline noise and interference from nearby peaks, then matched with the NIST library.

Compounds matched with the reference library were categorized into 15 groups, as listed in Table S1. Peak area calculation method was same as 3.2.5. A group of exclusive compounds for maize was identified by comparing pyrolysis products of non-labeled maize and control soils. Unique compounds exclusive for maize had a relative abundance of $>0.5\%$ of the total identified chromatograph peak areas of the maize material and were not detected in control soil samples. The

residual status of maize was evaluated by searching for these compounds in maize-treated soils after incubation.

4.2.5 Statistical Analyses

Spearman rank correlation coefficients were applied to assess relationships among climate data, soil properties, SOC saturation degree, and organic matter-related properties measured after incubation and SOC fractionation. Bray-Curtis distance-based PERMANOVA was applied to show the difference in SOM molecular composition between control and maize-treated soils. NMDS analysis was conducted using the “vegan” and “microeco” packages in R software (Liu et al., 2021; Oksanen et al., 2020). A two-tailed paired t-test was applied to assess differences between land uses (forest vs. agriculture) and between horizons (topsoil vs. subsoils). Linear regression was applied to determine numerical relationships between soil properties and the residual C derived from the added maize. PLS-PM was conducted to evaluate the effect of soil properties on stabilization of added maize using “SEMinR” package in R (Hair et al., 2021). Structural model was made based on Spearman correlation matrix. Variance inflation factor (VIF) was used to detect the severity of multicollinearity for factor selection. Bootstrapping PLS-PM was used to provide the significance of path coefficients (β) with confidence intervals. Statistical analyses were performed using R 4.1.1 and IBM SPSS ver. 24.0; significance levels are designated as *, **, and *** for $P < 0.05$, 0.01 and 0.001, respectively.

4.3 Results

4.3.1 Soil Properties and SOC Saturation Indices

Indices of SOC saturation degree and soil properties are shown in Table C4-1. Significant differences between forest and agriculture soils were only found in Fe_d and $\text{C-deficit}_{\text{Al}_o + \text{Fe}_o}$, which were lower in the forest versus agriculture soils (Tables C3-2 and C4-2). Section 3.3.1 demonstrates a gradual increase in active Al/Fe and clay and silt contents from the upper to lower IGP, accompanied by a decrease in pH (also shown in Table C4-1 and Fig. C4-1). $\text{C-deficit}_{\text{clay} + \text{silt}}$ displayed a negative correlation with $\text{SOC}/(\text{clay} + \text{silt})$ ($r_s > -0.99^{***}$), as did $\text{C-deficit}_{\text{Al}_o + \text{Fe}_o}$ and $\text{SOC}/(\text{Al}_o + \text{Fe}_o)$ ($r_s > -0.99^{***}$, Fig. C4-1). $\text{SOC}/(\text{clay} + \text{silt})$ and $\text{SOC}/(\text{Al}_o + \text{Fe}_o)$ were significantly higher, and $\text{C-deficit}_{\text{clay} + \text{silt}}$ and $\text{C-deficit}_{\text{Al}_o + \text{Fe}_o}$ were significantly lower in topsoil than subsoil horizons (Table C4-2). As C deficits (i.e., $\text{C-deficit}_{\text{clay} + \text{silt}}$ and $\text{C-deficit}_{\text{Al}_o + \text{Fe}_o}$) and carbon saturation indices (i.e., $\text{SOC}/(\text{clay} + \text{silt})$ and $\text{SOC}/(\text{Al}_o + \text{Fe}_o)$) displayed identical trends, I applied $\text{SOC}/(\text{clay} + \text{silt})$ and $\text{SOC}/(\text{Al}_o + \text{Fe}_o)$ to simplify the subsequent discussion.

Table C4-1 Soil physicochemical properties and SOC saturation indices

ID	pH	SOC	TN	IC	Clay	Clay + silt	CEC	Fe _o	Al _o	Fe _d	SOC/ (clay + silt)	SOC/ (Al _o + Fe _o)	C deficit clay + silt	C deficit Al _o + Fe _o
		g kg ⁻¹			%	%	cmol _c kg ⁻¹				g g ⁻¹	mol mol ⁻¹		
I1FT ^c	7.5	16.4	1.1	0.4	10	49	9.4	1.3	1.2	11.5	0.033	54.7	0.22	-0.82
I2FT	7.8	12.0	1.6	5.1	16	76	8.9	1.6	1.1	9.1	0.016	38.0	0.59	-0.27
I3FT	7.8	6.7	0.5	1.9	17	85	10.1	2.9	1.4	17.1	0.008	12.8	0.79	0.57
I4FT	8.8	28.9	1.3	18.4	21	92	8.0	2.2	1.7	8.8	0.031	60.9	0.14	-1.03
I5FT	6.4	15.9	1.3	0.3	20	81	9.2	7.0	5.1	13.8	0.020	10.9	0.48	0.64
I6FT	7.1	19.3	2.7	3.2	59	100	21.1	9.9	3.5	20.1	0.019	12.1	0.46	0.60
I1FS	8.5	3.3	0.2	0.0	15	45	5.8	2.1	1.3	17.9	0.007	8.3	0.83	0.72
I2FS	8.3	3.6	0.3	0.0	22	80	11.0	1.4	1.5	12.1	0.004	10.2	0.88	0.66
I3FS	7.7	3.6	0.2	0.0	21	85	11.9	3.8	1.7	24.4	0.004	5.4	0.89	0.82
I4FS	9.2	3.4	0.2	3.8	21	83	7.3	0.4	1.5	5.7	0.004	14.5	0.89	0.52
I5FS	6.0	3.3	0.2	0.0	15	86	4.9	4.5	3.4	15.4	0.004	3.4	0.90	0.89
I6FS	8.0	4.4	0.3	0.0	35	99	12.3	4.9	1.9	15.1	0.004	5.4	0.88	0.82
I1AT	8.2	12.6	0.9	0.2	15	68	6.6	1.4	1.7	11.2	0.019	33.4	0.52	-0.11
I2AT	9.9	4.7	0.4	0.1	13	62	5.7	1.3	1.0	8.3	0.008	16.9	0.81	0.44
I3AT	7.9	7.8	0.6	0.0	30	95	17.1	7.7	3.0	28.6	0.008	6.0	0.78	0.80
I4AT	8.6	16.4	0.9	16.6	26	87	7.1	2.0	1.6	10.0	0.019	38.2	0.49	-0.27
I5AT	6.4	11.1	1.0	0.1	19	80	8.2	4.5	3.5	14.8	0.014	11.4	0.63	0.62
I6AT	6.2	10.1	0.8	0.0	31	97	9.7	5.4	2.2	18.6	0.010	11.1	0.71	0.63
I1AS	8.5	3.6	0.3	0.0	26	81	9.3	2.4	2.0	24.9	0.004	6.7	0.88	0.78
I2AS	8.7	2.7	0.2	0.0	22	63	10.0	1.1	1.6	11.5	0.004	8.0	0.89	0.73
I3AS	8.2	5.7	0.4	0.0	33	98	23.3	6.5	3.1	40.8	0.006	4.9	0.84	0.84
I4AS	9.0	3.1	0.3	0.2	25	78	9.2	0.5	1.8	11.3	0.004	11.3	0.90	0.62
I5AS	8.0	2.8	0.1	0.0	13	90	4.7	3.4	2.2	19.2	0.003	4.3	0.91	0.86
I6AS	5.7	5.2	0.8	0.0	33	98	12.6	5.2	2.7	23.5	0.005	5.4	0.85	0.82

SOC, soil organic carbon; TN, total nitrogen; IC, inorganic carbon; CEC, cation exchange capacity; Fe_o and Al_o, active Fe and Al extracted by acid oxalate solution; Fe_d, dithionite-citrate extractable iron.

C deficit_{clay + silt}, C deficit indices calculated based on SOC and clay + silt contents; C deficit_{Al_o + Fe_o}, C deficit indices based on SOC and active Al and Fe contents

FT, FS, AT, AS, forest topsoil, forest subsoil, agriculture topsoil, and agriculture subsoil, respectively.

Table C4-2 SOC saturation indices and selected incubation results for topsoil and subsoil (mean $\pm$ standard deviation) and their statistical difference between topsoil and subsoil by paired t-test.

	unit	Topsoil	Subsoil	<i>P</i> value
SOC/(clay + silt)	g g ⁻¹	0.017 $\pm$ 0.009	0.005 $\pm$ 0.001	0.001
SOC/(Al _o + Fe _o)	mol mol ⁻¹	25.5 $\pm$ 18.8	7.3 $\pm$ 3.3	0.004
C deficit _{clay + silt}	%	56 $\pm$ 22	87 $\pm$ 5	0.001
C deficit _{Al_o + Fe_o}	%	16 $\pm$ 74	74 $\pm$ 11	0.004
M_Maize	mg C kg ⁻¹	215 $\pm$ 24	186 $\pm$ 29	0.003
M_Soil	mg C kg ⁻¹	1048 $\pm$ 366	390 $\pm$ 178	<0.001
M_Total	mg C kg ⁻¹	1263 $\pm$ 369	576 $\pm$ 190	<0.001
R_Maize	mg C kg ⁻¹	143 $\pm$ 28	160 $\pm$ 40	0.073
LF_M	mg C kg ⁻¹	24 $\pm$ 33	24 $\pm$ 18	0.985
HF_M	mg C kg ⁻¹	116 $\pm$ 28	136 $\pm$ 15	0.033
oPOM_M	mg C kg ⁻¹	11 $\pm$ 10	13 $\pm$ 8	0.094
MAOM_M	mg C kg ⁻¹	101 $\pm$ 20	104 $\pm$ 14	0.661

M_Maize, mineralized maize-derived C; M_Soil, mineralized original SOC; M_Total, sum of mineralized maize-derived C and SOC; R_Maize, residual maize-derived C in soil; LF_M, maize-derived C in light fraction; HF_M, maize-derived C in heavy fraction; oPOM_M, maize-derived C as occluded particulate organic matter in aggregates; MAOM_M, maize-derived C as mineral-associated organic matter.

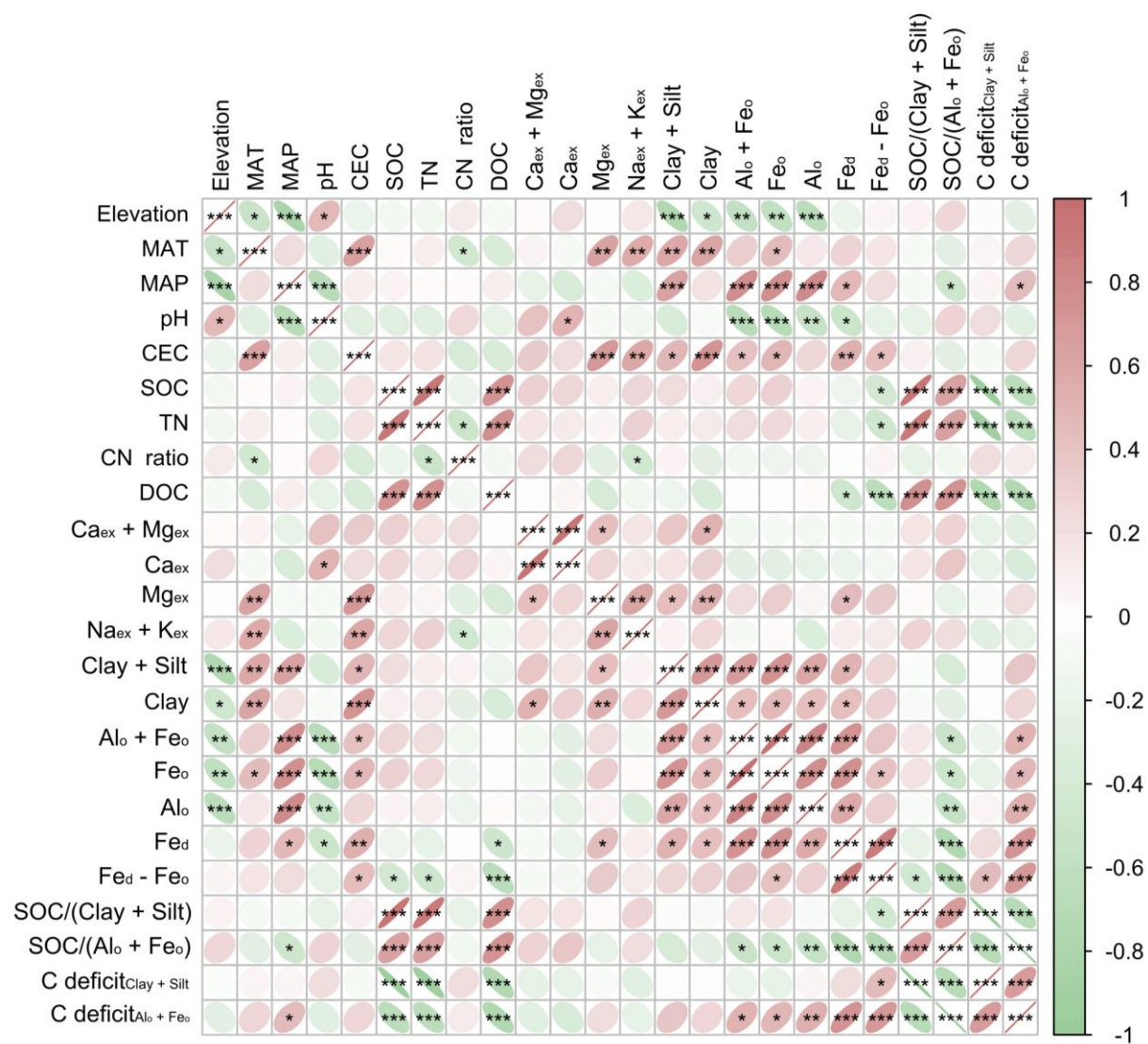


Fig. C4-1 Spearman correlation matrix (r_s) of environmental factors and soil properties. MAT, mean annual temperature; MAP, mean annual precipitation.

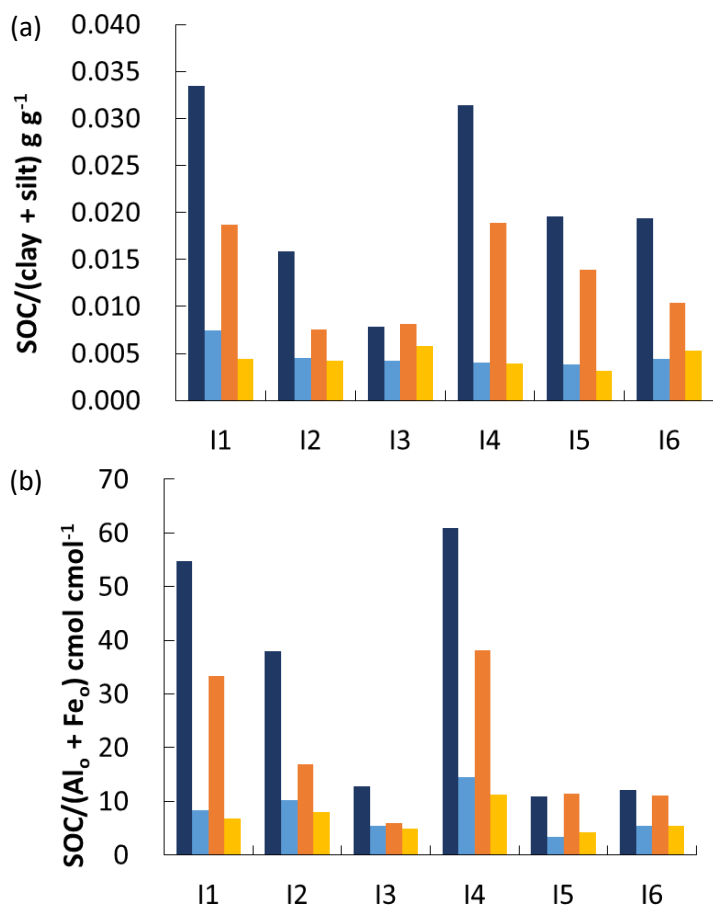


Fig. C4-2 Soil organic carbon (SOC) content and SOC saturation degree of studied soils

4.3.2 Long-term Soil Incubations

Mineralization of the added ^{13}C -labeled maize predominantly occurred during the first 70 days and dropped to very low levels by day 336 (Fig. C4-3), whereas mineralization of the SOC continued throughout the entire incubation period. The recovery of ^{13}C from the added maize (i.e., CO_2 trapped in alkaline solution + remaining in soil) was $84\% \pm 7\%$. The mean mineralized C from maize was 215 mg C kg^{-1} for topsoils and 186 mg C kg^{-1} for subsoils, corresponding to $51\% \pm 6\%$ and $44\% \pm 7\%$ of the added maize in topsoil and subsoil horizons, respectively.

