UNIVERSIDADE FEDERAL DE LAVRAS

HELEN CARLA SANTANA AMORIM

FROM GLOBAL TO LOCAL: TRENDS AND DRIVERS OF C:N RATIOS IN SOILS AND PARTICLE-SIZE FRACTIONS

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração Recursos Ambientais e Uso da Terra, para a obtenção do título de Doutora.

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FROM GLOBAL TO LOCAL: TRENDS AND DRIVERS OF C:N RATIOS IN SOILS AND PARTICLE-SIZE FRACTIONS

DO GLOBAL AO LOCAL: TENDÊNCIAS E FATORES QUE AFETAM AS RAZÕES C:N NOS SOLOS E NAS FRAÇÕES DE TAMANHO DE PARTÍCULA

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração Recursos Ambientais e Uso da Terra, para a obtenção do título de Doutora.

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2021

Aos meus pais e irmãos,
À todos aqueles que acreditam que a educação é o caminho,
DEDICO

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"Uma dúvida saudável é melhor que a arrogância da certeza."

(Yuri L. Zinn)

ABSTRACT

The balance between soil organic carbon (SOC) and N – assessed by C:N ratios – serves as a common indicator of soil organic matter (SOM) quality and decomposition. The potential mineralization of SOM also relates to the partition of total SOM across soil particle size fractions – sand, silt, clay - and its eventual association with such mineral components, which in turn can be affected by varying edaphic and environmental controls. Investigating the controlling factors of C:N ratios in soil and in soil particle-size fractions can provide insight into SOM stability and turnover, and thus into the potential for nutrient supply in agroecosystems, SOC sequestration and greenhouse gases emissions. Here, we aimed to assess the main trends and controlling factors of soil C:N ratios, by means of compiling global and local data on C:N ratios in bulk soils and in sand, silt, and clay fractions. For the global assessment, we compiled data from 74 studies and 30 countries. The selected studies contained data on C:N ratios in soils and in particle-size fractions (sand: Ø > 50 µm, silt: 2 < Ø < 50 µm, and clay: Ø < 2 µm), or SOC and N concentrations. Information on latitude, longitude, altitude, temperature and mean annual precipitation (MAP), Köppen climate classification, soil depth, land use (grassland, forest, and agriculture), soil pH, soil texture were also collected for modelling the C:N ratios. Global mean C:N ratio in soils was 13.2. Sand-sized SOM had the highest average (18.1) and broadest range of variation, while silt had a mean value of 14.3 and clay had the lowest mean (9.9) and the most narrow variation. C:N ratios in soils and in particle-size fractions were lower under arid climate, and higher under forests, particularly in temperate climates. Random Forest (RF) modelling suggests that sand content is the primary driver of global soil C:N ratios due to wide variability in C:N ratios of SOM associated with sands at a global scale. Köppen climate, sand content, and altitude were the most important explanatory variables for sand C:N ratios, whereas MAP, Köppen climate, and soil pH were the most relevant variables to explain variations in the global clay C:N ratios. In the local assessment, the effects of soil texture, mineralogical composition and fertility on C:N ratios in soils and in particle-size fractions were assessed under native vegetation in tropical highlands near Lavras, Brazil. Soil samples were collected in soils formed on eight contrasting parent materials at the 0-5, 30-40, and 90-100 cm depths. C:N ratios did not vary with depth (except for sand fractions), decreased with increasing silt contents, sum of exchangeable bases, and Mn oxide contents, and increased with clay, exchangeable Al^{3+} and H+Al contents. The inverse relationship between C:N ratios and soil fertility indicators in such soils is probably driven by higher retention of ammonium N forms either as cations or parts of organic molecules, particularly in the most fertile soil on itabirite. In contrary to the global trends, clay fractions were the most variable in C:N ratios, thus acting as the main driver of bulk soils C:N ratios, which was ascribed to the wide variation in clay mineralogy and activity sampled. Our findings evidenced the variables affecting C:N ratios in soils worldwide and, in the Lavras area, how C:N ratios are affected by soil texture and fertility. Such knowledge can support modeling the response of agroecosystems to changes in land use and climate at the global or regional scales. Locally, it can help designing management practices towards improved SOM accumulation and sustainable agriculture.

Keywords: soil organic matter, soil organic carbon, nitrogen, particle-size fractionation, soil texture, mineralogy

RESUMO

O balanço entre o carbono orgânico do solo (COS) e o N - avaliado pelas razões C:N - serve como um indicador da qualidade e decomposição da matéria orgânica do solo (MOS). A mineralização potencial da MOS também relaciona-se à partição da MOS total nas frações de tamanho de partícula do solo - areia, silte, argila - e sua eventual associação com esses componentes minerais, que por sua vez podem ser afetados por diferentes controles edáficos e ambientais. Investigar os fatores que controlam as razões C:N no solo e nas frações de tamanho de partícula pode fornecer uma visão sobre a estabilidade e a decomposição da MOS e, portanto, sobre o potencial fornecimento de nutrientes, sequestro de C, e emissão de gases de efeito estufa em agroecossistemas. O objetivo deste trabalho foi avaliar as principais tendências e fatores de controle das razões C:N do solo, por meio da compilação de dados globais e locais sobre as razões C:N em solos e nas frações areia, silte e argila. Para a avaliação global, compilamos dados de 74 estudos e 30 países. Os estudos selecionados continham dados sobre as razões C:N nos solos e frações granulométricas (areia: Ø> 50 µm, silte: 2 <Ø <50 µm e argila: Ø <2 µm) ou concentrações de COS e N. Informações sobre latitude, longitude, altitude, temperatura e precipitação média anual (MAP), classificação climática de Köppen, profundidade do solo, uso da terra (pastagem, floresta e agricultura), pH do solo, e textura do solo também foram coletadas para modelar as razões C:N. A média global da relação C:N nos solos foi de 13,2. A MOS de tamanho areia teve a maior média (18,1) e a mais ampla faixa de variação, enquanto o silte teve um valor médio de 14,3 e a argila teve a menor média (9,9) e a menor variação. As razões C:N nos solos e nas frações granulométricas foram menores em clima árido e maiores em florestas, particularmente de clima temperado. Os resultados de Random Forest (RF) sugerem que o teor de areia é o principal fator controlador das razões C:N do solo devido à ampla variabilidade nas razões C:N da MOS associada a fração areia em uma escala global. Clima de Köppen, conteúdo de areia e altitude foram as variáveis mais importantes para explicar as razões C:N da areia, enquanto MAP, clima de Köppen e pH do solo foram as variáveis mais relevantes para explicar as variações nas razões C:N da argila. Na avaliação local, os efeitos da textura, mineralogia e fertilidade do solo nas razões C:N nos solos e nas frações granulométricas foram avaliados sob vegetação nativa em terras altas tropicais perto de Lavras, Brasil. As amostras de solo foram coletadas em solos formados sobre oito diferentes materiais de origem nas profundidades de 0-5, 30-40 e 90-100 cm. As razões C:N não variaram com a profundidade (exceto para a fração areia), diminuíram com o aumento dos teores de silte, soma de bases trocáveis e teores de óxido de Mn, e aumentaram com os teores de argila, Al^{3+} trocável e H+Al. A relação inversa entre as razões C:N e os indicadores de fertilidade do solo em tais solos é provavelmente devida a maior retenção de formas amoniacais de N, tanto como cátions como parte de moléculas orgânicas, particularmente no solo mais fértil em itabirito. Divergindo das tendências globais, a fração argila teve a maior variação nas razões C:N, e portanto foi o principal fator afetando as razões C:N dos solos, o que se deve a ampla variação em mineralogia e atividade das argilas. Nossos resultados evidenciaram as variáveis que afetam as razões C: N em solos em todo o mundo e, para a área de Lavras, como as razões C:N são afetadas pela textura e fertilidade do solo. Esse conhecimento pode apoiar a modelagem da resposta dos agroecossistemas às mudanças no uso da terra e no clima em escala global ou regional. Localmente, pode ajudar a delinear práticas de manejo que promovam um melhor acúmulo de MOS e uma agricultura sustentável.