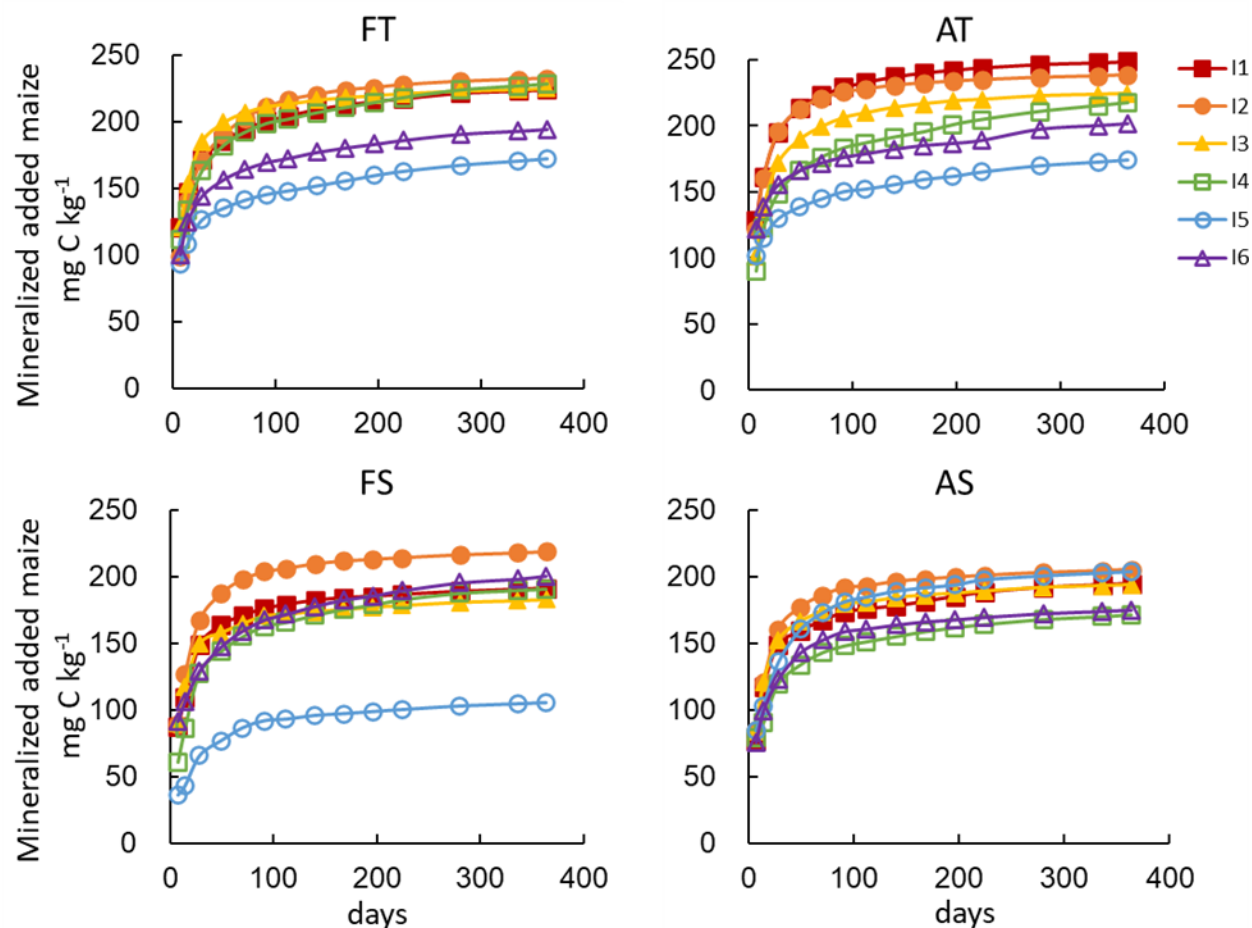


Fig. C4-3 Cumulative mineralized maize-derived C.

I1 and I2 in the upper, I3 and I4 in the middle, and I5 and I6 in the lower agro-climatic zone of IGP.

Mineralization rates of the added maize slowed as the incubation proceeded (Fig. C4-3) and were most rapid in the first 28 days. Mineralization rates of the added maize on the 7th day (0.13 to 0.52 mg C day⁻¹ sample⁻¹) were significantly correlated with SOC ($r_s = 0.50^*$) and Al_o ($r_s = -0.53^{**}$), and the mineralization rates on the 14th and 28th days (0.01 to 0.52 mg C day⁻¹ sample⁻¹) were negatively correlated with Al_o ($r_s = -0.64^{***}$ and -0.50^*) and Fe_o ($r_s = -0.43^*$ and -0.49^*), and positively correlated with pH ($r_s = 0.45^*$ and 0.52^{**}) (Fig. C4-4 and C4-5). Mineralization rates in the later stages (after 28th days) gradually decreased till the end of the incubation

(decreased from 0.01 to less than 8×10^{-3} mg C day⁻¹ sample⁻¹). Mineralization rates after 70th days were positively correlated with SOC, DOC, and SOC/(Al_o + Fe_o), and negatively correlated with Fe_d – Fe_o. For instance, the correlation coefficients for the mineralization rate versus SOC/(Al_o + Fe_o) at the 91st and 364th days were 0.63*** and 0.62**, respectively (Fig. C4-6). After the 91st day, the mineralization of added maize was very low ($<8 \times 10^{-3}$ mg C day⁻¹ sample⁻¹), especially for subsoil horizons ($3.1 \pm 1.3 \times 10^{-3}$ mg C day⁻¹ sample⁻¹) (Fig. C4-6).

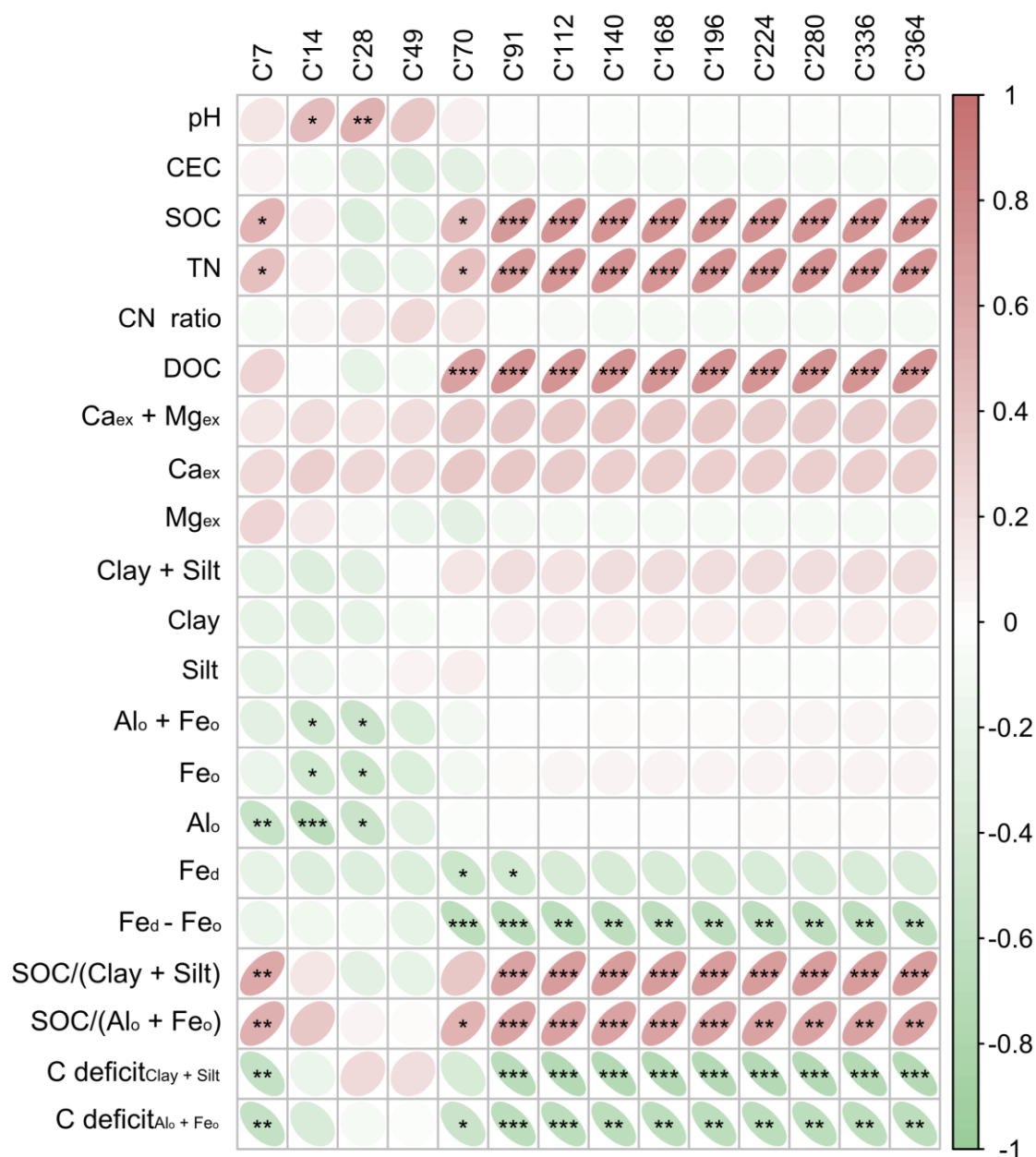


Fig. C4-4 Spearman correlation matrix (r_s) between soil physicochemical properties and mineralization rate of added maize on different days.

C'7 to C'364, the respiration rate of each sampling date. The green and red colored symbols represent the correlation coefficient of each paired property, referring to the bar on the right-hand side. The shape of colored symbols changes from a square circle to a straight line: a square circle of the colored symbols indicates a correlation coefficient of 0, and a straight line indicates a correlation coefficient of 1 or -1.

*, **, and *** indicate significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

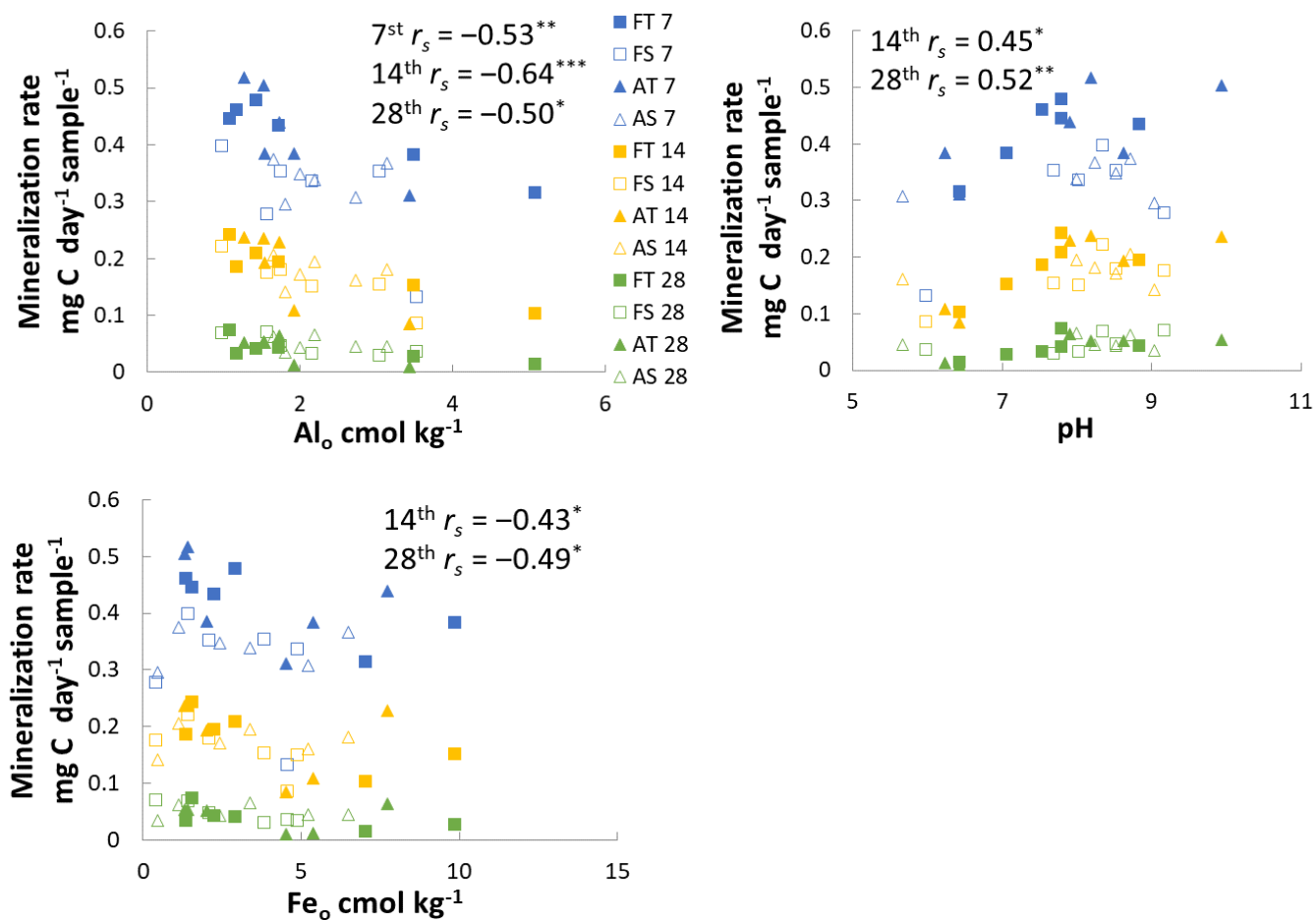


Fig. C4-5 Spearman correlation matrix (r_s) between soil physicochemical properties and mineralization rate of added maize on different days.

7, 14, 28, day 7, 14 and 28 of incubation, respectively. Only correlation coefficients of significant correlations were shown.

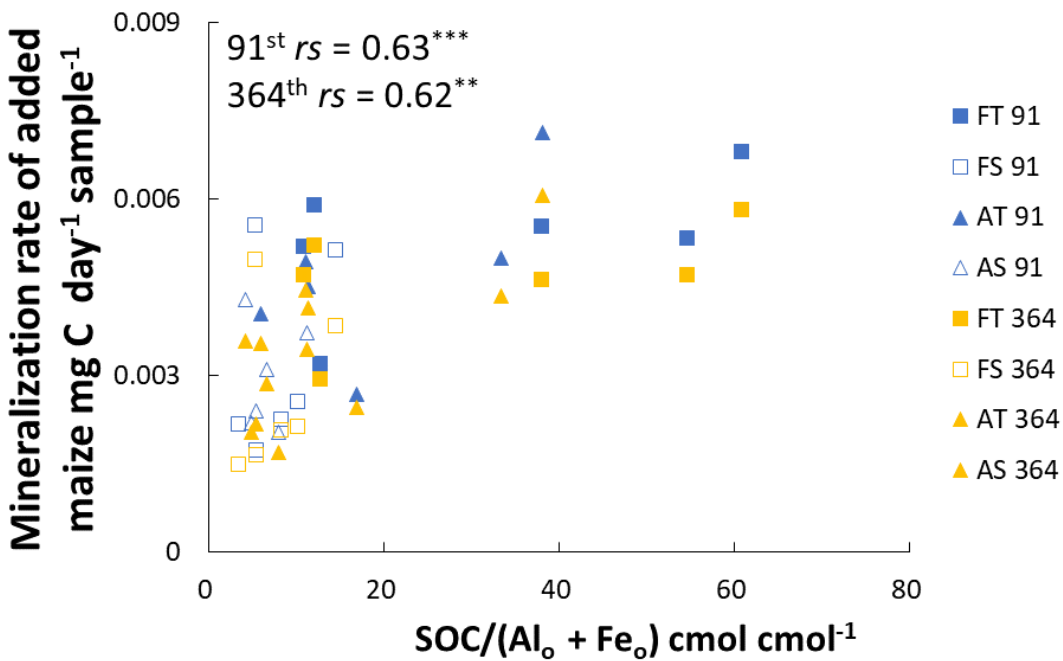


Fig. C4-6 Spearman correlation between mineralization rate of added maize material on days 91 and 364 of the incubation and carbon saturation degree.

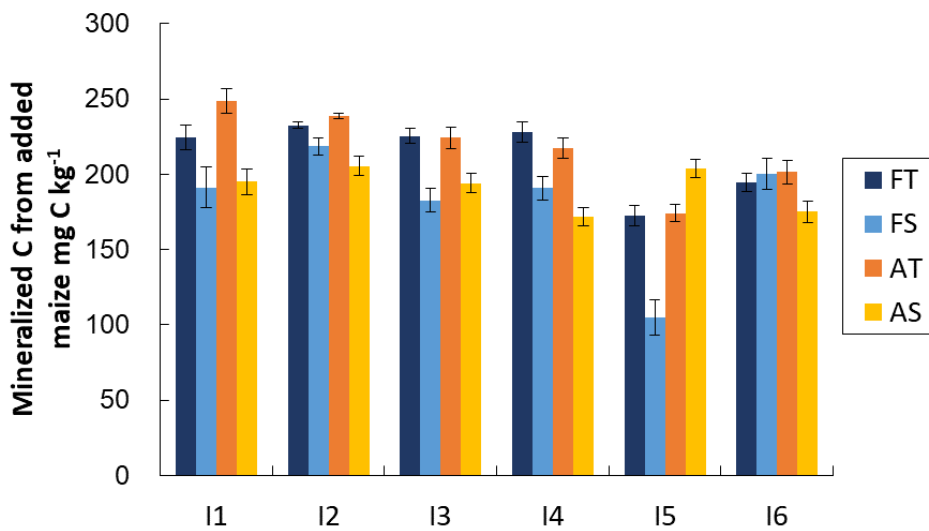


Fig. C4-7 Mineralized maize-derived C for different sites and horizons (mean $\pm$ standard deviation).

Mineralized C derived from maize in topsoil horizons was significantly higher than in subsoil horizons. The amount of mineralized maize tended to decrease from the upper to lower IGP,

especially for topsoil horizons (Fig. C4-7). Mineralized C from added maize displayed an increasing trend with increasing soil pH (Fig. C4-8a) and was positively correlated with SOC/(Al_o + Fe_o) ($r_s = 0.53^{**}$). The mineralized C from maize reached maximum values at a SOC to Al_o + Fe_o molar ratio of 15 or larger (Fig. C4-8b).

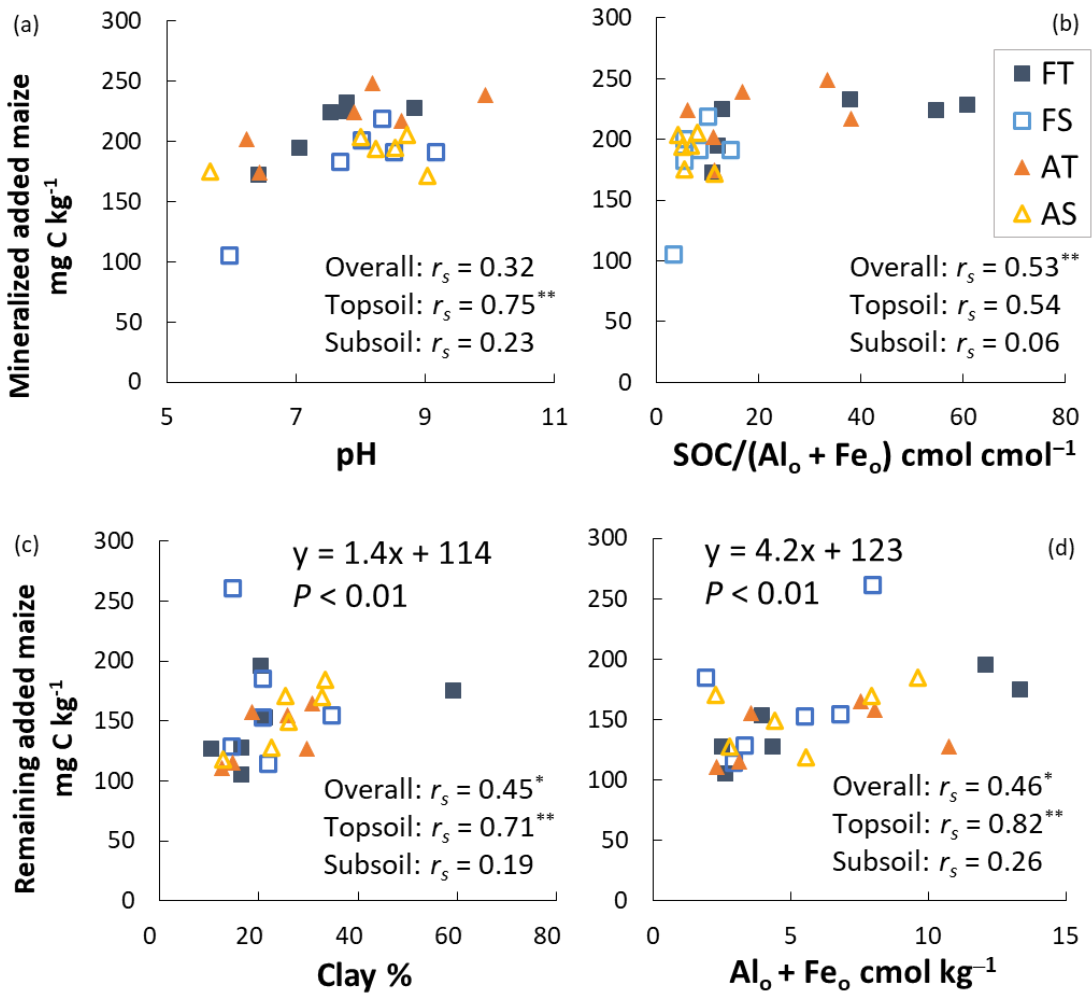


Fig. C4-8 Relationships (Spearman correlation (a–d) and linear regression (c and d)) between mineralized C from added maize and pH (a), carbon saturation degree (b), clay content (c) and active Al_o and Fe_o (d). Correlation between mineralized maize and pH was only significant for topsoils.

Residual maize C in topsoil and subsoil horizons was $143 \pm 28 \text{ mg C kg}^{-1}$ and $160 \pm 40 \text{ mg kg}^{-1}$, respectively (Table C4-2). The residual maize C was positively correlated with clay ($r_s = 0.45^*$), clay + silt ($r_s = 0.48^*$), and $\text{Al}_o + \text{Fe}_o$ ($r_s = 0.46^*$) across all sites (Fig. C4-9). Moreover, the intercept of regression formulas for the residual maize C versus clay content and $\text{Al}_o + \text{Fe}_o$ were $\sim 120 \text{ mg C kg}^{-1}$ (Intercept = 114^{**} and 123^{**} , respectively) (Figs. C4-8cd).

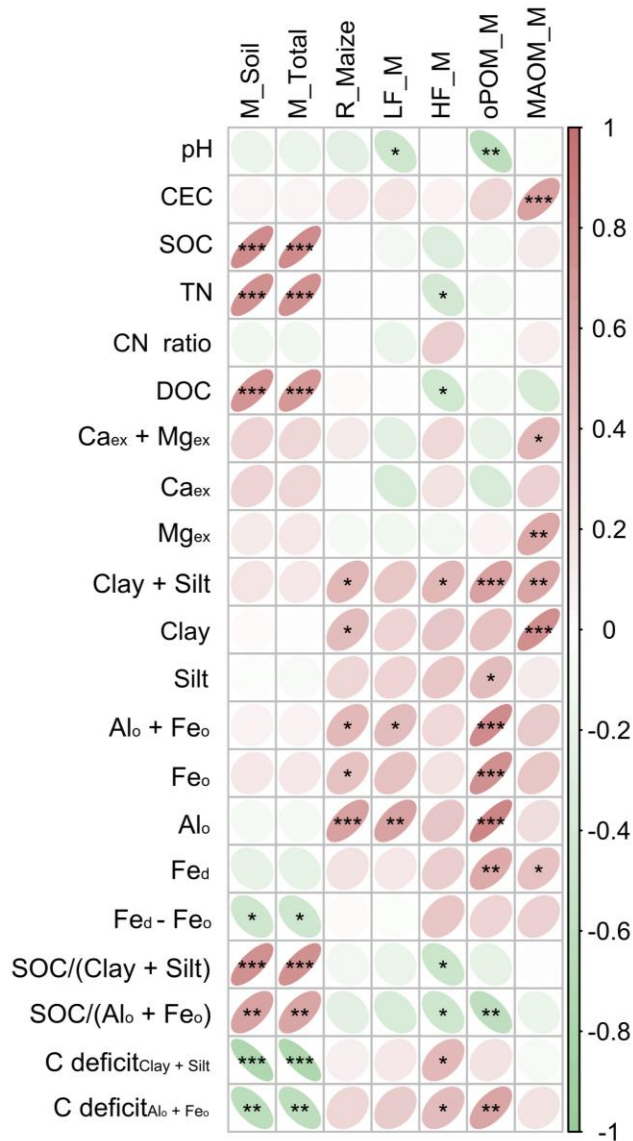


Fig. C4-9 Spearman correlation matrix (r_s) between soil physicochemical properties and selected incubation results across all soils.

4.3.3 SOM Fractionation

The amount of maize-derived C in different SOM fractions is shown in Fig. C4-10. More than 70% of residual maize-derived C was in relatively stable fractions, except for I5-FT (54%), that is, OM occluded in aggregates (oPOM, $8 \pm 4\%$) and associated with minerals (MAOM, $77 \pm 15\%$) (Fig. C4-10). Maize-derived C in HF was significantly higher in subsoil than topsoil horizons. Across sampling locations, lower IGP sites had the highest residual maize-derived C in both topsoil and subsoil horizons. Similarly, the amount of maize-derived C in LF and oPOM was higher in lower IGP sites (Fig. C4-10).

There was a significant negative correlation between the amount of maize-derived C in LF and pH ($r_s = -0.46^*$) (Fig. C4-11a). The maize-derived C in oPOM displayed correlations with pH ($r_s = -0.63^{**}$), clay + silt ($r_s = 0.64^{***}$), $Al_o + Fe_o$ ($r_s = 0.78^{***}$), and $SOC/(Al_o + Fe_o)$ ($r_s = -0.61^{**}$) (Figs. C4-9, C4-11b, C4-12). Also, maize-derived C in MAOM had a positive correlation with clay content ($r_s = 0.77^{***}$). These correlations related to maize-derived C in oPOM and MAOM were found even when topsoil and subsoil horizons were separately analyzed (Fig. C4-13).

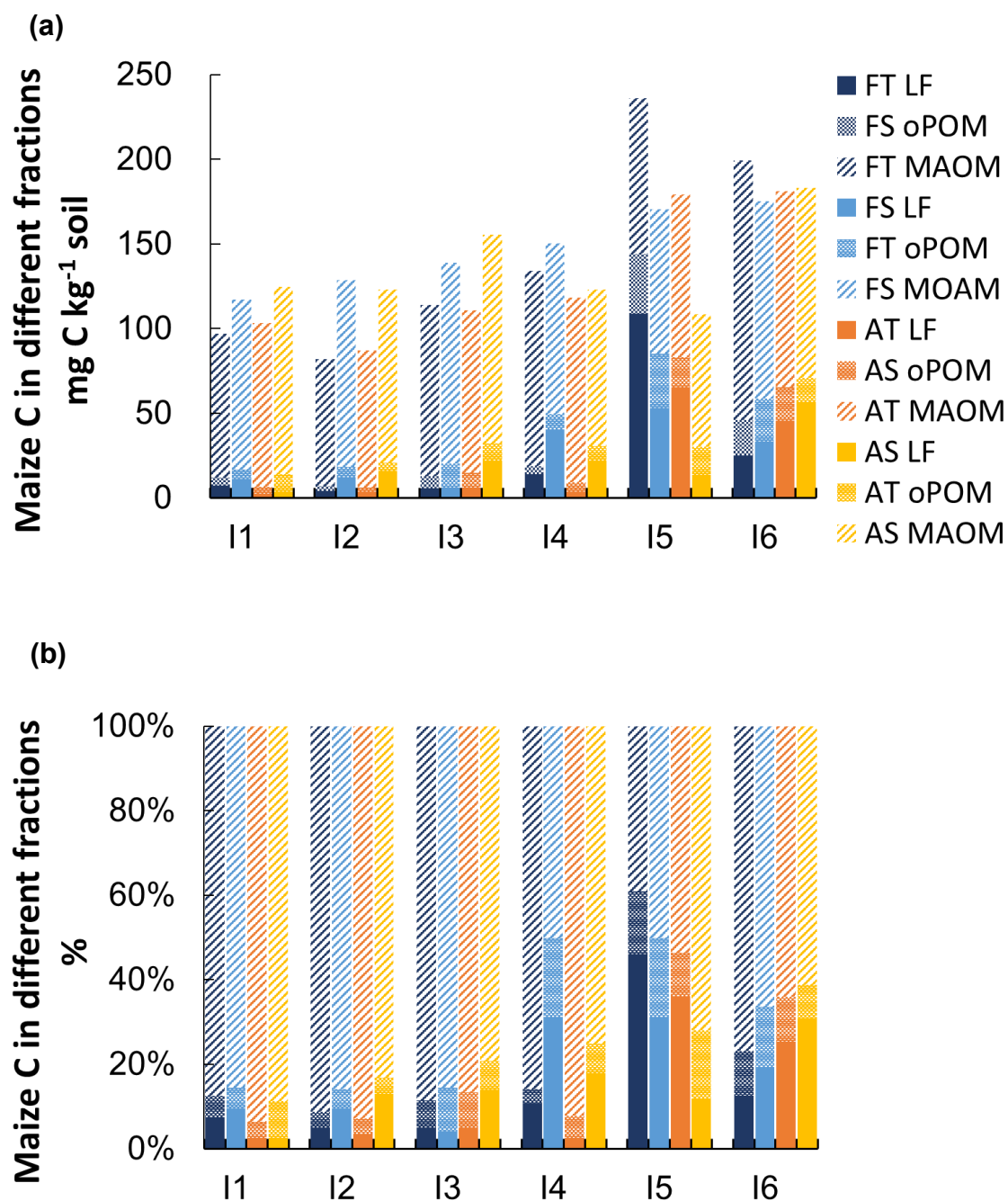


Fig. C4-10 Content of maize-derived C in soil (a) and proportion of maize-derived C in different SOM fractions (b) after one-year incubation.