Palavras-chave: matéria orgânica do solo, carbono orgânico do solo, nitrogênio, fracionamento de tamanho de partícula, textura do solo, mineralogia

CONTENTS

CHAPTER 1

INTRODUCTION

Soil organic matter (SOM) is a major component of the global C cycle, playing a primary role on nutrient cycling and availability in soils (Lal, 2009). SOM also improves soil structure and water retention, increases soil biodiversity, thus improving soil health, plant productivity, and ecosystem functioning. The chemical composition of SOM and its association with some mineral components in soils can control SOM stabilization decomposition in soils, and thus, soil organic C (SOC) mineralization (Baldock e Skjemstad, 2000; Rocci et al., 2021). Investigating the mechanisms and strength of the association of SOM with mineral components of each soil is critical to understand the impacts of land use change but also climate change on SOC sequestration or depletion potential, and nutrient supply, within agroecosystems (Ding et al., 2017; Eleftheriadis, Lafuente e Turrión, 2018; Liang et al., 2014).

Soils vary widely in their contents of the three particle-size fractions, namely sand, silt, and clay. Thus, SOM studies often employ soil particle-size fractionation and SOC analysis to assess SOM pools in these fractions, which have varying “quality” and thus turnover rates (Balesdent et al., 1998; Christensen, 1992; Spohn, 2020). The “quality” of a SOM pool or organic residue is often assessed by its C:N ratio, which refers to the mass proportion between total organic C and N, and serves as an indicator of its expected decomposability and potential to mineralize of immobilize N and other nutrients (Kopittke et al., 2020; Post et al., 1985). C:N ratios in particle-size fractions of most surface soils decrease with decreasing particle size (Anderson et al., 1981; Tiessen e Stewart, 1983). Sand-sized SOM, which often have higher C:N ratios, commonly comprises plant tissues only partly decomposed, and thus is more decomposable and affected by changes in land use and soil management than clay-associated organic fractions, which tend to be more stable despite lower C:N ratios (Christensen, 1992, 2001).

SOM properties and turnover are affected by the chemical and functional composition of organic inputs, biological activity, and environmental conditions (Burke et al., 1989; Jenny, 1994). Thus, such factors can be also expected to control C:N ratios in bulk soils and in soil particle-size fractions. Effects of land use and management changes on SOC and N concentrations in bulk soils and soil particle-size fractions have been widely studied in the global literature (Baah-Acheamfour et al., 2014; Bayer et al., 2006; El-Naggar et al., 2018;

Gelaw, Singh e Lal, 2015; Nicolás, Hernández e García, 2012), although the overall results for C:N ratios are highly variable and general trends are not much clear. Studies including climate and edaphic controls on both SOC and N concentrations in soil particle-size fractions are limited, and rarely explore variations in C:N ratios (Amelung et al., 1998; He et al., 2014). Moreover, the relevance of the controlling factors may vary within spatial scales, i.e., factors that drive C:N ratios, and thus, SOM dynamics, in a local or regional scale can be ineffective on a global dimension, and vice-versa.

The present work aimed to assess factors affecting soil C:N ratios, by means of compiling global and local data on C:N ratios in bulk soils and in sand, silt, and clay fractions. We aim to provide a better perspective on how this indicator of SOM quality can help to understand SOC sequestration or depletion, and to serve as a proxy to assess sustainability of land use systems and potential nutrient supply in terrestrial ecosystems. This study was divided into two parts. The first part is a global assessment of the literature, on which the main trends on C:N ratios were identified, and the effects of various soil, geography, and environmental factors on C:N ratios in soils and particle-size fractions were investigated. The second part is a local assessment, on which the effects of soil texture, mineralogy, and fertility indicators on C:N ratios in bulk soils and particle-size fractions were investigated in tropical highlands near Lavras, Brazil, in soils derived from varying parent materials.

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CHAPTER 2

GENERAL CONSIDERATIONS

The proportion between soil organic carbon (SOC) and soil N concentrations – C:N ratios – reflect the potential of soil organic matter (SOM) mineralization or stabilization, which affects nutrient supply and SOC sequestration in terrestrial ecosystems. In soils, SOM stability and turnover is greatly affected by its association with primary particles – sand, silt, and clay. Thus, investigating the environmental and edaphic factors that affect C:N ratios in such SOM size pools can provide insight into bulk SOM mineralization and response upon changes in climate and land use. In the present study, we investigated the main trends and factors affecting C:N ratios, by means of compiling global and local data on C:N ratios in bulk soils and in sand, silt, and clay fractions, and soil characterization data.

Globally, we demonstrated that C:N ratios in the sand-sized SOM have the highest mean values and are highly variable, whereas clay C:N ratios have the lowest mean values and variability. This trend was consistent across soil depths, climate types, and land uses. Overall, the lowest C:N ratios occurred under arid climates, likely due to low primary productivity and, consequently, limited SOM accumulation. Forest soils had the highest C:N ratios within land uses, due to greater SOM inputs, particularly in the sand-sized fraction. Random Forest (RF) modelling showed that soil C:N ratios are strongly affected by sand contents, due to increased variability in sand C:N ratios. Köppen climate, clay contents, and altitude were the most relevant variables to explain sand C:N ratios, whereas MAP, Köppen climate, and soil pH are the main drivers of global clay C:N ratios. The interaction of such variables determine C:N ratios in SOM of bulk soils and particle-size fractions, and thus can be used in modelling SOM stability and turnover upon land use and climate changes, in global or regional scales.

However, the relevance of such environmental and edaphic variables as affecting factors of C:N ratios can vary across scales. Thus, we also investigated the local scale, assessing the effects of soil texture, mineralogy, and fertility indicators on C:N ratios in soils and particle-size fractions in native forest soils formed from contrasting parent materials in tropical highlands near Lavras, Minas Gerais, Brazil,. Overall, C:N ratios were unaffected by depth, except for the sand-sized SOM, most likely due to the presence of buried charcoal fragments. C:N ratios in soils and in particle-size fractions were inversely correlated with exchangeable bases and Mn oxide contents, and positively correlated with exchangeable Al^{3+} and H+Al contents. The inverse relationship between C:N ratios and soil fertility indicators in the studied soils is likely due to the increased N contents in SOM, particularly in the soil on itabirite, which unexpectedly presented very high natural fertility. Unlike the reported in a global scale, C:N ratios in clay fractions were much more variable than those in the sand sizes, due to the contrasting clay mineralogy in the Lavras area. Future studies should investigate the microbial and plant diversity in such soils, as well as organo-mineral interactions, as they can be relevant drivers of SOC dynamics, nutrient cycling, and availability in these environments.

Our study evidenced the main trends and affecting global factors of soil C:N ratios, and, for the first time, C:N ratios in particle-size fractions. This understanding is critical to predict the behavior of C and N cycles upon a global warming scenario. Although their cycling is coupled via SOM, C is more easily lost in the ecosystem than N, which tends to be more efficiently immobilized in soils. Therefore, changes in C:N ratios can evidence the decoupling of these cycles through SOM mineralization and potential CO_2 and N losses in agroecosystems. Further studies using different statistical approaches and including biological indicators may expand our results and help building a set of variables to model global SOM dynamics and SOC sequestration.

CHAPTER 3

ARTICLE 1 - Global trends and drivers of C:N ratios in soils and particle-size fractions

Running title: *Global C:N ratios in soils and size-fractions*

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Abstract

The proportions between soil organic carbon (SOC) and soil N concentrations – C:N ratios – reflect the potential of soil organic matter (SOM) mineralization or stabilization, which affects nutrient supply and SOC sequestration in terrestrial ecosystems. Soil C:N ratios depend on those of its particle-size fractions (sand, silt, and clay), which differ considerably, and the global drivers of C:N ratios for soil and particle-size fractions remain poorly understood to date. Here, we evidenced such controlling factors by compiling the global literature on soil C:N ratios and, for the first time, on soil particle-size fractions. Soil C:N ratios varied between 7.3 and 43, with a mean value of 13.2, and were lower under arid climate (10.5) and grasslands (11.1). Sand C:N ratios had the highest variation (4.4 to 46) and mean value (18.1), whereas clays had the lowest range (5 to 17.6) and mean (9.9), and silt was intermediate. Low C:N ratios in clays and their stability under land-use change result from mineral-organic associations, which lack in sand fractions. Random forest modelling suggests that sand content is the primary driver of global soil C:N ratios due to wide variability in sand C:N ratios. Climate, soil texture, and altitude are the main drivers of sand and silt C:N ratios, whereas the C:N ratio of the clay-sized fraction is driven by climate and soil pH. Such variables can be used to model C:N ratios in soils and particle-size fractions and the global or regional response of SOM dynamics to land-use conversion and climate change.

Keywords: soil organic matter, particle-size fractionation, SOC sequestration, land use change, soil texture, climate.

Introduction

Soil organic matter (SOM) is inextricably linked to nutrient cycling and availability in soils (R. Lal, 2009), thus on terrestrial ecosystems productivity and functioning (Rattan Lal, 2016). Soil organic carbon (SOC) comprises most of SOM mass, and is a major component of the global C cycle. The biogeochemical cycles of C and N are closely coupled within SOM and affect its fate, due to the elemental stoichiometry between these elements in plant biomass, its alteration by microorganisms (Kirkby et al., 2011), and eventual stabilization in soil pools. The mass proportion between SOC and N ─ C:N ratios ─ serves as a proxy of SOM decomposability and potential to mineralize or immobilize N and other nutrients. Investigating patterns and controlling factors of global soil C:N ratios is critical to understand the impacts of land use and climate changes on SOC sequestration or depletion, as well as nutrient supply in agroecosystems (Chen et al., 2019; F. Ding et al., 2019; Eleftheriadis et al., 2018).

Soil C:N ratios are relatively stable at regional and global scales (Ge et al., 2019; Tian et al., 2010; Zinn et al., 2018), but can be significantly affected by land use change according to patterns thus far unclear. Increasing temperatures and decreasing precipitations can increase or decrease soil C:N ratios in arid regions, and this decoupling in C and N cycles impairs primary production in such environments (Delgado-Baquerizo et al., 2013; Jiao et al., 2016; Wang et al., 2020). In temperate regions, soil C:N ratios tend to increase with increasing precipitation, altitude, and under conifer forests (Marty et al., 2017; Tashi et al., 2016), but it is difficult to isolate the effects of climate from vegetation. Tree species are even reported as the key variable explaining soil C:N variations in temperate forest soils, whereas soil type and climate play a secondary role (Cools et al., 2014). Effects of soil texture and pH on soil C:N ratios are often overridden by climate (Jiao et al., 2016; Wang et al., 2020), altitude, and vegetation (Marty et al., 2017), despite their pivotal role in SOC retention (Yuri L. Zinn et al., 2007a) and stabilization mechanisms (Rasmussen et al., 2018).

Soils vary widely in contents of the three particle-size fractions ─ sand, silt, and clay ─ due to different parent materials and weathering. Thus, particle-size fractionation and SOC analyses are often used to assess the partition of SOM within particle-size pools, which have different stabilization mechanisms and turnover rates (Balesdent et al., 1998; Bent T. Christensen, 1992; Spohn, 2020). For example, sand-sized SOM mostly comprises partially decomposed plant tissues, has higher C:N ratios, and is more decomposable and vulnerable to changes due to land use and soil management than clay-sized SOM, typically with lower C:N ratios and more stable (B. T. Christensen, 2001; Bent T. Christensen, 1992; Guggenberger et al., 1994). However, studies on how C:N ratios in soil particle-size fractions respond to land use and climate changes are scarce compared to bulk soils, results and trends are highly variable (Amelung et al., 1998; Baah-Acheamfour et al., 2014a; Chen et al., 2019; Eleftheriadis et al., 2018; He et al., 2014), and a global perspective is lacking. Investigating which factors control C:N ratios in bulk soils and soil particle-size fractions can help elucidating unclear patterns and provide a more comprehensive understanding on soils response to land use and climate change. Thus, we compiled the global literature on C:N ratios in bulk soils and in sand, silt, and clay fractions, and hypothesized that i) C:N ratios in bulk soils and particle-size fractions are affected by selected geographic, climate and edaphic factors (H1), and ii) C:N ratios in coarser fractions (sand- and silt-sized) will be the most affected by land use (H2).

Material and Methods

Data collection

Our database was built from a worldwide literature survey of peer-reviewed publications and dissertations showing data on C:N ratios, SOC and N concentrations in both bulk soil and particle-size fractions, the latter much rarer than the former. The bibliographic searches were performed through the databases Scopus and Google Scholar. The following search parameters were initially used: ‘C:N ratio’, ‘particle-size fraction’, and ‘soils’. The terms added to the initial keywords identified studies by the soil dispersion method for the particle-size fractionation, which included ultrasonic dispersion or sonication. To narrow down our results, we applied the advanced search tool of the Google Scholar database, including the terms ‘ultrasonic’ and ‘ultrasound’ and excluding the terms ‘aggregate’, ‘particulate’ and ‘polytungstate’ (a chemical used for density-based separations). The terms excluded from the initial keywords prevented studies of C:N ratios in soil aggregates and SOM density separates, which are not relevant to our purposes. Then, we consistently searched for studies on specific world regions and countries with diverse land uses and climate, and we only selected studies that included field data. Incubation studies were only selected when data from original soil samples were available. The whole database and its summary, collated according to climate, depth class, and land use, are listed in Supplemental Information 1 and Extended Data Table 1, respectively.

We chose reports that included data on bulk soil, sand, silt or clay C:N ratios, or SOC and total N data on sand, silt or clay, and then calculated the C:N ratios. Total N, and alternatively C:N data were calculated, when only SOC and C:N or SOC and N data were presented, respectively. Analytical methods included dry or wet (dichromate) combustion for SOC, and Dumas combustion or Kjedahl distillation for total N. The dispersion methods before the particle-size fractionation included chemical dispersion with sodium and shaking, and sonication or ultrasonication. Particle-size fractionation methods included dry or wet sieving to isolate the sand fraction, and sedimentation following Stoke's law or centrifugation to separate the silt and clay fractions. As the selected papers presented variable particle size classifications, the following particle-sizes were used to ensure data comparability: 2,000-50 µm (sand), 50-2 µm (silt), and <2 µm (clay). Data on mixed particle size separates (i.e. clay + silt, <20 or 50 µm) were not included. Particle-size fractions subdivided in coarse, medium, and fine fractions were summed into one single fraction, weighed by the percentage of each sub-fraction, and had their C:N ratios averaged to a single value.

Along with the bulk soil and soil particle size C:N ratios, information on environmental settings of study sites was also collected. The following data were recorded: latitude, longitude, altitude (m), mean annual temperature (MAT; °C), mean annual precipitation (MAP; mm), Köppen-Geiger climate (Peel et al., 2007), soil classification, soil sampling depth (cm), land use, soil pH, particle size distribution (%), soil organic carbon (SOC; g kg^{-1}), and soil N (g kg^{-1}). When the report did not provide the geographical coordinates of the study, we chose the nearest named site using the Google Maps platform (https://www.google.com/maps) to fulfill the missing information. Figure 1 shows the location of the selected studies. The website *https://en.climate-data.org/*, based on the Copernicus Climate Change Service data source, was used to search for missing data on MAT, MAP, and Köppen climate. For missing information on altitude, data from the nearest meteorological station were recorded using the Google Earth database. As the reports had varying soil classification systems, we correlated the given information into soil orders following the FAO-WRB Legend to ensure data comparability.

Data Analysis

Our database was divided into three nominal categories (climate, land use, and soil depth). The "climate" category consisted of A (tropical), B (arid), C (temperate), and D (cold)

Köppen-climate types as described by the original reports, based on their MAT and MAP information, or per the *https://en.climate-data.org/* database. The varying land uses found in the original reports were broadly grouped into ‘grassland’, which included native grasslands and intensively managed pastures; ‘forest’, including native and plantation forests, agroforestry systems, and fruit orchards; and ‘cropland’ included annual and some perennial crops, such as corn, soybean, wheat, rice, cotton, cassava, beans, barley, sorghum, among others. Land use systems described as ‘native vegetation’, without mention to grasslands, were included in the ‘forest’ land use. Soil depths were arbitrarily divided into ‘topsoil’, which included soil layers within the upper 30 cm, as well as layers originally described as ‘A’ or ‘Ap horizon’, and ‘plow layer’, whereas ‘subsoil’ included soil layers below 30 cm. Descriptive statistics were calculated (Supplemental Material 3) for all the numerical variables (soil depth, sand, silt, and clay contents, soil pH, latitude, MAT, MAP, SOC and TN concentrations, and C:N ratios in soil and in particle-size fractions (Extended Data Table 2), and by category for selected variables (SOC, TN, and soil and particle-size fractions C:N ratios; Table 1). The Kruskal-Wallis test ($p < 0.05$) was performed to compare the SOC and N concentrations and C:N ratios within each category.