LF, light fraction; oPOM, occluded particulate organic matter in aggregates; MAOM, maize-derived C as mineral-associated organic matter.

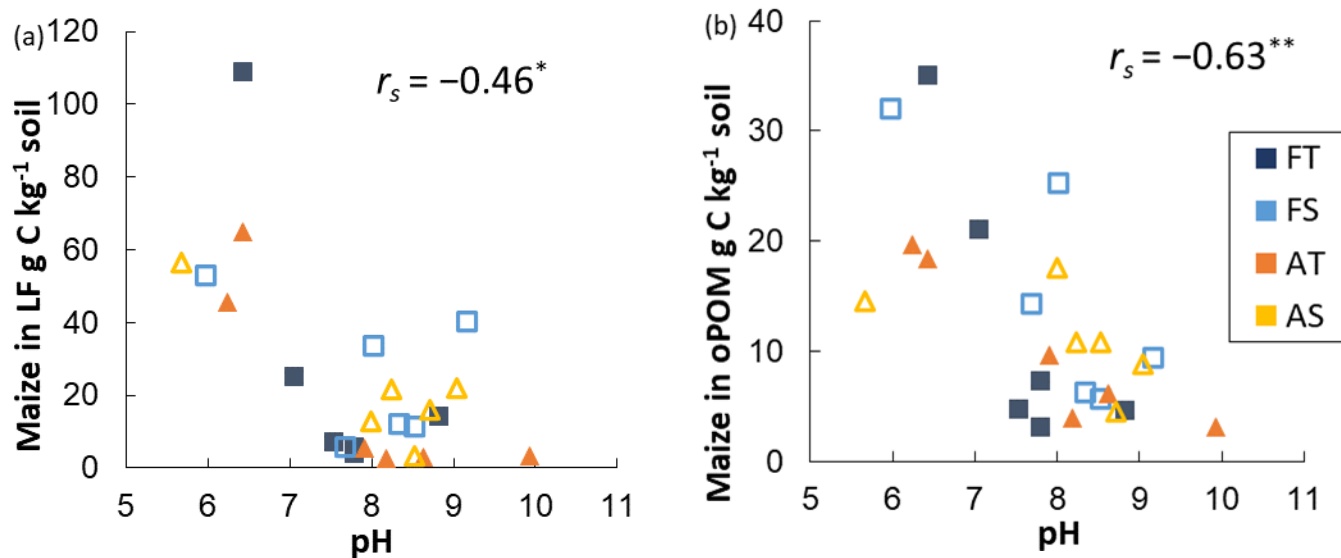


Fig. C4-11 Spearman correlation between pH (H_2O) and the maize-derived C in LF (a) and occluded particulate organic matter (oPOM) (b)

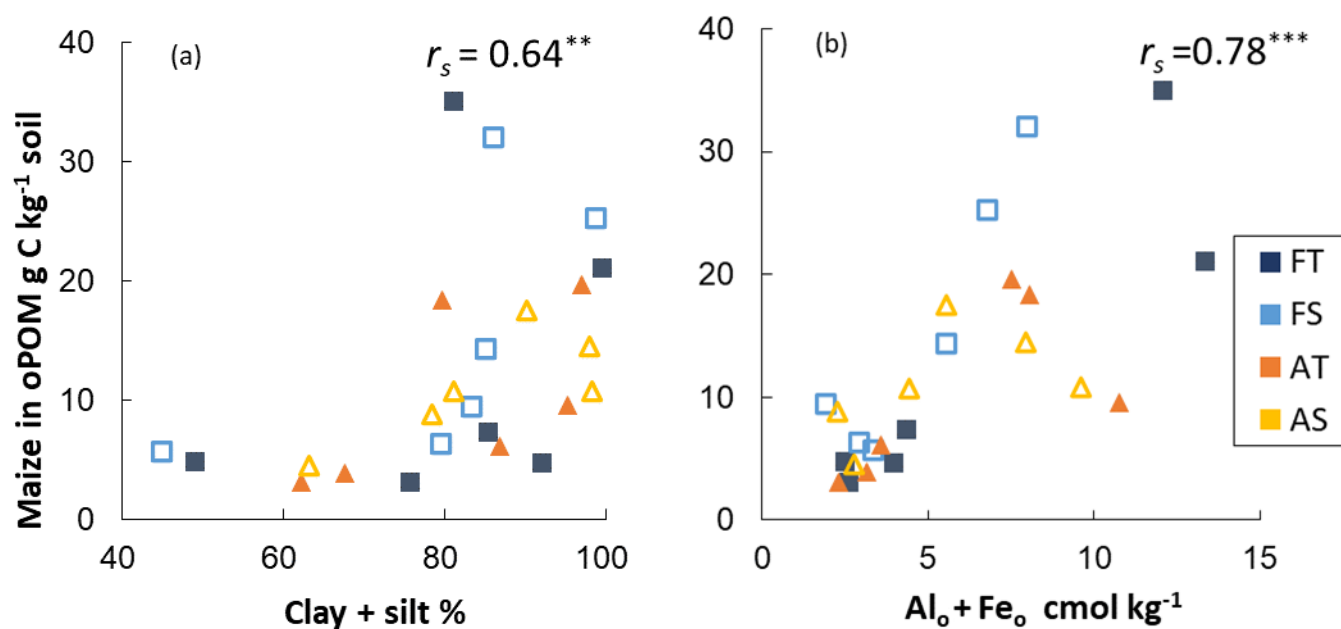


Fig. C4-12 Spearman correlations between occluded particulate organic matter (oPOM) and clay + silt (a) and active Al and Fe ($\text{Al}_0 + \text{Fe}_0$) (b)

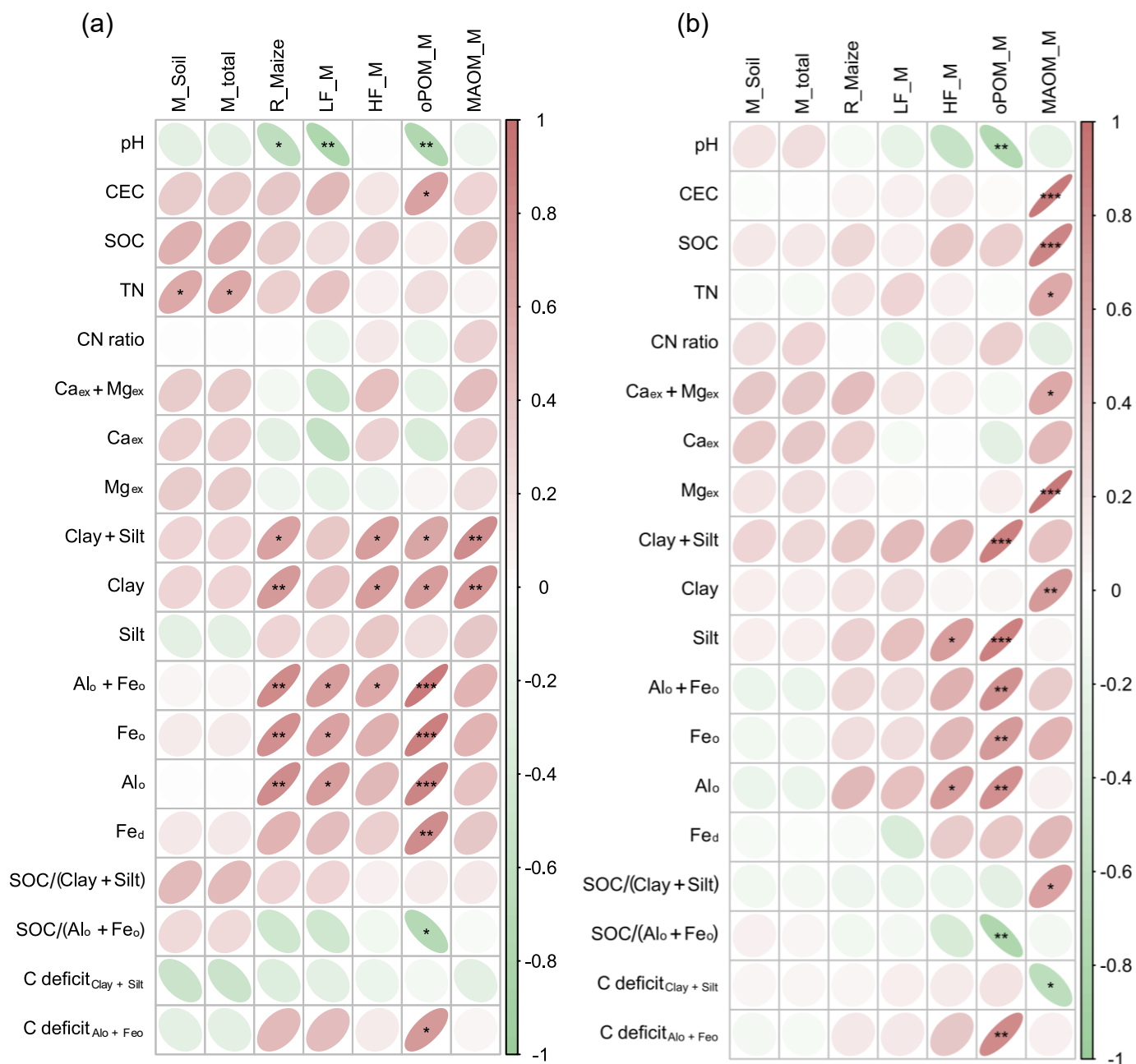


Fig. C4-13 Spearman correlation matrix (r_s) between soil physicochemical properties and selected incubation results for topsoil and subsoil horizons.

Partial least squares path modeling (PLS-PM) was utilized to evaluate the contribution of soil properties, which were influenced by climate, river (lower basin has finer particles), and soil

horizon, on stabilization of added maize (Fig. C4-14). Maize-derived C in oPOM was well explained ($r^2 = 0.56$) with direct contribution by $Al_o + Fe_o$ ($\beta = 0.62^{***}$) and $SOC/(Al_o + Fe_o)$ ($\beta = -0.22^*$). Clay plus silt indirectly contributed to maize-derived C in oPOM by cooperating with $Al_o + Fe_o$ ($\beta = 0.46^*$). Maize-derived C in MAOM was also well explained ($r^2 = 0.74$) and was only directly and positively contributed by clay content ($\beta = 0.81^{**}$), which was more abundant in lower IGP. The contributions of CEC and exchangeable Ca and Mg to both maize-derived C in oPOM and MAOM were not significant in the model.

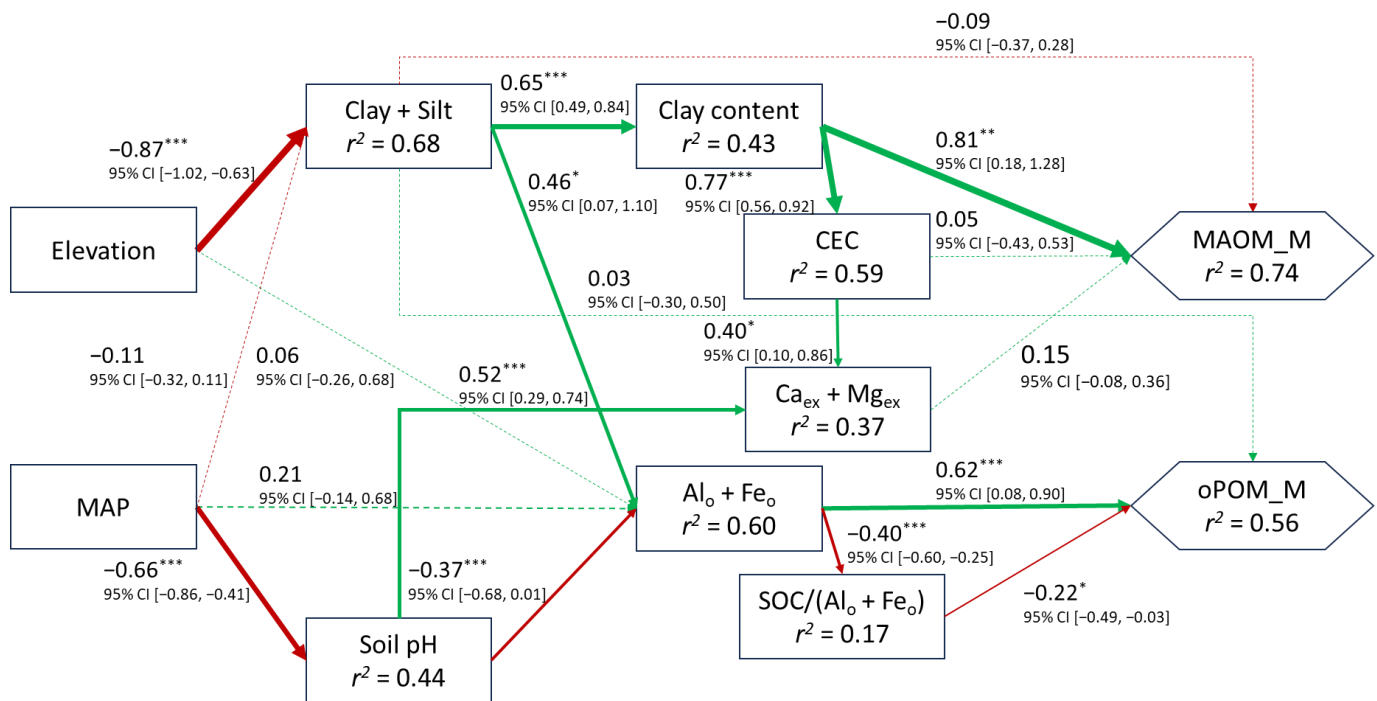


Fig. C4-14 Partial Least Squares Path Modeling (PLS-PM) for evaluating the effect of soil properties on the stabilization of added maize.

Solid and dotted arrows represent the significant and non-significant contributions, respectively; green and red colors represent the positive and negative contributions, respectively; the number above the arrow line represents the normalized path coefficient (β). The significance of each path coefficient and the confidence interval (CI) of the path coefficient were provided by model bootstrapping.

4.3.4 *Py-GC/MS Analyses*

A total of 15 unique compounds (peaks) specific to maize plant material (23% of the total identified peak area) was found when comparing the Py-GC/MS chromatographs of maize (Fig. C4-15) with the control soils. Those peaks had a relative abundance of >0.5% for maize material in the chromatograph but were not present in the 24 control soils (Table C4-3). Of the 15 unique peaks identified for maize plant materials, I found only two of these peaks, namely 2-cyclopenten-1-one and 2-methoxy-4-vinylphenol in the maize-incubated soils (Table C4-4). The 2-cyclopenten-1-one is derived from polysaccharides (Buurman et al., 2007; Chen et al., 2018), and 2-methoxy-4-vinylphenol is derived from partially-degraded lignin (Ansari et al., 2019; Nakagawa-Izumi et al., 2017). None of the 15 unique maize peaks were found for most of the maize-incubated soils (18 of 24 soils). For instance, pyrolysis products of lignocellulose and cellulose, such as acetol, 2-hydroxy-3-methyl-2-cyclopenten-1-one and 3-ethyl-2-hydroxy-2-cyclopenten-1-one- (Ansari et al., 2019; Wang et al., 2012), were not found in maize-incubated soils. Also, peaks of plant metabolites and steroids from maize, such as phytol, vitamin E and beta-sitosterol acetate were absent (Ahn et al., 2016) (Tables C4-3 and S1).

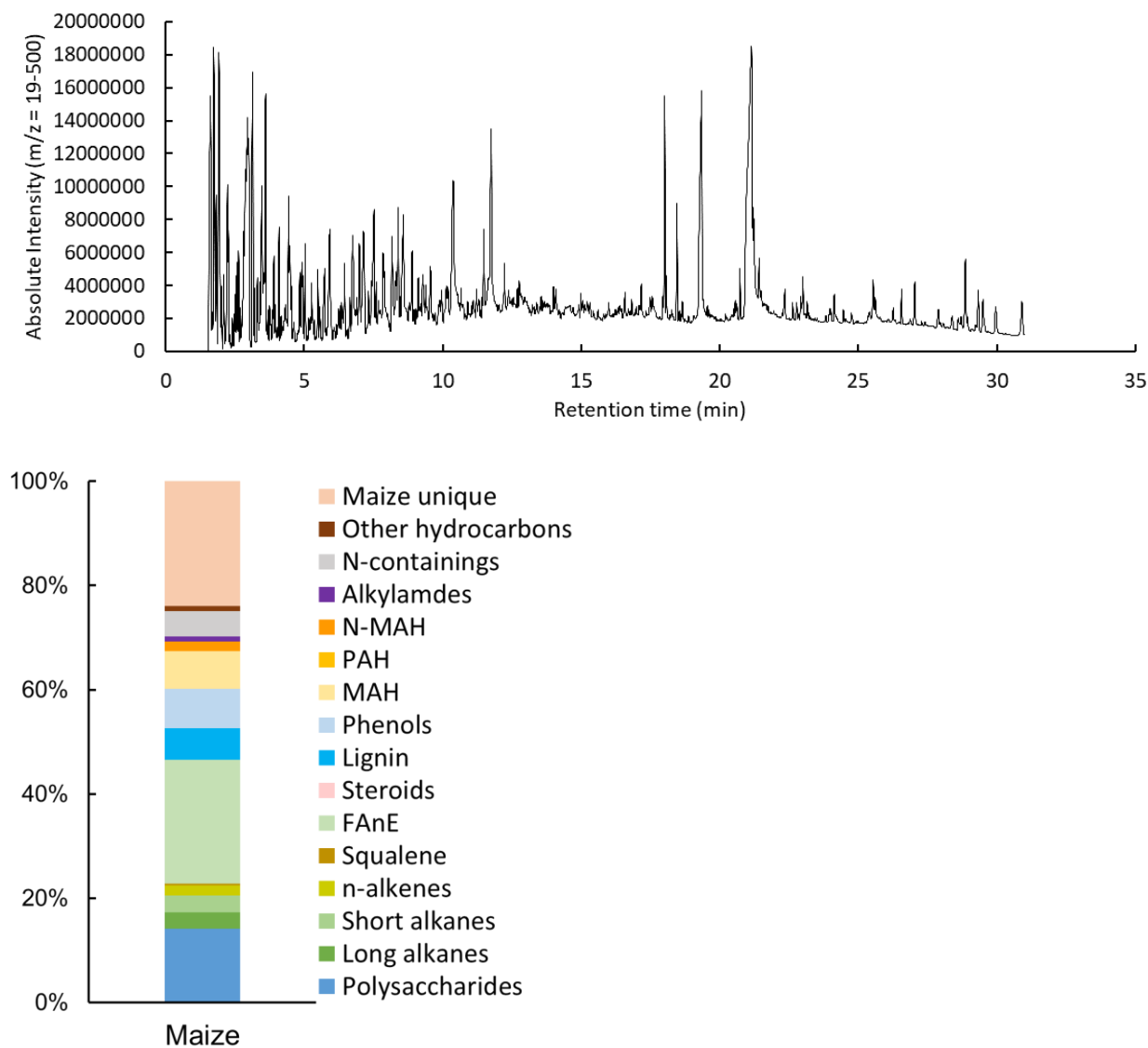


Fig. C4-15 Chromatogram and relative abundance of the most abundant 16 organic molecule groups for maize material added to the soil incubations as determined by pyrolysis-gas chromatography/mass spectroscopy (PY-GC/MS)

Long alkanes (C23 to C33); short alkanes (C7 to C22); FAnE, fatty acids and their esters; MAH, monocyclic aromatic hydrocarbons; PAH, polycyclic aromatic hydrocarbons; N-MAH, N containing MAH; N-containing, non-aromatic N containing compounds.

Table C4-3 Unique compounds in maize plant material used for incubation experiment.

Name	Retention time s	Relative abundance in total identified peak areas %
Acetol	3.14	3.6
2-Methoxy-4-vinylphenol	11.82	3.3
2,4-Dimethyl-2-oxazoline-4-methanol	7.17	2.8
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	7.52	2.6
Neophytadiene	18.03	2.4
3-Amino-s-triazole	6.99	1.8
2-Cyclopenten-1-one	4.57	1.3
Neophytadiene	18.46	1.1
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	8.90	1.0
Cholest-8(14)-en-3-ol, 4-methyl-, (3.beta.,4.alpha.,5.alpha.)-	30.91	0.7
Vitamin E	29.50	0.7
Phenol, 2,6-dimethoxy-	12.30	0.6
beta-Sitosterol acetate	29.33	0.6
Phytol	20.73	0.5
Phenol, 2-methoxy-4-methyl-	10.08	0.5
Total		23.0

Table C4-4 Unique compounds of maize plant material found in maize-treated soils after one-year incubation.

Type	Name	I4FT ^a	I5FS	I5AT	I5AS	I6FT	I6AT
Cellulose	2-Cyclopenten-1-one	-	0.91%	2.03%	0.23%	-	2.09%
Lignin	2-Methoxy-4-vinylphenol	0.32%	-	0.07%	-	0.08%	0.29%

^a Unique compound for maize plant material (Table C4-1) was absent from soils not listed in this table.

The effects of maize material treatment on organic matter composition were evaluated by comparing the maize-treated and control soils after incubation (Table C4-6, Fig.C4-16). About 67% of pyrolysis peaks (66% ±11% for treatment soils and 68% ±16% for control soils) were quantified and categorized into 15 compound groups, while the remaining 33% were mostly small molecules

(e.g., butene) that were not attributable to the original organic compounds before pyrolysis. As an example, pyrolysis peaks of I5 are shown in Fig.C4-17. The majority of lignin-derived compounds in maize, 2-methoxy-4-vinylphenol (42% of maize lignin) and 2-methoxy-phenol (47% of maize lignin) were not found in subsoil horizons. However, four (2-methoxy-phenol found in I4-FT, I5-AT, I6-FT and I6-AT; Table C4-4) and six (2-methoxy-phenol found in I1-FT, I1-AT, I2-FT, I4-FT, I6-FT and I6-AT) topsoil horizons did contain lignin-derived compounds. In those topsoil horizons, 2-methoxy-4-vinylphenol and 2-methoxy-phenol proportions were significantly higher in the maize-incubated than in control soils ($P < 0.05$).

Table C4-5 Relative abundance of organic compounds (mean $\pm$ standard deviation, $n = 24$) in maize-treated and control soils at the end of the incubation period and their statistical difference between maize-treated and control soils by t-test.

Name	Mean abundance in maize-treated soil %	Mean abundance in control soil %	<i>P</i> value
Polysaccharides	10.2 $\pm$ 7.0	8.8 $\pm$ 4.0	0.115
Long alkanes	2.6 $\pm$ 1.7	1.8 $\pm$ 1.8	0.147
Short alkanes	2.0 $\pm$ 0.9	1.3 $\pm$ 0.9	0.007
n-alkenes	3.3 $\pm$ 1.9	1.8 $\pm$ 1.0	<0.001
Squalene	3.8 $\pm$ 6.3	2.3 $\pm$ 3.8	0.285
FAnE	17.9 $\pm$ 17.2	4.9 $\pm$ 6.6	<0.001
Steroids	0.9 $\pm$ 1.2	1.7 $\pm$ 5.0	0.371
Lignin	0.5 $\pm$ 0.6	0.5 $\pm$ 0.9	0.918
Phenols	3.1 $\pm$ 3.2	1.9 $\pm$ 2.4	0.003
MAH	11.4 $\pm$ 4.2	17.0 $\pm$ 9.7	0.028
PAH	1.5 $\pm$ 0.9	1.5 $\pm$ 1.2	0.868
N-MAH	2.0 $\pm$ 1.1	2.2 $\pm$ 1.0	0.199
Alkylamides	0.3 $\pm$ 0.5	0.4 $\pm$ 0.5	0.688
N-containing	39.3 $\pm$ 15.2	52.5 $\pm$ 17.8	0.005
Other hydrocarbons	1.2 $\pm$ 1.2	1.3 $\pm$ 1.5	0.795
Total	66 $\pm$ 11	68 $\pm$ 16	0.507

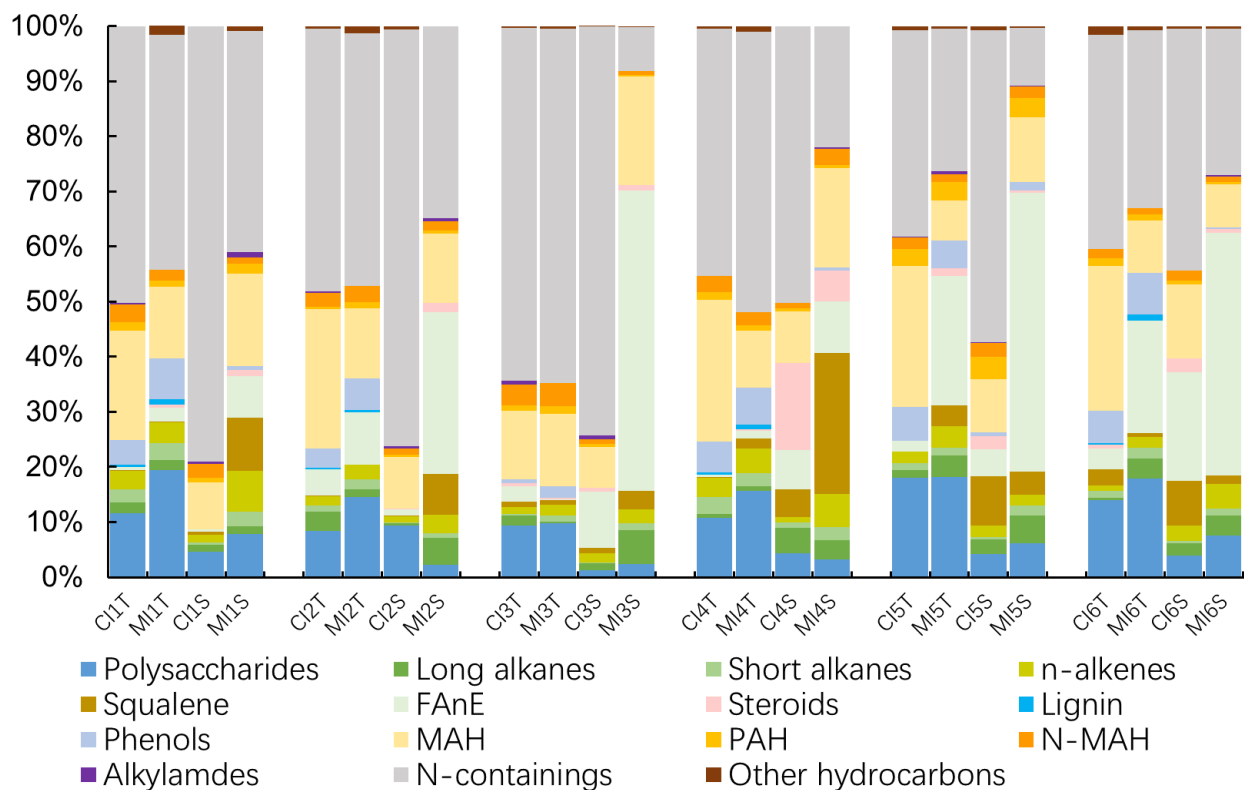
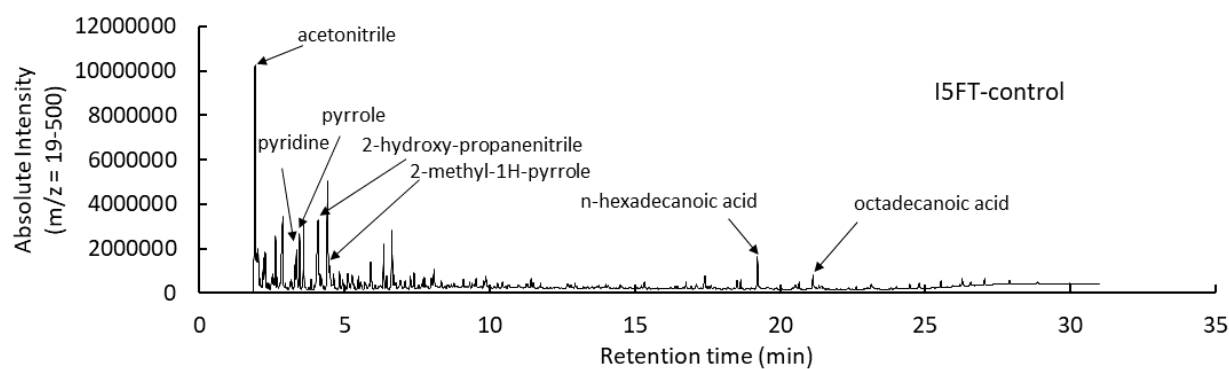


Fig. C4-16 Relative abundance of 15 organic molecule groups for soils after incubation between control (C) and maize-treated (M) soils as determined by pyrolysis-gas chromatography/mass spectroscopy (PY-GC/MS)