We computed Pearson’s correlation coefficients for the selected numerical variables vs. the soil and particle-size C:N ratios. The relative importance of each of the different predictors for C:N ratios was quantified by the machine-learning technique ‘random forest with conditional inference trees’ - *regr.cforest* (Strobl et al., 2007). The models created by this technique are mostly resistant to overfitting and do not require assumptions about the relationship between the predictors and the target variable. Furthermore, random forests can model non-linear relationships as linear relationships (Prasad et al., 2006). To achieve optimal model convergence, an ensemble of random forests was created using the *baggingwapper* method of the *mlr* package. The number of trees and the *min split* criterion was optimized for each predictor (soil, sand, silt or clay C:N) using the *tuneParams* function of the *mlr* package, and we calculated tenfold cross-validated R^2. After fitting the model, we evaluated the conditional importance of each parameter for the model. Additionally, partial dependence plots (PDPs) were used to test effects between the predicted C:N ratios and independent controls across the range of possible values that were included in the regression forest modeling. PDPs can illustrate the average marginal effect of one or more independent variables on the predicted outcome of a machine learning model across a specific range of values (Friedman, 2001), and show whether the relationship between the predicted variable and an independent control is linear, monotonic or complex (Elith et al., 2008). Data compilation was performed using an electronic spread-sheet. Statistical analyses and models were performed in the R software (R Core Team, 2020) with the *party* and *mlr* packages (Hothorn, Buhlmann, et al., 2006; Hothorn, Hornik, et al., 2006; Strobl et al., 2007, 2008).

Results

Database summary

The data set comprised 317 observations from 74 studies published between 1980 and 2019, covering 29 countries (Fig. 1). Most studies were performed in North America (19) and Western Europe (25). The greatest number of observations refers to Brazil (n = 48), Canada (n = 45), and the USA (n = 36). The temperate Köppen climate (C) was the most common type (Extended Data Table 1), especially Cfb (temperate oceanic) and Cfa (humid subtropical). Most information on C:N ratios in bulk soils and soil particle-size fractions refer to topsoil (n = 251). The most common land use was cropland (n = 139), followed by 102 observations from forests, and 66 from grasslands (Table S1). We were unable to retrieve information on land use for 10 reports.

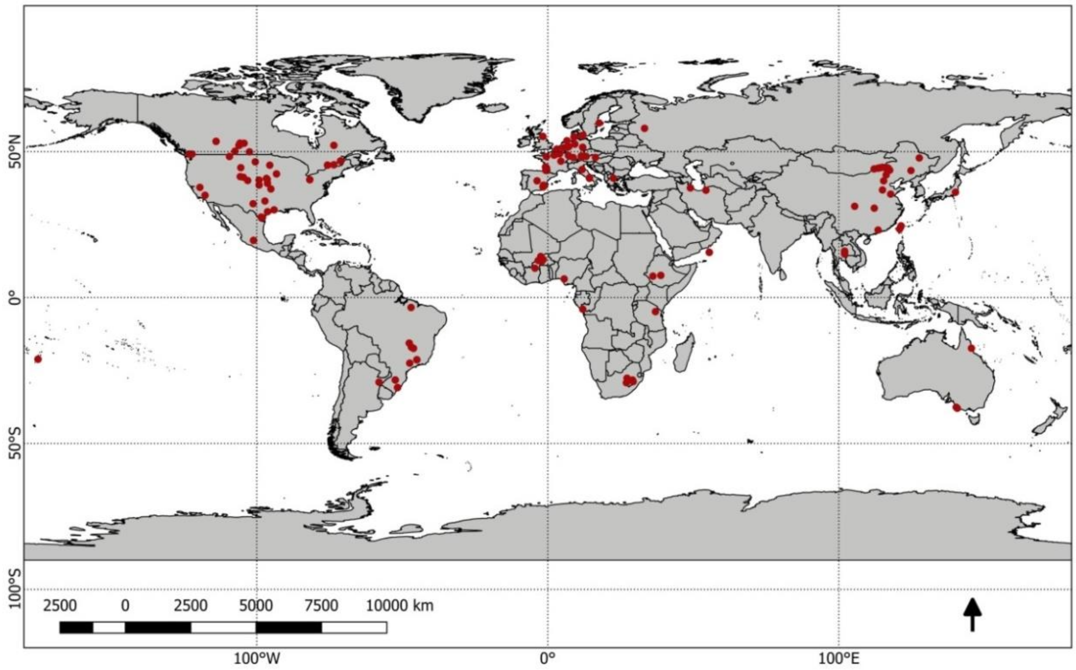

Fig. 1 World map showing the location of the surveyed sites compiled from the literature.

Our database is comprehensive and representative of the large soil and climate variability in the world. Data on C:N ratios of soil and particle-size fractions refer to samples collected at depths between 2.25 and 100 cm, with a mean value of 23.1 cm and modal value of 10 cm (Table S2). Soil texture varied accordingly, with sand contents varying between 2.1 and 96.9%, silt varying between 0.6 and 83.5%, and clay ranging from 1.5 to 82.7%. Soil pH ranged between 3.0 and 8.5, with mean of 5.7 and modal value of 4.0. The studied sites were located between 2 and 3,200 m a.s.l. MAT ranged from 0.5 to 29°C and MAP varied between 140 to 4,100 mm. Mean SOC and N concentrations were 25.2 and 1.95 g kg^{-1}, respectively. SOC concentrations ranged between 0.5 and 145 g kg^{-1}, whereas N ranged between 0.07 and 9.7 g kg^{-1}. As expected, higher SOC and N concentrations occurred in the top 30 cm depth, corresponding to the upper quartiles of 32 g SOC kg^{-1} and 2.53 g N kg^{-1}.

C:N ratios varied much less than SOC and TN concentrations (Table S2). Soil C:N ratios ranged between 7.3 and 43.0, with a mean value of 13.2, median of 11.7, and modal value of 11.0. Mean C:N ratios in particle-size fractions decreased with particle size (Fig. 2).

Sand C:N ratios varied between 4.4 and 46.0, i.e. more widely than bulk soils, with a mean of 18.1. Although bulk soil and sand C:N ratios had similar modal values, sand had a higher median (16.7) and upper quartile (21.3) compared to bulk soil. Silt C:N ratios showed a narrower range (7.0 to 41.8), with lower mean (14.3) and median (13.1). Remarkably, clay C:N ratios showed the narrowest range (5.0 to 17.6), and much lower mean and median (9.9 and 9.6, respectively). The wider variability in sand C:N ratios (Fig. 2), aside with the lower ranges for silt and especially clay, suggests that sand contents exert a primary control on C:N ratios for bulk soils.

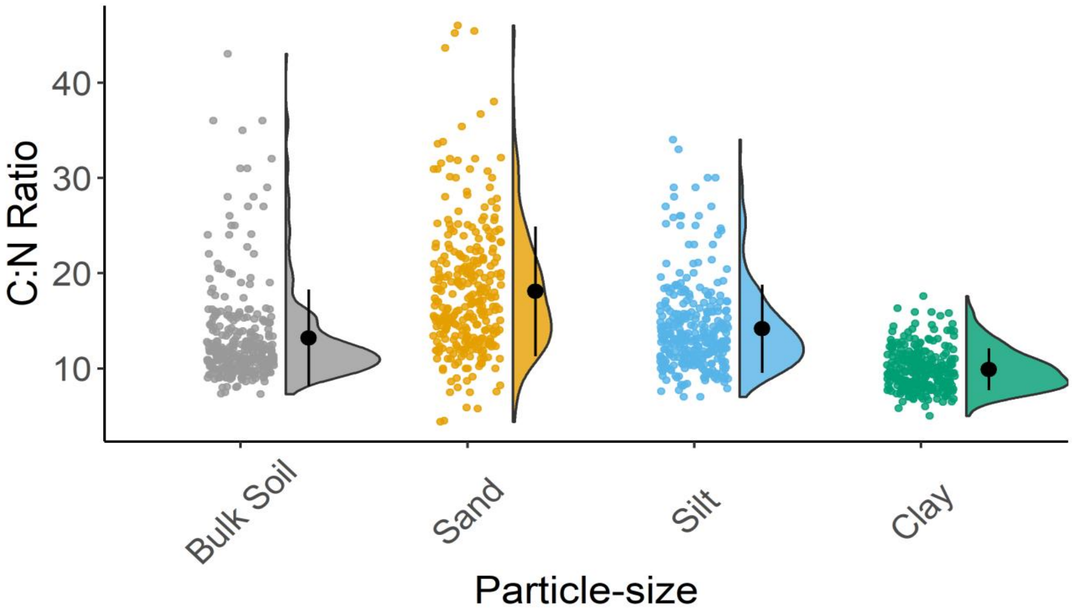

Fig. 2 Raincloud plot of C:N ratios in bulk soil and soil particle-size fractions (clay, silt, and sand). The plot displays the C:N ratio (horizontally jittered), the density distribution, its mean and 95% confidence interval.