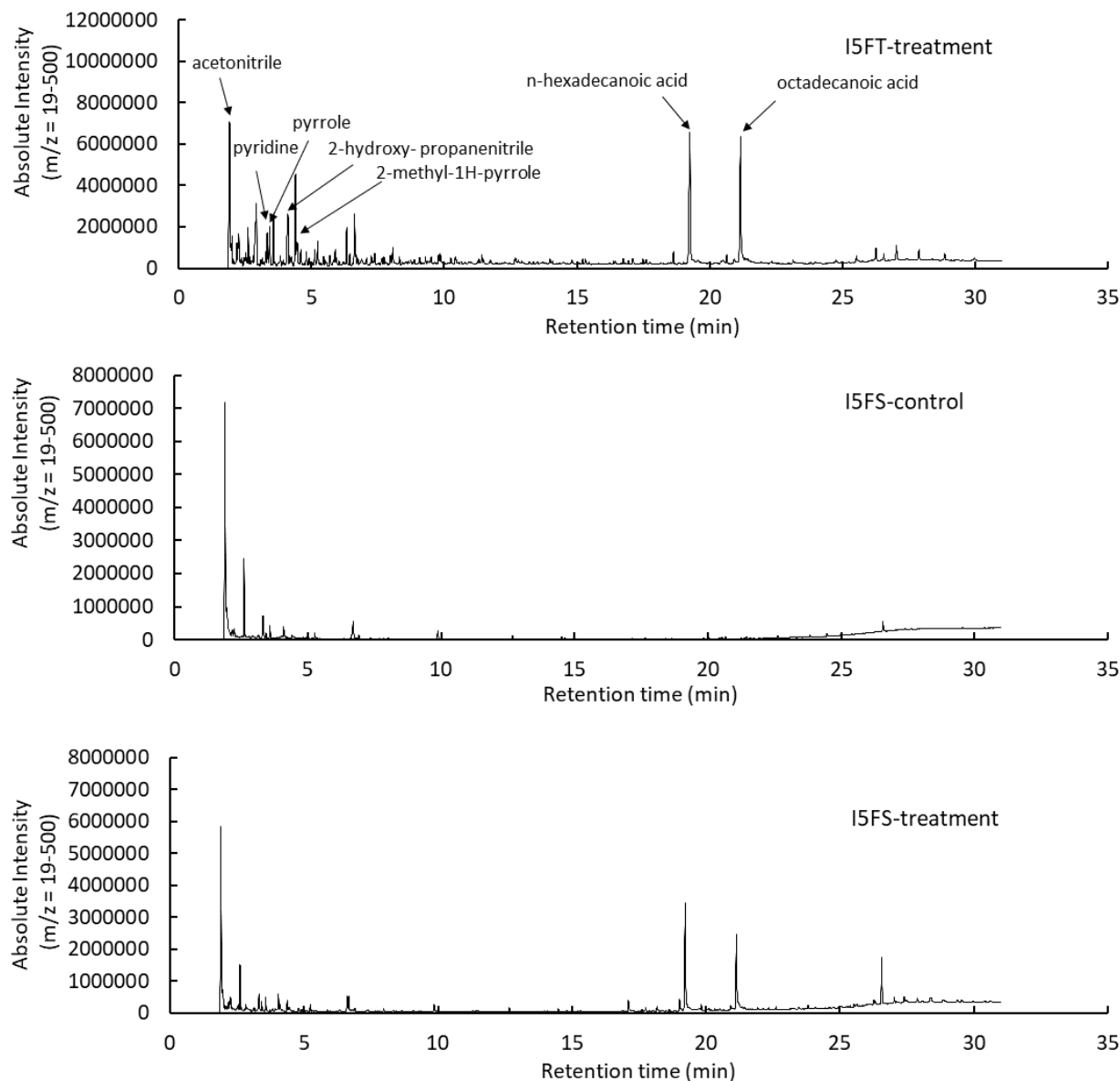
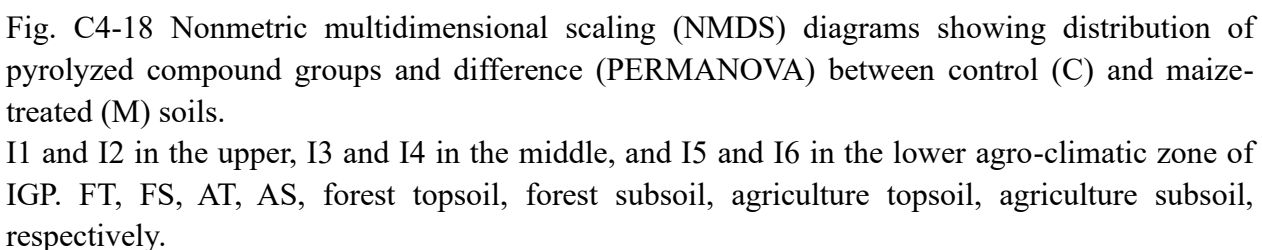


Fig. C4-17 Results of pyrolysis-gas chromatography/mass spectroscopy (Py-GC/MS) of topsoils and subsoils after incubation with and without maize plant material for a representative site (I5 forest).

Acetonitrile, pyridine, pyrrole, 2-hydroxy-propanenitrile, and 2-methyl-1H-pyrrole are N-containing; n-hexadecanoic acid and octadecanoic acid are fatty acids. Details of the compounds are provided in Table S1.

A significant difference in SOM composition between the maize-treated and the control soils after incubation was identified using NMDS ordination and PERMANOVA testing (Fig. C4-18).

As decomposition products of lignin, phenols (i.e., phenol and alkylphenol) were significantly higher in soils with maize addition across all sites and horizons (Table C4-6, Fig. C4-16). A predominant difference was soils with maize addition had a significantly higher proportion of FAnE than control soils (Table C4-6, Fig. C4-17). The increase of FAnE in maize-incubated soils was mainly due to the increase of n-hexadecanoic acid and octadecanoic acid, which are the main components or derivatives of fatty acids and their esters in maize plant materials. Short alkanes and n-alkenes were also higher in the maize-incubated soils than in the controls (Table C4-6). In contrast, N-containing, non-aromatic hydrocarbons (N-containing) were significantly lower in the maize-incubated soils (Table C4-6, Figs. C4-16 and C4-17). The effects of maize addition on the increased FAnE and decreased N-containing components were more evident in subsoil horizons (Fig. C4-16).



4.4.1 Factors Regulating Mineralization of Maize Plant Material

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mineralization rates of the added maize were negatively correlated with Al_o , and positively correlated with original SOC content and SOC saturation degree ($SOC/(Al_o + Fe_o)$ & $SOC/(silt + clay)$) (Fig. C4-4). Aggregation can occur quickly during wet-dry cycles and/or physical disturbance events (Oades and Waters, 1991; Tisdall, 2020), which is similar to the process of rewetting and maize incorporation at the beginning of the incubation. Although the absolute values of Al_o and Fe_o are considered low compared to acidic soils, they appear to be highly effective binding agents active in formation of soil aggregates (Wagai et al., 2020). Initially formed aggregates may break down in later incubation stages (i.e., after day 196) and release some of the occluded maize for microbial utilization. Alternatively, some of these aggregates are likely reinforced by microbially generated organic materials derived from maize, thereby contributing to OM occlusion within aggregates (Coleman et al., 2018) (Fig. C4-9). The correlation between mineralization rate and Al_o , rather than Fe_o (or Fe_d) (Fig. C4-4 and C4-5), may result from Al being more soluble than Fe across a wide range of pH values rendering it more interactive with organic matter (Ashida et al., 2021).

A higher microbial energy supply from soils with higher SOC can support a more active microbial community with a larger abundance of copiotroph microbes (Koch, 2001), which were previously demonstrated to have a strong response to organic matter additions (Dungait et al., 2011; Wu et al., 2020). Higher carbon saturation degrees indicate that a larger proportion of the stabilization capacity is filled, thereby decreasing its ability to stabilize additional SOC (Rodrigues et al., 2022b) and leading to greater microbial respiration. A higher microbial activity/respiration occurred in soils with higher SOC, DOC, and higher SOC saturation as demonstrated by their

strong positive correlations (Fig. C4-4). This resulted in a greater amount of mineralized C from the original SOC, as well as the total mineralized C (i.e., C from original SOC + added maize) (Fig. C4-9).

After initiation of maize mineralization by quick-response microbes, decomposition of maize during later incubation stages appears to be influenced by pH-regulated enzyme activities, as well as the stabilization of maize material (probably both aggregation and direct stabilization) by Al_o (and Fe_o) (Figs. C4-4 and C4-5). The ability of active Al and Fe to immobilize extracellular enzymes (Allison, 2006; Koji, 1989) may also retard the decomposition of organic matter with high-molecular-weight compounds in fresh plant material. Higher pH in drier upper IGP can make some components of the added maize more soluble (Curtin et al., 2016), with the DOM being more accessible to microbes (Figs. C4-4 and C4-9). Additionally, utilization and decomposition of fresh plant materials, such as lignin and fatty acid esters, require the production of extracellular enzymes (i.e., laccase and acetylsterase) (Perez et al., 2002) that are more effective at near-neutral pH values (Fujii et al., 2020; Puissant et al., 2019). Hence, I posit that the positive correlations between mineralization rates on the 14th and 28th days and soil pH demonstrate that a higher pH promotes the decomposition of plant material possibility by enhancing enzyme activities (Figs. C4-4 and C4-5).

Once the increase in total respiration due to maize addition settled after 70 days of incubation, the original SOC, DOC, and SOC saturation degree appear to regain control of the mineralization rate for added maize. The positive correlations between the mineralization rates of added maize and indices of carbon saturation after 70 days (Figs C4-4 and C4-6) infer that soils with lower SOC

saturation have greater SOC stabilization by active Al/Fe and crystalline clay minerals (Kleber et al., 2007; Lyu et al., 2021a; Stewart et al., 2008). These stabilization processes can preserve decomposition products from the primary decomposition stage, thereby retarding further mineralization. For soils with high SOC and DOC, continued high mineralization rates after 70 days of incubation may suggest a more limited stimulus by the maize addition (i.e., 420 mg C kg⁻¹ soil for maize addition) as the maize addition did not permanently change the microbial community (Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010). After stabilization of respiration rates (Fig. C4-3), energy from the original SOC and primary decomposition products weakly associated with soil minerals, as suggested by the higher SOC saturation degrees, can cause higher microbial activity and associated higher mineralization rates. Moreover, higher mineralization rates for maize-derived C occurred in topsoil horizons with higher SOC contents and higher SOC saturation degrees (Fig. C4-6) than subsoil horizons during the later incubation stage. This further suggests that differences in the original SOC and SOC saturation degrees could regulate organic matter mineralization by controlling microbial activities through substrate accessibility/availability.

4.4.2 Controls for SOC Stabilization and Fates of Maize-derived C

Active Al/Fe and fine mineral particles promoted the accumulation of maize-derived C as inferred by the positive correlations of residual maize-derived C with Al_o, Fe_o and clay (+ silt) (Figs. C4-9 and C4-8). Because carbon-use efficiency by microbes is about 0.4–0.7 (Manzoni et al., 2012; Saifuddin et al., 2019), the 48 ± 7% of cumulative maize mineralization (Fig. C4-3, Table

C4-2) suggests that most of the added maize was processed by microorganisms. Higher clay (+ silt) content and Al_o (+ Fe_o) (Table C4-1) in the lower IGP sites appear to have contributed to greater stabilization of microbially processed C from the added maize (Fig. C4-14). Interestingly, the stronger correlation between remaining maize content and Al_o suggests that Al_o might be more effective at stabilizing OM compared to Fe_o (Fig. C4-9). The positive impact of active Al/Fe on enlarging SOC of neutral soils was also proved by Ashida et al., using a large dataset crossing 4 countries (Ashida et al., 2021). The lower amount of stabilized maize-derived C in topsoil horizons (Fig. C4-10a) is ascribed to less efficient stabilization of OM in topsoil versus subsoil horizons due to its higher SOC saturation degree (Fig. C4-2, Table C4-2). The positive correlation between cumulative mineralization of maize and $\text{SOC}/(\text{Al}_\text{o} + \text{Fe}_\text{o})$ and the limited release of CO_2 derived from maize after one year of incubation indicate that the SOC saturation degree may be important in controlling the mineralization of added organic matter (McNally et al., 2018; McNally et al., 2017). Under lower SOC saturation conditions, organic matter is more strongly stabilized by soil minerals via inner sphere interactions (Kleber et al., 2007), which attenuates further organic matter decomposition. Notably, the OM stabilization capacity seems to have a limit, as the mineralized maize-derived C reached a threshold when the ratio of SOC to $\text{Al}_\text{o} + \text{Fe}_\text{o}$ reached ~ 15 (Fig. C4-8b).

Because the active Al/Fe ($5.6 \pm 1.0 \text{ cmol kg}^{-1}$) and Fe_d ($16.4 \pm 7.9 \text{ cmol kg}^{-1}$) contents were low, OC stabilization by direct adsorption or complexation with active Al/Fe or Fe (hydr)oxides is expected to play only a minor role. Moreover, the non-zero intercepts for plots of mineralized C with active Al/Fe and clay (Figs. C4-8cd) imply that even without clay minerals or active Al/Fe present, the added maize would not completely mineralize within a year. This suggests that the

accumulation of added maize in the soil is not totally dependent on clay or active Al/Fe contents. For instance, soil pH (Fig. C4-8a), recalcitrance of some organic maize components (e.g., lignin), and microbial accessibility may also limit mineralization (Dungait et al., 2012; Marschner et al., 2008; Rousk et al., 2009). As the total mineralization of added maize was close to 50% and further release of maize-derived CO₂ was nearly undetectable after 336 days (Fig. C4-3), the residual maize-derived C was considered predominantly stabilized (West and Marland, 2003). Hence, fractionation of organic C after incubation was investigated further to assess stabilization mechanisms for the residual maize-derived C.

Based on SOM fractionation, maize-derived C was mainly stabilized by soil minerals (i.e., clay, silt, and active Al/Fe) and preserved as MAOM and oPOM (Fig. C4-10). The MAOM fraction was the major destination of maize-derived C ($77 \pm 15\%$ of residual maize C), which is controlled primarily by clay. The predominant contribution of clay content to maize-derived MAOM ($\beta = 0.81^{**}$; Fig. C4-14) infers that clay minerals control the majority of organic matter stabilization. The negative charge provided by clay minerals (e.g., vermiculite) can contribute to the preservation of organic matter as MAOM by adsorbing polar or charged functional groups (Kleber et al., 2007). This premise is further supported by the significant positive correlation between CEC and maize-derived MAOM ($r_s = 0.64^{***}$). Multivalent exchangeable Ca²⁺ and Mg²⁺ (Fig. C4-9), which were more abundant in the higher pH soils of drier upper-to-middle IGP (Table C3-1, Fig. C4-14), can provide a bridge connecting the negatively charged mineral surfaces and organic matter (Rowley et al., 2018), thereby stabilizing the added organic matter from microbial utilization. However, this bridging effect seemed not predominant in stabilizing added maize as MAOM, as revealed by its

insignificant contribution to maize-derived MAOM ($\beta = 0.15$; Fig. C4-14). On the other hand, maize-derived MAOM had no clear relationship with active Al/Fe, which may be due to their very low contents in the IGP soils, as compared to volcanic soils (Garrido and Matus, 2012; Lyu et al., 2022; Ugolini and Dahlgren, 2002) and acidic soils (Ashida et al. 2021). Additionally, the weak positive correlation between Fe_d and maize-derived MAOM ($r_s = 0.42^*$) suggests that free Fe (hydr)oxides may also partially retain organic matter by surface adsorption (Zhao et al., 2020). However, the insignificant correlation between $\text{Fe}_d - \text{Fe}_o$ and maize-derived MAOM challenges the direct role of crystalline Fe (hydr)oxides in stabilizing SOM.

The oPOM fraction was a minor destination for maize-derived C ($8 \pm 4\%$ of residual maize C). A significant relationship between SOC and $\text{Al}_o + \text{Fe}_o$ in non-volcanic soils with pH greater than 6 (Ashida et al., 2021) and a significant contribution of Al_o to a linear mixed model predicting SOM stabilization mechanisms (Rasmussen et al., 2018) imply a role for active Al/Fe in stabilizing SOC even in neutral-to-alkaline soils. The predominant contribution of $\text{Al}_o + \text{Fe}_o$ to maize-derived oPOM ($\beta = 0.62^{***}$; Fig. C4-14) infers a role for active Al/Fe in organic matter stabilization. Fe (hydr)oxides (Fe_d), such as ferrihydrite, goethite and hematite, and active Al/Fe constituents (Al_o and Fe_o) are especially active in preserving SOM (Lützow et al., 2006; Xue et al., 2020). Rather than direct sorption by (hydr)oxides (Kleber et al., 2007; Kogel-Knabner et al., 2008), an indirect stabilization of organic matter through the promotion of soil aggregate formation is considered an important mechanism (Wagai et al., 2020). Active Al/Fe acts as adhesive agents, which can work even in small quantities, binding larger particles (i.e., clay and silt, positively correlated with maize-derived oPOM) to form stable soil aggregates that contribute to preservation of maize

transformation products (Figs. C4-9 and C4-14). This preservation would be retarded when active Al/Fe is more saturated in topsoil (Table C4-2), as revealed by the negative contribution of SOC/(Al_o + Fe_o) to maize-derived oPOM ($\beta = -0.22^*$). I assume that the maize-derived oPOM is largely contributed by active Al/Fe, with the contribution of C stabilized as oPOM about 10% that of MAOM (Fig. C4-10). This contribution is consistent with a previous study that predicted the relative contribution of active Al to SOM stabilization in neutral-to-slightly alkaline soils (Rasmussen et al., 2018).