High C:N ratios in the sand- and silt-sized fractions (Table S2) also occurred when data were stratified by climate, land use, and depth intervals (Table 1). Sand-sized fractions always had the highest C:N ratios (14.1 - 21.0). Clay had the lowest variation (8.7 - 10.8) and silt showed intermediate values (10.8 - 15.7). In bulk soil and all size fractions, the lowest

C:N ratios occurred under arid climates. Temperate and cold climates had increased SOC and TN concentrations compared to tropical regions, yet their C:N ratios in soil and particle-size fractions were not different ($p < 0.05$). Silt C:N ratios under tropical climates were even higher than those under cold climates. Despite the contrast in SOC and N concentrations between topsoil and subsoil, C:N ratios in bulk soils and particle-size fractions did not differ with depth intervals. Among land uses, forest had higher SOC and N concentrations than cropland, and the highest soil and sand C:N ratios. Grasslands had the lowest numerical values of C:N ratios, and the lowest silt C:N ratio. Thus, we accept H2, as C:N ratios in the sand and silt fractions differed within land uses.

Table 1. SOC and N concentrations (g kg^{-1}) of bulk soils, and mean C:N ratios ± mean standard error of the means for bulk soil and particle-size fractions per category (climate, soil depth, and land use).

Variable	**Climate**				**Soil Depth**		**Land Use**		
	Tropical	Arid	Temperate	Cold	Topsoil	Subsoil	Cropland	Forest	Grassland
SOC	15.2b±2.5	10.1b±1.4	28.9a±1.9	35.2a±2.8	28.3a±1.5	9.8b±1.2	17.6b±1.0	33.1a±2.8	23.6ab±2.6
TN	1.3c±0.2	0.8c±0.1	2.1b±0.1	2.9a±0.2	2.2a±0.1	0.8b±0.1	1.4b±0.1	2.4a±0.2	2.1ab±0.2
Soil C:N	12.0a±0.4	10.5b±0.2	14.6a±0.5	12.1a±0.4	13.5a±0.3	12.0a±0.5	12.8b±0.4	15.0a±0.7	11.1b±0.2
Sand C:N	20.9a±1.6	14.1b±0.8	18.7a±0.5	17.6a±0.6	17.6a±0.4	20.3a±1.3	17.2b±0.5	21.0a±0.8	15.9b±0.8
Silt C:N	15.7a±0.7	10.8c±0.3	15.4ab±0.4	13.2b±0.4	14.3a±0.3	14.2a±0.8	14.8a±0.4	15.4a±0.6	11.9b±0.3
Clay C:N	10.8a±0.3	8.7b±0.2	9.8a±0.2	10.5a±0.4	9.8a±0.1	10.1a±0.4	9.9a±0.2	10.3a±0.3	9.4a±0.2

Different letters indicate significant differences by the Kruskal-Wallis test ($p < 0.05$) within the same row and within the same category. Topsoil refers to 0-30 cm depths, and subsoil refers to > 30 cm depths. SOC: soil organic C (g kg^{-1}); TN: total N (g kg^{-1}).

Table S3 lists the Pearson's correlation coefficients between climate, geography, and soil properties in our database. SOC and N contents were negatively correlated with depth (r = -0.39 and -0.40, respectively). Latitude had a strong, negative correlation with MAT (r = -0.73), and moderate, negative correlations with MAP (r = -0.45) and clay content (r = -0.43). MAT was also correlated with MAP (r = 0.39) and clay content (r = 0.50), and negatively correlated with silt content (r = -0.34), SOC, and TN (r = -0.22 for both). MAP was positively correlated with clay content (*r* = 0.30), SOC (*r* = 0.43) and TN (*r* = 0.35), and negatively correlated with soil pH (*r* = -0.32). Soil pH also had a negative correlation with sand content (*r* = -0.33). Total N was positively correlated with silt (*r* = 0.31) and negatively correlated with sand content (*r* = -0.31). Lastly, SOC and TN were highly and positively correlated (*r* = 0.94).

Controlling factors of C:N ratios in soil and particle-size fractions

The moderate Pearson's correlation coefficients between sand and silt contents with bulk soil and silt C:N ratios, and between soil pH and clay C:N ratios (Table S3) suggest a possible control of soil texture and pH on C:N ratios. However, the correlations between other variables and C:N ratios in soil and particle-size fractions were generally poor. Thus, modeling C:N ratios as a function of selected variables was attempted to better assess their effects on C:N ratios. The model for bulk soil C:N ratios had a relatively high R^2 (0.69, Fig. 3). Sand and silt contents were the most important explanatory variables, confirming the trends discussed earlier, followed by Köppen climate. Particularly, sand content accounted for almost 50% of the relative importance. Partial dependence plots (PDPs) showed that soil C:N ratios increase steeply for sand contents > 75% (Fig. S1). These soils were mostly Spodosols under temperate climate (Table S4), in which most SOC is uniquely retained as Fe- and Al-chelates.

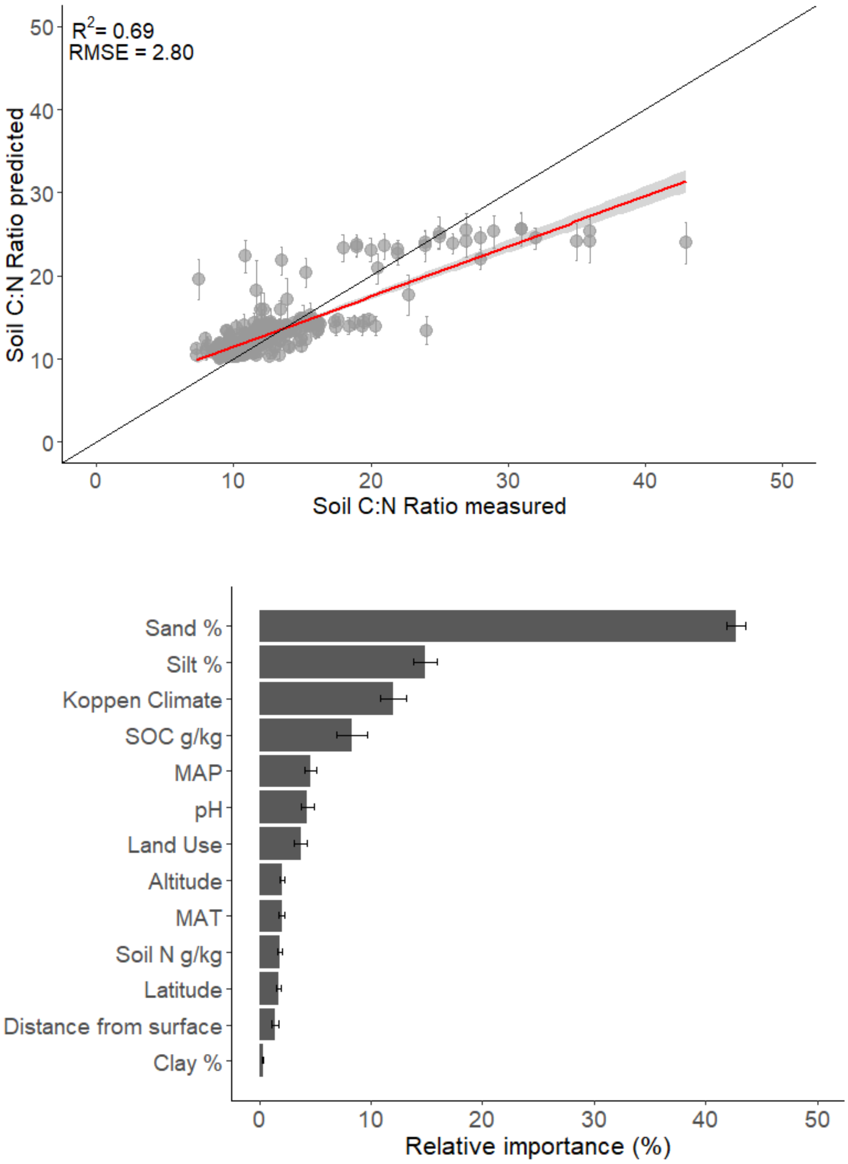

Fig. 3 Soil C:N predictive model and importance of the explanatory variables. The red line indicates the model fit and the gray shadow indicates the 95% confidence interval. MAP, mean annual precipitation; MAT, mean annual temperature; Distance from surface, soil depth.

Sand fractions issued the most scattered plot (R^2 = 0.50, RMSE = 4.78, Fig. 4), in consequence of the wider variability in sand C:N ratios (Table S2). Köppen climate, clay contents, altitude, and land use were the most important explanatory variables. Sand C:N ratios were generally higher under forests (as demonstrated earlier), at pH < 5.0 and at altitudes < 1,000 m (Fig. S2). In addition, sand C:N ratios increased when clay contents were

> 60% (Fig. S2). The silt C:N ratio model followed more closely the 1:1 measured vs. predicted line, thus presenting a better R^2 of 0.62 and lower RMSE (3.05) (Fig. 5). The most important variables to explain global silt C:N ratios were Köppen climate, silt and sand contents. Similar to soil C:N ratios, increased silt C:N ratios occurred in soils with sand contents higher than 75% and low silt content (Fig. S3).

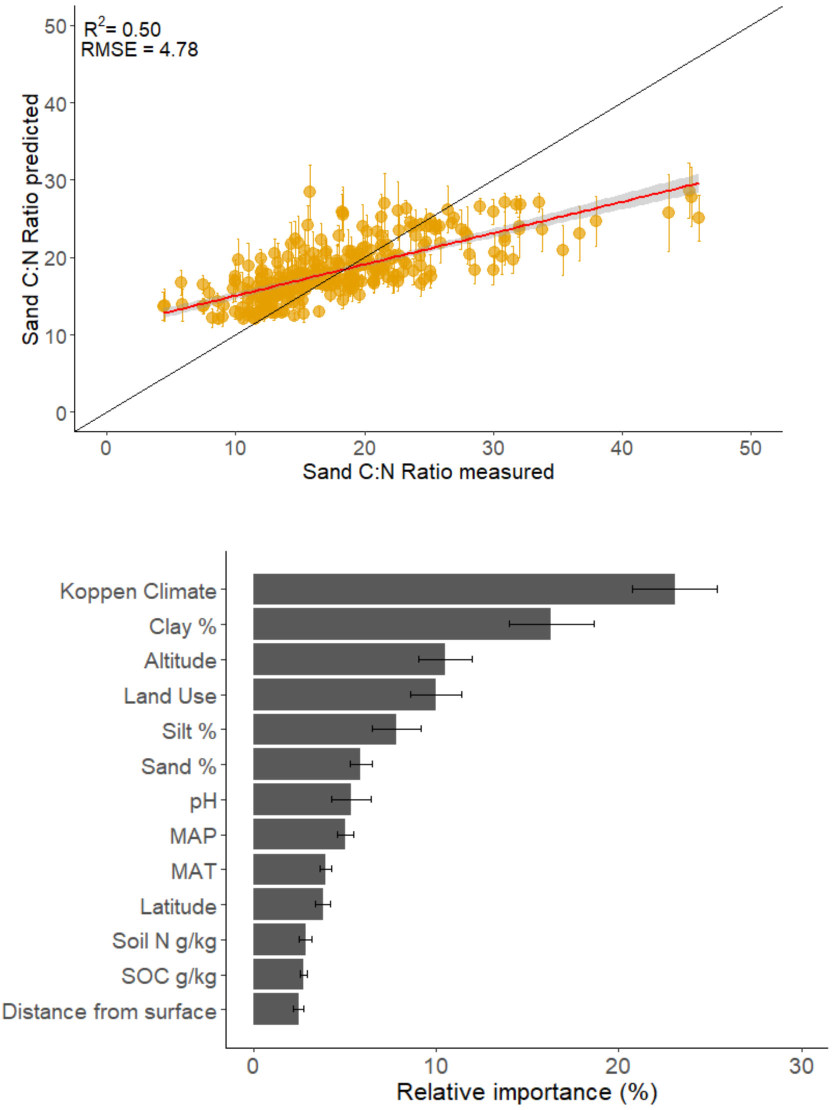

Fig. 4 Sand C:N predictive model and importance of the explanatory variables. The red line indicates the model fit and the gray shadow indicates the 95% confidence interval. MAP, mean annual precipitation; MAT, mean annual temperature; Distance from surface, soil depth.

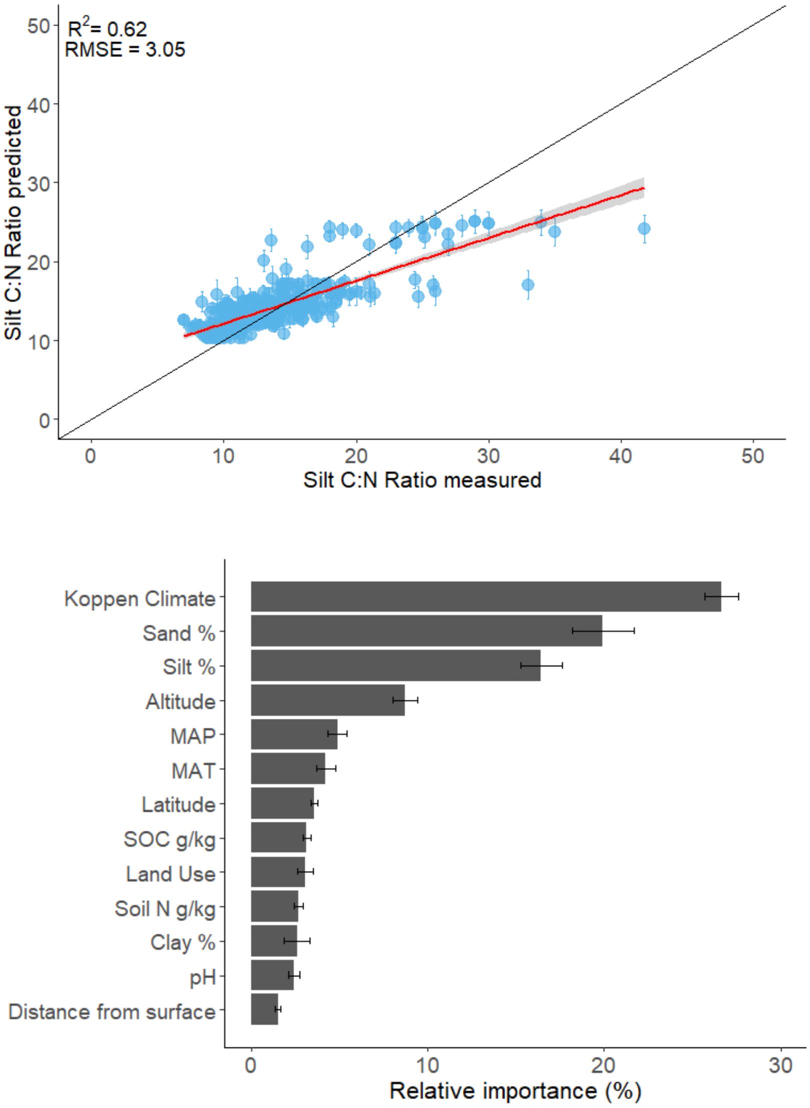

Fig. 5 Silt C:N predictive model and importance of the explanatory variables. The red line indicates the model fit and the gray shadow indicates the 95% confidence interval. MAP, mean annual precipitation; MAT, mean annual temperature; Distance from surface, soil depth.

The clay C:N ratio model was the least scattered (RMSE = 1.63). Remarkably, the apparent slope near zero (Fig. 6) caused a low R^2 of 0.44, and reflected the overall homogeneity of C:N ratios in clay fractions. MAP, Köppen climate, and soil pH were the most important explanatory variables, reflecting the effect of arid climates lowering C:N ratios as discussed earlier. Indeed, PDPs showed that clay C:N ratios tend to values of 10.25 for MAP > 1,500 mm, and < 9.5 for MAP < 1,000 mm (Fig. S4).