The negative correlation of pH with LF and oPOM (Figs. C4-9 and C4-11) may suggest attenuation of decomposition due to reduced activity of exoenzymes and microbes (Cao et al., 2016; Perez et al., 2002); however, it does not explain the near cessation of maize-derived C mineralization during the later incubation period (Fig. C4-3). Active Al/Fe, elevated in lower pH, moister soils of lower IGP, in conjunction with accumulated clay and silt fractions deposited downstream (lower IGP), appear to serve as the basic framework for soil aggregate formation (Asano and Wagai, 2014; Churchman et al., 2020; Six et al., 2002), (Table C3-1 and C4-1, Fig. C4-14). Hence, soil aggregate formation appears to preferentially preserve the less-degraded maize components as oPOM.

4.4.3 Identification of Maize-derived Organic Compounds by Py-GC/MS

Most maize-derived organic compounds are considered to be microbially processed and stabilized in the soil based on the molecular compositions determined by Py-GC/MS. Peaks for acetol, 2-hydroxy-3-methyl-2-cyclopenten-1-one, and 3-ethyl-2-hydroxy-2-cyclopenten-1-one

(Table C4-3) are considered pyrolysis products of cellulose (Melligan et al., 2012; Wang et al., 2020; Zhang et al., 2011) and were identified as unique peaks for the maize plant material. These peaks disappeared by the end of the one-year incubation, indicating decomposition of the lignocellulose structure. Even though some metabolites (e.g., neophytadiene in the maize material) have a broad-spectrum antimicrobial ability (Nishanbaev et al., 2019), the plant metabolites and steroids from the maize were effectively decomposed (Tables C4-3 and C4-4).

Although lignin is generally considered a recalcitrant substance (Marschner et al., 2008), lignin derived from the maize was absent in subsoil horizons and present in low quantities in topsoil horizons (Table C4-4). This indicates intensive decomposition of lignin during the incubation. The significant increase of phenols (Table C4-6, Fig. C4-16), a decomposition product of lignin and tannin (Leinweber and Schulten, 1999), further confirms decomposition of these recalcitrant materials. Lignin decomposition was enhanced under the C-limited conditions (i.e., low SOC content, Table C4-1) of subsoil horizons, as exhibited by the disappearance of lignin derived from maize in subsoil horizons relative to its presence in topsoil horizons. This aligns with prior work showing oligotrophic bacteria (e.g., Actinomycetes and Chloroflexi) and lignin-degrading fungi (e.g., Ascomycota) are abundant in subsoil horizons, adapting to utilize recalcitrant organic compounds like lignin in C-limited conditions (Abdel-Hamid et al., 2013; Fierer et al., 2007).

Maize addition improved the quality of soil organic matter as a substrate for microbial utilization, as indicated by an increased proportion of FAnE and decreased N-containing compounds (Table C4-6, Figs. C4-16 and C4-17). For example, fatty acids and their esters are

highly susceptible to decomposition (Jansen and Wiesenberg, 2017) due to their high energy density (about 38 kJ g⁻¹) (Sadava et al., 2009). Py-GC/MS identified a large proportion of FAnE (17.9%) with a major contribution from n-hexadecanoic acid and octadecanoic acid in maize-treated soils (Table C4-6, Fig. C4-17). Because of the long incubation period and the virtual cessation of CO₂ release from the added maize near the end of the incubation (Fig. C4-3), the remaining fatty acids and their esters derived from the added maize are considered to be stabilized by soil mineral interactions, thereby reducing their accessibility/availability as a microbial substrate. The fatty acids and their esters, which microbes would preferentially utilize, appear to be stabilized through association with mineral surfaces and/or occlusion in soil aggregates (Yang et al., 2020).

Nitrogen-containing organic matter originates mainly from microbially-derived organic matter (Buurman and Roscoe, 2011; Carr et al., 2013). Thus, the high relative abundance of N-containing compounds in pyrolysis products of control soil indicates recycling of organic matter by the microbial community (high degree of degradation). In addition to the significant increase of FAnE in maize-treated soils, increases of other labile compounds (e.g., short alkanes) and a decrease of N-containing compounds (Table C4-6) indicate that the added maize underwent microbial processing and enhanced the quality of SOM. The degraded products were then stabilized by associations with soil minerals (i.e., mainly as MAOM), preventing them from further degradation (Yan et al., 2021). The stronger impact of maize addition on the composition of organic compounds in subsoil versus topsoil horizons is likely due to its lower SOC content and stronger relative stimulation of the microbial community and C cycling by the added carbon substrates.

4.5 Summary

Higher active Al/Fe contents appeared to initially facilitate aggregation, which inhibited the decomposition of newly introduced maize organic matter. In contrast, higher pH, especially in the upper IGP, promoted C mineralization during the first month of incubation possibly through enhancing microbial accessibility and exoenzyme activities. Higher SOC content and SOC saturation degree, as the main difference between topsoil and subsoil, stimulated mineralization of the added maize by meeting microbial energy demand and limiting stabilization. Residual C from the added maize was mainly stabilized as MAOM ($77 \pm 15\%$) and oPOM ($8 \pm 4\%$) underwent microbial processing, as indicated by the disappearance of the unique organic compounds found in maize and increased short alkanes. Since the major of residue decomposition occurs within 3 months. The summer rainy season, from July to September, provides optimal soil moisture that may mirror incubation conditions and promote rapid microbial decomposition. Higher clay contents directly stabilized more degraded maize as MAOM. Active Al/Fe along with Fe (hydr)oxide contributed to the stabilization of maize-derived C, primarily as oPOM, by binding clay and silt as a framework for stable aggregate formation. Maize addition improved SOM quality, as indicated by the increased proportion of fatty acids and decreased N-containing compounds.

CHAPTER 5 GENERAL DISCUSSION AND CONCLUSION

5.1 Importance of Active Al/Fe in Soil Organic Carbon Stabilization

In neutral to alkaline soils, despite minimal levels, active Al/Fe plays a pivotal role in SOC stabilization, overshadowing the roles of Ca/Mg. Active Al/Fe positively impacts SOC stabilization, which is evident in the stability of the original SOC and the stabilization of newly added plant materials. This effect is observed not only in soils of volcanic regions but also in neutral-to-alkaline soils, where lower SOC pools might be attributed to their reduced active Al/Fe content.

Active Al/Fe enhances SOC stability, as evidenced by its predominant contribution to the mean residence time of the intermediate pool, which controls SOC stability within years to decades in both topsoil and subsoil. Active Al/Fe also stabilizes microbially processed carbon from added plant materials, such as maize-derived carbon. This is observed through the lower mineralization rates of added maize in soils with lower SOC saturation of active Al/Fe and a positive correlation between remaining maize content and active Al/Fe. In the more C deficient IGP soils, the higher EP and lower pH environment favor the formation of active Al/Fe, which collaborates with fine mineral particles accumulated downstream to preserve OM and extend the mean residence time of SOC. These preservations and stabilizations are primarily achieved through promoting organo-mineral interactions and aggregation. This process remains effective even with low quantities of active Al/Fe values in neutral-to-alkaline soils. In detail, active Al/Fe can act as bonding agents between organic matter and mineral surface (e.g., clays), as well as facilitating aggregation (i.e., binding clay and silt). Given the small quantities of active Al/Fe, oPOM formation, rather than

direct association, is more essential in preserving added plant materials, as revealed by the predominant contribution of active Al/Fe to maize-derived oPOM. Meanwhile, Ca and Mg cations can work as bridging agents for SOC stabilization in alkaline soils. However, Ca/Mg bridging is less intense than that of active Al/Fe, as revealed by the significant correlation between C_s and active Al/Fe rather than exchangeable Ca^{2+}/Mg^{2+} .

Active Al/Fe also distinctly affects SOM composition by stabilizing less-degraded organic components like fatty acids and saccharides and reducing N-containing compounds resulting from SOM degradation. The importance of clay and exchangeable Ca^{2+}/Mg^{2+} in SOM composition seems marginal compared to the significant impact of active Al/Fe. Regarding stabilizing newly added plant materials, active Al/Fe contributes to the stabilization of microbially processed maize-derived carbon.

In summary, active Al/Fe is crucial for stabilizing both original SOC and newly added plant materials. This dual role underscores its importance in soil carbon cycle dynamics, particularly in different soil environments, ranging from volcanic regions to neutral-to-alkaline soils. These results align with existing literature on the impact of active Al/Fe on SOC sequestration, providing a plausible explanation for the observed low SOC levels in neutral-to-alkaline soils. The effectiveness of active Al/Fe in promoting organo-mineral interactions and soil aggregate formation emerges as a key mechanism in SOC stabilization.

5.2 Factors Regulating SOM Molecular Composition

Soil organic matter in the Indo-Gangetic Plain exhibits significant degradation, characterized

by a high proportion of nitrogen-containing compounds and a lack of less-degraded components like undegraded polysaccharides. In the plant additive incubation experiment, Py-GC/MS analysis also reveals that most added plant materials in IGP soil are microbially processed and stabilized. Specific pyrolysis products of cellulose, 2-hydroxy-3-methyl-2-cyclopenten-1-one, and 3-ethyl-2-hydroxy-2-cyclopenten-1-one, identified as unique to maize plant material, diminish over a one-year incubation, indicating lignocellulose structure decomposition. Despite antimicrobial components in maize metabolites, both the metabolites and steroids undergo effective decomposition. The advanced SOM degradation in IGP soils, including the added plant materials, surpasses that in tropical volcanic regions and is attributed to near neutral soil pH, which promotes SOM decomposition, and low content of active Al/Fe, a key component regulating SOM stabilization, in neutral-to-alkaline IGP soils.

Regarding SOM composition, active Al/Fe has a notable impact, stabilizing less-degraded and less-recalcitrant organic matter, like fatty acids and saccharides, while diminishing the proportion of nitrogen-containing compounds. This contrasts with the minimal influence of clay and exchangeable $\text{Ca}^{2+}/\text{Mg}^{2+}$ on SOM composition. Near neutral pH conditions in the IGP enhance the activities of extracellular enzymes, leading to increased SOM degradation and a rise in N-containing compounds as degradation residues. While agricultural activities accelerate SOM degradation, their impact seems relatively minor in the IGP. Despite fewer weakly degraded plant-derived materials, agricultural soils' overall SOM composition and SOC-related properties are similar to those in forest soils. Yet, the resemblance between agricultural and forest soils may reveal an underlying, extensive human impact, including on forest areas.

In these soils, lignin, typically resistant to decomposition, shows extensive degradation, particularly in the subsoil, where SOM is more degraded than in the topsoil. The subsoil's SOM composition is distinct, with elevated levels of microbial derivatives and fewer less degraded compounds, such as lignin and phenols. Significant lignin decomposition from added maize, particularly in subsoil horizons with low SOC content, was also observed. This aligns with findings that oligotrophic bacteria and lignin-degrading fungi, capable of breaking down recalcitrant compounds and prevalent in subsoil, adapt to utilize recalcitrant compounds in carbon-limited conditions.

Maize addition underwent microbial processing and improved soil organic matter quality for microbial utilization, indicated by increased fatty acids and their esters and decreased N-containing compounds. The remaining fatty acids and their esters from maize, which microbes would preferentially utilize, appear to be stabilized through association with mineral surfaces and/or occlusion in soil aggregates. The aggregation and organo-mineral association reduce the microbial accessibility of maize-derived organic materials, particularly in subsoil with lower SOC content. The more substantial impact of maize material addition on the composition of organic compounds in subsoil versus topsoil horizons is likely due to its lower SOC content, lower SOC saturation and stronger relative stimulation of the microbial community and C cycling by the added carbon substrates.

This discussion synthesizes the highly degraded status of SOM and the various factors influencing SOM molecular composition in the neutral-to-alkaline soils of IGP, highlighting the roles of active Al/Fe, soil pH, microbial activity, agricultural practices, horizontal difference, and

the addition of external organic materials in shaping the nature and stability of SOM.

5.3 Differences between topsoil and subsoil

In IGP, subsoil total SOC is notably influenced by active Al/Fe and clay, contrasting with topsoil where no distinct impact of climatic and geochemical attributes on total SOC was observed. The significantly higher SOC content in topsoil ($13.3 \pm 6.7 \text{ g kg}^{-1}$ compared to subsoil's $3.7 \pm 0.9 \text{ g kg}^{-1}$) is attributed to its greater MAOM content. Unlike the topsoil, subsoil shows a higher proportion of non-hydrolyzable MAOM, indicating a predominance of intensively degraded and/or directly stabilized OM.

Topsoil SOC storage is more dependent on the intermediate SOC pool, with larger sizes and proportions than in subsoil. In contrast, subsoil SOC stability leans more towards its stable SOC fraction, evidenced by a higher proportion of stable SOC pool. The soil mineral reactivity in subsoil is more pronounced, with MAOM enlarged in the presence of active Al/Fe, clay, and exchangeable $\text{Ca}^{2+}/\text{Mg}^{2+}$. This contrasts with topsoil where these factors do not significantly enlarge MAOM. The stabilization role of active Al/Fe is more pronounced in subsoil, attributed to subsoil minerals' less carbon-saturated nature and a higher propensity for inner-sphere interactions. In topsoil, exchangeable $\text{Ca}^{2+}/\text{Mg}^{2+}$ shows a positive but inconsistent impact on SOC pools. High EP in the IGP likely causes carbon leaching and clay movement from topsoil to subsoil, increasing the stable pool size in subsoil. Subsoil SOM is more degraded than topsoil, with elevated levels of N-containing compounds and MAH, a result of more enhanced microbial processing and reduced fresh litter input.

From the viewpoint of stabilizing newly induced plant materials, maize-derived carbon in topsoil shows less efficient stabilization compared to subsoil, possibly due to higher SOC saturation in topsoil. Active Al/Fe in subsoil forms stable soil aggregates, aiding in the preservation of maize transformation products, but this process is less efficient in topsoil. Enhanced lignin decomposition under carbon-limited conditions of subsoil horizons is exhibited by the disappearance of maize-derived lignin in subsoil compared to its presence in topsoil. The stronger impact of maize addition on SOM composition in subsoil versus topsoil is likely due to lower SOC content and stronger microbial community stimulation in subsoil by added plant materials.

In summary, the topsoil and subsoil in the IGP display distinct differences in SOC content, stability, and degradation, influenced by active Al/Fe, clay, SOC saturation status, soil pH, microbial activity, and the response to the addition of external organic materials.

5.4 Recommendation

Focusing on the importance of climatic and soil properties in SOC stabilization and SOM molecular composition in IGP, some suggestions to improve SOC quantity and quality can be made:

- 1) Given the crucial role of active Al/Fe in stabilizing both the original SOC and newly added plant materials, strategies to increase the quantity of active Al/Fe in IGP soils could be beneficial. This might involve applying amorphous aluminum hydroxides, which are widely used in the water industry, as specific soil amendments that supply or enhance the activity of Al.
- 2) Since neutral-to-alkaline pH conditions in the IGP enhance SOM degradation, adjusting

the soil pH through applying Fe sulfate not only lowers soil pH but also adds iron, which is beneficial for plant growth and C stabilization. Lowering the pH could enhance the activity of active Al/Fe and reduce microbial activities, which is critical in stabilizing less-degraded organic components.

- 3) Applying organic matter could gradually lower soil pH and increase SOC directly. These materials release organic acids, which naturally decrease the pH when leaching is obvious. Application of OM also prevents the crystallization of Al/Fe oxides and increases active Al/Fe.
- 4) Tailoring approaches for soil horizons. Recognizing the different SOC characteristics in topsoil and subsoil. Strategies effective in topsoil, which has a greater dependency on the intermediate SOC pool, may not be as effective in subsoil, where SOC stability leans more towards the stable SOC fraction. For instance, a massive application of fresh residues into subsoil horizon may not be appropriate.
- 5) Promoting and maintaining the microbial activity to a stable status that favors SOC accumulation, possibly through a continuous application of biofertilizers and maintaining soil moisture, can enhance SOC levels.

5.5 Conclusion

In neutral to alkaline soils of IGP, despite minimal levels, active Al/Fe plays a crucial role in SOC stabilization. The stability of SOC in these soils is predominantly governed by the intermediate SOC pool, closely associated with MAOM. Active Al/Fe are the key determinants of

this intermediate SOC pool's stability in both topsoil and subsoil, overshadowing the influence of clay content and exchangeable $\text{Ca}^{2+}/\text{Mg}^{2+}$. In the subsoil, which exhibits lower carbon saturation, the carbon preservation effect of active Al/Fe is more pronounced. Active Al/Fe facilitates the stabilization of carbon from newly added organic matter, primarily as oPOM, by binding clay and silt as a framework for stable aggregate formation, along with the positive effect of clay in preserving organic matter as MAOM. In contrast, the higher soil pH, particularly in the upper regions of the IGP, tends to promote carbon mineralization.

The study also highlights the intense degradation of SOM in these soils, likely due to the notably low levels of active Al/Fe and partially attributed to the high soil pH. As soil depth increases, both the proportion of the stable SOC pool and the degree of SOM degradation increase. Higher precipitation, which lowers soil pH, enhances active Al/Fe contents and retards microbial activity, thereby slowing SOM decomposition and potentially facilitating organic matter translocation from topsoil to subsoil. Active Al/Fe is instrumental in retaining bioactive components like long-chain fatty acids and weakly degraded polysaccharides. In contrast, exchangeable $\text{Ca}^{2+}/\text{Mg}^{2+}$ and clay content, while positively affecting the size of the intermediate SOC pool, do not significantly alter the overall SOM composition. Agricultural activity, though it accelerates SOM degradation and diminishes the light fraction, may have a minimal impact on the general SOM composition and the majority of SOC pools. Although the degradation of newly added residues is intensive, the microbially processed organic matter can be substantially stabilized as MAOM or oPOM, particularly in the deeper soil horizons where carbon saturation levels are lower, thereby improving SOC quality.

In conclusion, the high soil pH and notably low active Al/Fe content in neutral-to-alkaline soils in IGP contribute to reduced mid to long-term SOC stability and enhanced SOM degradation, potentially explaining the low SOC levels in these soils. Additional research utilizing comprehensive ranges of soils and soils from natural forests in the IGP and applying in situ incubation experiments are warranted to assess the general applicability of this finding. The application of plant residues, such as mixing residues to soils and applying organic mulches, combined with strategies to lower soil pH and enhance active Al/Fe levels using industrial products, could be promising strategies for increasing both the quantity and quality of SOC in these regions and in other neutral-to-alkaline soils.

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Table S1 List of organic compounds identified by pyrolysis-gas chromatography/mass spectroscopy (PY-GC/MS)

Type	No.	Name	Retention time (min)
Undegraded polysaccharides (Undegraded PS)			
UPS	1	Levoglucosenone	8.83
UPS	2	Levoglucosan	15.21
Degraded polysaccharides (Degraded PS)			
PS	1	Cyclopentene	2.14
PS	2	Furan,2-methyl-	2.35
PS	3	Furan,3-methyl-	2.41
PS	4	Furan,2,3-dihydro-	2.59
PS	5	Cyclopentene,3-methyl-	2.6
PS	6	Cyclopentane,methylene-	2.8
PS	7	Cyclopentane,1,2-dimethyl-,cis-	2.86
PS	8	Furan,2,5-dimethyl-	3.06
PS	9	2,4-Dimethylfuran	3.11
PS	10	Aceticacid	3.17
PS	11	2-Vinylfuran	3.23
PS	12	Furfural	4.21
PS	13	3-Furaldehyde	4.52
PS	14	3-Cyclopentene-1-acetaldehyde,2-oxo-	4.53
PS	15	2-Cyclopenten-1-one	4.57
PS	16	2-Cyclopenten-1-one,2-methyl-	5.62
PS	17	Ethanone,1-(2-furanyl)-	5.67
PS	18	2(5H)-Furanone	5.87
PS	19	2-Cyclopenten-1-one,2-hydroxy-	6.02
PS	20	2-Furancarboxaldehyde,5-methyl-	6.48
PS	21	2-Cyclopenten-1-one,3-methyl-	6.6
PS	22	2-Cyclopenten-1-one,2,3-dimethyl-	7.68
Long alkane (C>22)			
Long alkane	1	C23	22.45
Long alkane	2	C24	23.3
Long alkane	3	C25	24.11
Long alkane	4	C26	24.89
Long alkane	5	C27	25.64
Long alkane	6	C28	26.38
Long alkane	7	C29	27.15
Long alkane	8	C30	28.02
Long alkane	9	C31	28.99
Long alkane	10	C32	30.14
Long alkane	11	C33	31
Short alkane (C<=22)			
Short alkane	1	C7	2.97
Short alkane	2	C8	4.06
Short alkane	3	C9	5.49
Short alkane	4	C10	7.04
Short alkane	5	C11	8.6
Short alkane	6	C12	10.11
Short alkane	7	C13	11.53
Short alkane	8	C14	12.9
Short alkane	9	C15	14.21
Short alkane	10	C16	15.44
Short alkane	11	C17	16.58

Short alkane	12 C18	17.66
Short alkane	13 C19	18.71
Short alkane	14 C20	19.71
Short alkane	15 C21	20.66
Short alkane	16 C22	21.53

n-alkene

n-alkene	1 C7-	2.9
n-alkene	2 C8-	3.93
n-alkene	3 C9-	5.49
n-alkene	4 C10-	6.91
n-alkene	5 C11-	8.48
n-alkene	6 C12-	9.97
n-alkene	7 C13-	11.42
n-alkene	8 C14-	12.79
n-alkene	9 C15-	14.11
n-alkene	10 C16-	15.34
n-alkene	11 C17-	16.5
n-alkene	12 C18-	17.59
n-alkene	13 C19-	18.64
n-alkene	14 C20-	19.65
n-alkene	15 C21-	20.61
n-alkene	16 C22-	21.51
n-alkene	17 C23-	22.41
n-alkene	18 C24-	23.26
n-alkene	19 C25-	24.07
n-alkene	20 C26-	24.86
n-alkene	21 C27-	25.6
n-alkene	22 C28-	26.29
n-alkene	24 C29-	26.99
n-alkene	25 C30-	27.88
n-alkene	26 C31-	28.88
n-alkene	27 C32-	30.03

Squalene

Squalene	1 Squalene	26.45
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Fatty acids and their esters (FAnE)

FAnE	1 Nonanoicacid	11
FAnE	2 n-Decanoicacid	12.36
FAnE	3 Dodecanoicacid	14.92
FAnE	4 Hexanethioicacid,S-methylester	15.14
FAnE	5 Aceticacid,3,7,11,15-tetramethyl-hexadecylester	16.93
FAnE	6 Isodecylmethacrylate	17.02
FAnE	7 Tetradecanoicacid	17.19
FAnE	8 Sebacicacid,but-2-enylpropylester	18.18
FAnE	9 Pentadecanoicacid	18.29
FAnE	10 Palmitoleicacid	19.12
FAnE	11 n-Hexadecanoicacid	19.3
FAnE	12 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	21.15
FAnE	13 Octadecanoicacid	21.22
FAnE	14 Myristicacidglycidylester	22.45
FAnE	15 Octanoicacid,hexadecylester	23.93
FAnE	16 Hexanoicacid,2-ethyl-,anhydride	25.09
FAnE	17 Myristylmyristate	27.75
FAnE	18 Octylpalmitoleate	29.53
FAnE	19 Tetradecanoicacid,hexadecylester	29.76

Steroids		
S	1 gamma.-Sitosterol	23.45
S	2 Cholesta-3,5-diene	26.44
S	3 Cholesta-4,6-dien-3-ol,(3.beta.)-	27.34
S	4 Cholest-3,5-diene	27.52
S	5 Cholesterol	29.68

Lignin		
LIG	1 Aceticacid,phenylester	8.01
LIG	2 Acetophenone	8.11
LIG	3 Phenol,2-methoxy-	8.55
LIG	4 Cinnamaldehyde,(E)-	8.71
LIG	5 Phenol,4-Methyl-2-methoxy-	10.08
LIG	6 Phenol,4-ethyl-2-methoxy-	11.32
LIG	7 2-Methoxy-4-vinylphenol	11.82
LIG	8 Phenol,2,6-dimethoxy-	12.3
LIG	9 trans-Isoeugenol	13.63
LIG	10 Vanillicacid	14.14

Phenols		
Ph	1 Phenol	6.78
Ph	2 Phenol,2-methyl-	7.92
Ph	3 Phenol,3-methyl-	8.23
Ph	4 Phenol,2,3-dimethyl-	9.32
Ph	5 Phenol,4-ethyl-	9.55

Monocyclic aromatic hydrocarbons (MAH)		
MAH	1 Benzene	2.71
MAH	2 Toluene	3.7
MAH	3 Ethylbenzene	4.95
MAH	4 o-Xylene	5.07
MAH	5 Styrene	5.39
MAH	6 p-Xylene	5.43
MAH	7 Benzene,propyl-	6.36
MAH	8 Benzene,(1-methylethyl)-	6.48
MAH	9 Mesitylene	7
MAH	10 Benzene,1,2,4-trimethyl-	7.01
MAH	11 Benzofuran	7.05
MAH	12 Benzene,1,2,3-trimethyl-	7.47
MAH	13 Benzene,1-ethynyl-4-methyl-	7.82
MAH	14 Benzene,(1-methylpropyl)-	7.89
MAH	15 Benzene,n-butyl-	7.96
MAH	16 Benzene,propoxy-	8
MAH	17 Benzofuran,2-methyl-	8.82
MAH	18 Benzene,(1-methyl-2-cyclopropen-1-yl)-	9.44
MAH	19 2-Methylindene	9.52
MAH	20 1H-Inden-1-one,2,3-dihydro-	11.37

Polycyclic aromatic hydrocarbons (PAH)		
PAH	1 Azulene	9.59
PAH	2 Naphthalene	10.01
PAH	3 Naphthalene,1-methyl-	11.61
PAH	4 Naphthalene,2-methyl-	11.86
PAH	5 Naphthalene,2-ethenyl-	12.75
PAH	6 Naphthalene,2,3-dimethyl-	13.12
PAH	7 Naphthalene,2,6-dimethyl-	13.33

PAH	8 2H-1-Benzopyran-2-one,3-methyl-	14.42
PAH	9 Dibenzofuran	14.6
PAH	10 Fluorene	15.42
PAH	11 Naphthalene,2,3,6-trimethyl-	15.5
PAH	12 9H-Fluorene,9-methyl-	16.88
PAH	13 Naphthalene,1,2,3,4-tetramethyl-	17.19
PAH	14 9H-Fluorene-9-one	17.23
PAH	15 Dibenzothiophene	17.41
PAH	16 Phenanthrene	17.72
PAH	17 Anthracene	17.85
PAH	18 Pyrene	20.62
PAH	19 Benzo[b]naphtho[2,3-d]furan	21.2
PAH	20 Indeno[2,1-b]chromene,	21.36
PAH	21 7H-Benz[de]anthracene-7-one	22.73
PAH	22 Benzo[b]naphtho[2,1-d]thiophene	23.5
PAH	23 Naphthalene,1-(phenylmethoxy)-	23.93
PAH	24 Chrysene	24.16
PAH	25 Benzo[k]fluoranthene	26.6

N-containing monocyclic aromatic hydrocarbons (N-MAH)

N-MAH	1 Benzonitrile	6.83
N-MAH	2 Benzene,isocyanato-	7.95
N-MAH	3 Benzonitrile,2-methyl-	8.18
N-MAH	4 Benzene,1-isocyano-2-methyl-	8.49
N-MAH	5 Benzene,1-isocyano-3-methyl-	8.71
N-MAH	6 Benzoxazole,2-methyl-	8.89
N-MAH	7 Benzonitrile	9.24
N-MAH	8 p-Tolylhydroxylamine	9.68
N-MAH	9 Quinoline	10.79
N-MAH	10 Indole	11.52
N-MAH	11 Indole,3-methyl-	12.8
N-MAH	12 Naphthalene,1-isocyano-	14.65

Alkylamides

A	1 2-Aminocyclohexanecarboxamide	4.1
A	2 N,N-Dimethylacetamide	5.56
A	3 Hexadecanamide	21.42
A	4 Palmitoleamide	23.02
A	5 Octadecanamide	23.23
A	6 Docosenamide	26.29

Other N-containing compounds (N-containing)

N	1 Acetonitrile	1.99
N	2 Propenenitrile	2.1
N	3 Propanenitrile	2.27
N	4 Isobutyronitrile	2.48
N	5 2-Butenenitrile	2.64
N	6 Butanenitrile,3-methyl-	3.27
N	7 1H-Pyrrole,1-methyl-	3.36
N	8 Pyridine	3.44
N	9 Pyrrole	3.54
N	10 Proline	3.95
N	11 2-Furancarbonitrile	4.07
N	12 Pyridine,2-methyl-	4.31
N	13 Propanenitrile,2-hydroxy-	4.37
N	14 Propanenitrile,3-hydroxy-	4.38
N	15 dl-Proline	4.39
N	16 Pyrrolidine,2-butyl-1-methyl-	4.42

N	17	1H-Pyrrole,2-methyl-	4.61
N	18	1H-Pyrrole,3-methyl-	4.74
N	19	Pyridine,3-methyl-	4.94
N	21	1H-Pyrrole,1-ethyl-	5.89
N	22	Pyridine,2,5-dimethyl-	5.98
N	23	1H-Pyrrole-2-carboxaldehyde	7.22
N	24	4-Pyridinecarbonitrile	8.12
N	25	3-Acetamidofuran	10.55
N	26	Methenamine	10.57
N	27	Valylvaline,N,N'-dimethyl-n-butoxycarbonyl-,isobutylester	10.59
N	28	Pentadecanenitrile	18.64
N	29	Hexadecanenitrile	18.76
N	30	Octadecanenitrile	20.76

Other hydrocarbons

OH	1	1,3-Cyclopentadiene	2.08
OH	2	1,4-Cyclohexadiene	2.56
OH	3	1,3-Cyclopentadiene,1-methyl-	2.59
OH	4	Cyclohexanone,4-methyl-	3.93
OH	5	Cyclopropane,pentyl-	3.95

Maize unique

M	1	Acetol	3.14
M	2	3-Amino-s-triazole	6.99
M	3	2,4-Dimethyl-2-oxazoline-4-methanol	7.17
M	4	2-Cyclopenten-1-one,2-hydroxy-3-methyl-	7.52
M	5	Maltol	8.79
M	6	2-Cyclopenten-1-one,3-ethyl-2-	8.9
M	7	Benzofuran,2,3-dihydro-	10.38
M	8	Phenol,4-ethyl-2-methyl-	10.61
M	9	Phenol,4-ethenyl-2,6-dimethoxy-	14.99
M	10	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	17.08
M	11	Neophytadiene	18.02
M	12	Neophytadiene	18.46
M	13	Hexadecanoicacid,methylester	18.9
M	14	Heptadecanoicacid	20.24
M	15	Phytol	20.73
M	16	beta-Ocimene	22.78
M	17	Hexadecanoicacid,2-hydroxy-1-(hydroxymethyl)ethylester	24.14
M	18	gamma-Tocopherol	28.62
M	19	beta-Sitosterolacetate	29.33
M	20	VitaminE	29.5
M	21	Cholest-8(14)-en-3-ol,4-methyl-(3.beta.,4.alpha.,5.alpha.)-	30.91