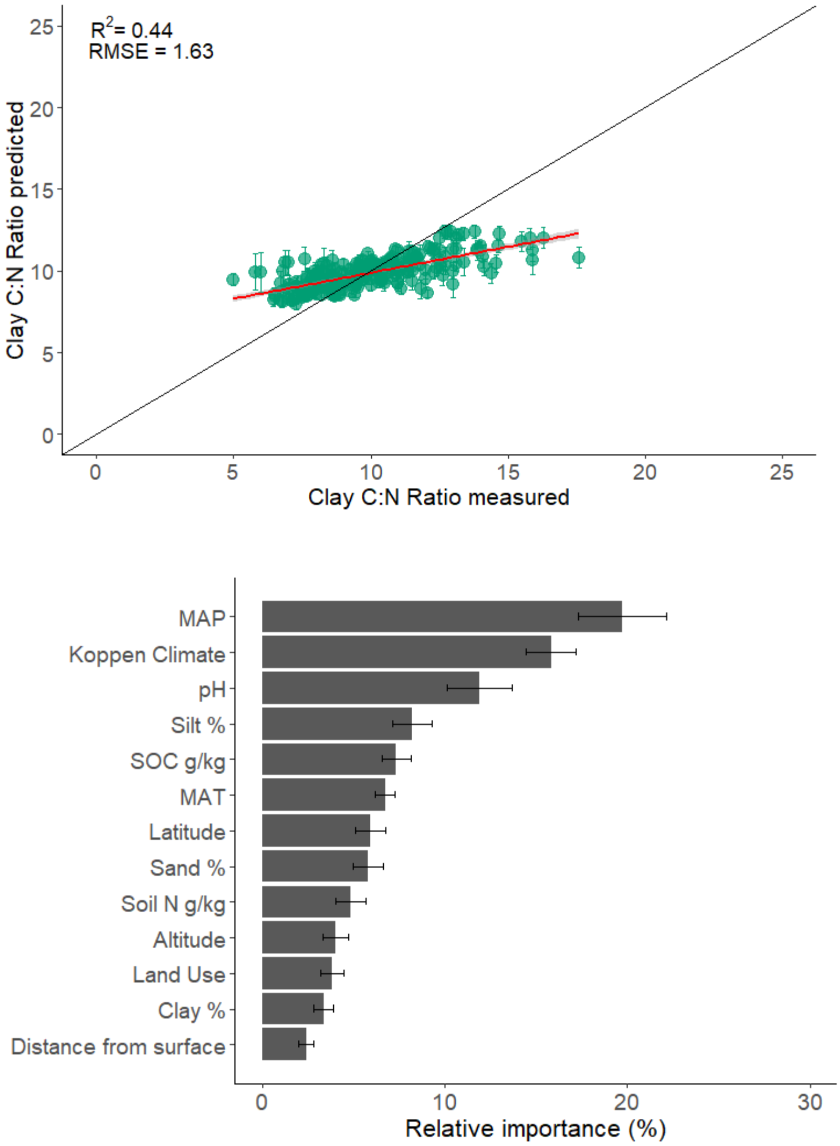

Fig. 6 Clay C:N predictive model and importance of the explanatory variables. The red line indicates the model fit and the gray shadow indicates the 95% confidence interval. MAP, mean annual precipitation; MAT, mean annual temperature; Distance from surface, soil depth.

Discussion

Trends in soil properties and global C:N ratios

The most common effects of geography and climate on key soil properties were evident in our database. Clay content, a key indicator of soil development, was negatively correlated with latitude (*r* = -0.43) and positively correlated with MAT and MAP (*r* = 0.50

and 0.30, respectively), thus reflecting the trend for increased clay contents in tropical, weathered soils. Soil pH was negatively correlated with MAP, due to increased base leaching (Slessarev et al., 2016), and also increased plant biomass and production of organic acids, which contribute to soil acidification. The positive correlations between MAP and SOC (r = 0.43) and MAP and TN (r = 0.35) indicate the positive relationship between precipitation and primary productivity. MAT was negatively correlated with SOC and TN (r = -0.22 for both correlations), owing to lower SOM decomposition rates at lower temperatures (Jenny, 1994). SOC and N were negatively correlated with soil depth (r = -0.39 and -0.40, respectively), owing to increased organic inputs to the soil surface. Although often reported in a regional scale (Ge et al., 2019; Zinn et al., 2007a), clay contents did not correlate either with SOC or TN, likely due to the overarching controls exerted by climate and geography in a global scale. Finally, SOC and TN were highly and positively correlated (r = 0.94), due to their coupled, but not fixed, cycling as SOM.

Our study confirmed that C:N ratios decrease with decreasing particle-size. C:N ratios in clays are generally lower than whole soils, whereas C:N ratios of silt and sand are higher, since the sand fraction contains is mostly comprised by particulate organic matter (Bent T. Christensen, 1992). Low C:N ratios in the clay-sized fraction are traditionally ascribed to abundant, intermediate SOM decomposition products generated by microbial activity, including N-rich amino-sugars and amino acids (Angst, Mueller, et al., 2021; Guggenberger et al., 1994; Knicker et al., 2000). In fact, a study using nano-scale secondary ion mass spectrometry (NanoSIMS) and labelled residue demonstrated that N-rich microbial products originating from OM decomposition attach directly to mineral surfaces, forming new organo-mineral associations and enhancing SOM stabilization (Kopittke et al., 2018). Besides, the clay fraction can contain more mineral-fixed ammonium-associated forms than the silt and size separates (Bent T. Christensen, 1992). Alternatively, it can also reflect an optimal ammonium N/C proportion favoring SOM sorption onto clay surfaces, which deserves investigation.

The low C:N ratios under arid climates have been noted earlier (Delgado-Baquerizo et al., 2013) in bulk soils, although its underpinning causes and effects on SOM turnover are not well understood. In mesic soil temperature regimes, low soil C:N ratios are generally attributed to low primary production and SOC concentrations (Wang et al., 2020). Here, this trend is partially associated with lower C:N ratios under grasslands, which included steppes from arid regions. Most croplands and pastures in the original reports received N through mineral fertilization or manure, which could possibly reduce C:N ratios in soils and particle-size fractions compared to forest soils, or promote SOM priming with a similar lowering effect on C:N ratios (Kuzyakov, 2010). The main products of SOM mineralization are CO_2, NO_3^- and NH_3, but the latter can easily accept a proton and sorb onto clays as NH_4^+, or can be taken up by the microorganisms, also as NO_3^-. On the other hand, CO_2 is preferably lost from soils (Kuzyakov, 2010; Spohn, 2020), resulting in the decoupling of SOC and TN via partial N conservation and thus in lower C:N ratios.

More abundant and frequent organic inputs are the apparent cause for generally higher soil and sand C:N ratios under forests (Ge et al., 2019), as evidenced by the higher SOC concentrations (Table 1). Changes in sand C:N ratios have been ascribed to rapid but disproportional depletion (or increase) in SOC compared to N after land use change (Baah-Acheamfour et al., 2014b; Ding et al., 2017; Eleftheriadis et al., 2018; Nicolás et al., 2012; Solomon et al., 2002). Besides, the sand-sized fraction is mostly particulate organic matter, which is not stabilized by organic mineral complexes (Guggenberger et al., 1994; Solomon et al., 2000) and is only partially occluded within soil mineral aggregates, thus delaying the decomposition by soil fauna (Zinn et al., 2007b). Compared to other particle-size fractions, sand-sized organic matter is more susceptible to changes associated to land use, although microbial-derived decomposition by-products can also be affected, which would potentially affect the silt- and especially clay-sized fractions C:N ratios (Ding et al., 2017; Guggenberger et al., 1995; Zhang et al., 1999).

Effects of soil properties, climate and geography on global C:N ratios

Soil particle-size distribution played a pivotal role in controlling global C:N ratios. Particularly, sand content was the most relevant variable to explain soil C:N ratios, which is primarily due to the wide variability in sand C:N ratios (Table S2). Previous studies have demonstrated the influence of soil texture on soil organic matter mineralization and SOC storage, especially for silt and clay (Angst et al., 2021; Wiesmeier et al., 2019; Zinn et al., 2007a), due to the physical protection and chemical stabilization of organic matter (Hassink, 1997; Six et al., 2002). Luo et al. (2021) indicated that sand content is one of the soil attributes that most affects global SOC stocks. Sand content has effects on the formation and transformation of soil aggregates, which in turn regulate SOC stability, soil porosity, and thus, oxygen availability for the microbial degradation of organic matter (Dungait et al., 2012). Similar to our findings, Wang et al. (2020) observed that soil C:N ratios increased with sand content in drylands of northern China. However, the authors argued that silt and clay contents were more important than sand in driving variations in soil C:N:P ratios due to greater nutrient retention and chemical stabilization of organic matter.

Sand C:N ratios had the widest range of variation compared to the silt- and clay-sized fractions, and were mostly affected by climate, clay contents, altitude, and land use. Sand-sized organic matter (50-2,000 µm) encompasses partially degraded plant residues, with varying degrees of alteration, which explains the wider variability in C:N ratios compared to the other particle-size fractions. The relevance of altitude as a controlling factor of SOC and N dynamics in soils has been demonstrated regionally, but thus far no altitudinal effects on C:N ratios of particle-size fractions were reported on a global scale. Lower temperatures at higher altitudes slow organic matter decomposition, leading to increased SOC content (Tashi et al., 2016; Y. L. Zinn et al., 2018). Light fraction SOM pools can be more sensitive to altitude-induced climate changes than the heavy, finer fractions, associated the mineral matrix (Wagai et al., 2008), which may explain the control exerted by altitude on sand C:N ratios.

Similar to the soil C:N ratio model, climate and soil texture were the most relevant variables affecting silt C:N ratios. The pattern of increasing C:N ratios with increased sand contents (> 75%) was also observed for the silt fraction, which probably reflects the Spodosols in our database. Because silt is an intermediate fraction between sand and clay, it has properties common to both fractions, and not surprisingly, the controlling factors of its C:N ratios resemble those of bulk soil. As discussed, sand content affects the formation and stability of aggregates, and thus the mineralization of organic matter in soils. Silt content is one of the best predictors of soil C:N ratios in China northern drylands (Wang et al., 2020) due to greater organic matter stabilization compared to the sand fraction, which affects nutrient stoichiometry in soil particle-size fractions. Along with sand contents, silt was pointed out as one of the most important controls of global SOC stocks (Luo et al., 2021).

Lastly, C:N ratios in clays were driven by climate and soil pH. Particularly, increased clay C:N ratios for MAP > 1,500 mm and lower values for MAP < 1,000 mm confirm the lowering effect of arid climates on C:N ratios. Accordingly, SOC retention by clay minerals increases with MAP across a range of parent materials and biomes (Kramer & Chadwick, 2018). Soil pH has been indicated as one of the primary drivers of changes in C:N ratios in arid ecosystems (Jiao et al., 2016; Wang et al., 2020), as it reflects the status of nutrient availability in soils and the type of organo-mineral bonds, which in turn determine the mechanisms of organic matter stabilization in soils (Rasmussen et al., 2018). In addition, soil pH controls microbial activity and diversity (Shen et al., 2013; Xu et al., 2020), which directly affect organic matter decomposition. In our study, the trend for decreasing clay C:N ratios with increasing soil pH is likely a result of low organic inputs and soil alkalinity in arid regions, with subsequent slow decomposition and stabilization of organic matter in clay fractions.

Although only marginally important to explain soil C:N ratios, Köppen climate affected C:N ratios in all particle-size fractions. The higher variability in C:N ratios within temperate climates, aside with low values for arid climates, probably explains the relevance of Köppen climate types when modelling global C:N ratios. Climate affects primary productivity and microbial activity (Kramer & Chadwick, 2018; Marty et al., 2017), and controls SOM degradation or accumulation, particularly in topsoils (Luo et al., 2021). Soil clay contents control colloidal SOM stabilization through adsorption, but texture is rarely included in global modeling since its effects are often secondary compared to climate. Here, we also demonstrated that soil texture, particularly sand contents, exert a strong control on soil C:N ratios and thus should be considered in SOM stability prediction models.

Conclusions

We evidenced the controlling factors and trends of global C:N ratios in soils and particle-size fractions by compiling global literature and applying a random forest modelling approach. Sand C:N ratios have the highest mean values and are highly variable, clay C:N ratios have the lowest mean values and variability, and silt C:N ratios are intermediate. This trend is consistent within varying soil depths, climate types, and land uses. Overall, the lowest C:N ratios occurred under arid climates. Forest soils had the highest C:N ratios within land uses. Due to the high variability in sand C:N ratios, the sand fraction exerts a strong control on soil C:N ratios globally. Sand C:N ratios are affected mainly by Köppen climate types, clay contents, and altitude. MAP, Köppen climate, and soil pH are the main drivers of global clay C:N ratios. Such variables determine soil and particle-size fractions C:N ratios, and can be potentially useful in modelling efforts to predict SOM stability and turnover upon land use and climate changes in global or regional scales.

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Conflict of Interest

The authors have no conflict of interest to declare.

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Supplemental Information

Table S1. Number of observations according to climate, soil depth and land use system within the surveyed papers. Topsoil refers to 0-30 cm depths, and subsoil refers to + 30 cm depths.

Climate	**Soil Depth**	**Land Use**				***n***
		Cropland	Forest	Grassland	Unreported	
(A) Tropical	Topsoil	9	12	1	0	22
	Subsoil	8	5	0	0	13
(B) Arid	Topsoil	10	6	22	1	39
	Subsoil	1	0	8	0	9
(C) Temperate	Topsoil	73	45	21	8	147
	Subsoil	5	20	0	0	25
(D) Cold	Topsoil	29	13	13	1	56
	Subsoil	4	1	1	0	6
n		139	102	66	10	317

Table S2. Descriptive statistics of soil, climate, and geographical factors, as well as C:N ratios in soil and particle-size fractions of the surveyed sites.

Data Description	**Soil, Climate, and Geography Properties**											**C: N ratios**			
	Depth	Sand	Silt	Clay	pH	LAT	ALT	MAT	MAP	SOC	TN	Soil	Sand	Silt	Clay
	cm		------- % -------			DD	m	°C	mm	------ g kg^{-1} ------					
n	285	236	243	213	170	317	284	317	317	276	270	304	296	309	255
Mean	23.1	46.0	30.6	28.5	5.7	26.37	618.0	13.0	961.5	25.20	1.95	13.2	18.1	14.3	9.9
Std. Deviation	20.5	27.2	19.8	19.4	1.2	28.49	699.0	7.3	559.2	21.86	1.63	5.1	6.8	5.0	2.2
Mode	10.0	27.0	50.0	23.0	4.0	-21.33	80.0	20.4	1460.0	32.00	1.00	11.0	10.0	16	8.0
Minimum	2.25	2.1	0.6	1.5	3.0	- 37.95	2.0	0.5	140.0	0.50	0.07	7.3	4.4	7.0	5.0
1st Quartile	10.0	24.9	13.2	14.3	4.7	13.55	80.0	7.7	601.0	10.89	0.90	10.3	13.2	11.0	8.2
Median	20.0	41.2	28.3	23.0	5.8	40.40	326.0	12.7	868.0	18.55	1.45	11.7	16.7	13.1	9.6
3rd Quartile	26.0	67.5	44.0	36.6	6.8	46.82	920.0	19.4	1300.0	32.00	2.53	14.2	21.3	15.9	11.2
Maximum	100.0	96.9	83.5	82.7	8.5	59.82	3200.0	29.0	4100.0	145.00	9.70	43.0	46.0	41.8	17.6

Sand: 50-2,000 µm; silt: 2-50 µm; clay <2 µm; LAT, latitude; ALT, altitude; MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic C; TN, total N.

Table S3. Pearson's correlation coefficients between soil, climate, geography variables, and C:N ratios in soil and particle-size fractions.

Variable	Depth	Latitude	Altitude	MAT	MAP	pH	Clay %	Silt %	Sand %	SOC g kg^{-1}	TN g kg^{-1}
Depth	1	-0.11	-0.10	0.18**	0.03	0.09	0.14	-0.05	-0.06	-0.39***	-0.40***
Latitude		1	-0.27***	-0.73***	-0.45***	-0.08	-0.43***	0.23***	0.21**	0.10	0.07
Altitude			1	-0.01	0.16**	-0.01	-0.05	-0.05	-0.02	0.13*	0.14*
MAT				1	0.39***	0.23**	0.50***	-0.34***	-0.11	-0.22***	-0.22***
MAP					1	-0.32***	0.30***	-0.05	-0.18**	0.43***	0.35***
pH						1	0.22*	0.24**	-0.33***	-0.26**	-0.10
Clay %							1	-0.21**	-0.66***	0.07	0.11
Silt %								1	-0.65***	0.20**	0.31***
Sand %									1	-0.13	-0.31***
SOC g kg-^{1}										1	0.94***
TN g kg-^{1}											1
Soil C: N	-0.08	0.14*	-0.18***	-0.01	0.11	-0.35*	-0.09	-0.36***	0.52***	0.19*	-0.08
Sand C: N	0.13	-0.06	-0.28***	0.11	0.18**	-0.32***	0.35***	-0.21**	0.01	0.07	0.13
Silt C: N	0.00	0.08	-0.27***	0.17**	0.15**	-0.27***	0.13***	-0.41***	0.37***	0.12*	0.00
Clay C: N	0.08	-0.18**	-0.05	0.12	0.23***	-0.40***	0.10	0.14	0.05	0.08	0.08

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. MAT: mean annual temperature; MAP: mean annual precipitation; Sand: 50-2,000 μm; silt: 2-50 μm; clay <2 μm; SOC, soil organic C; TN, total N.

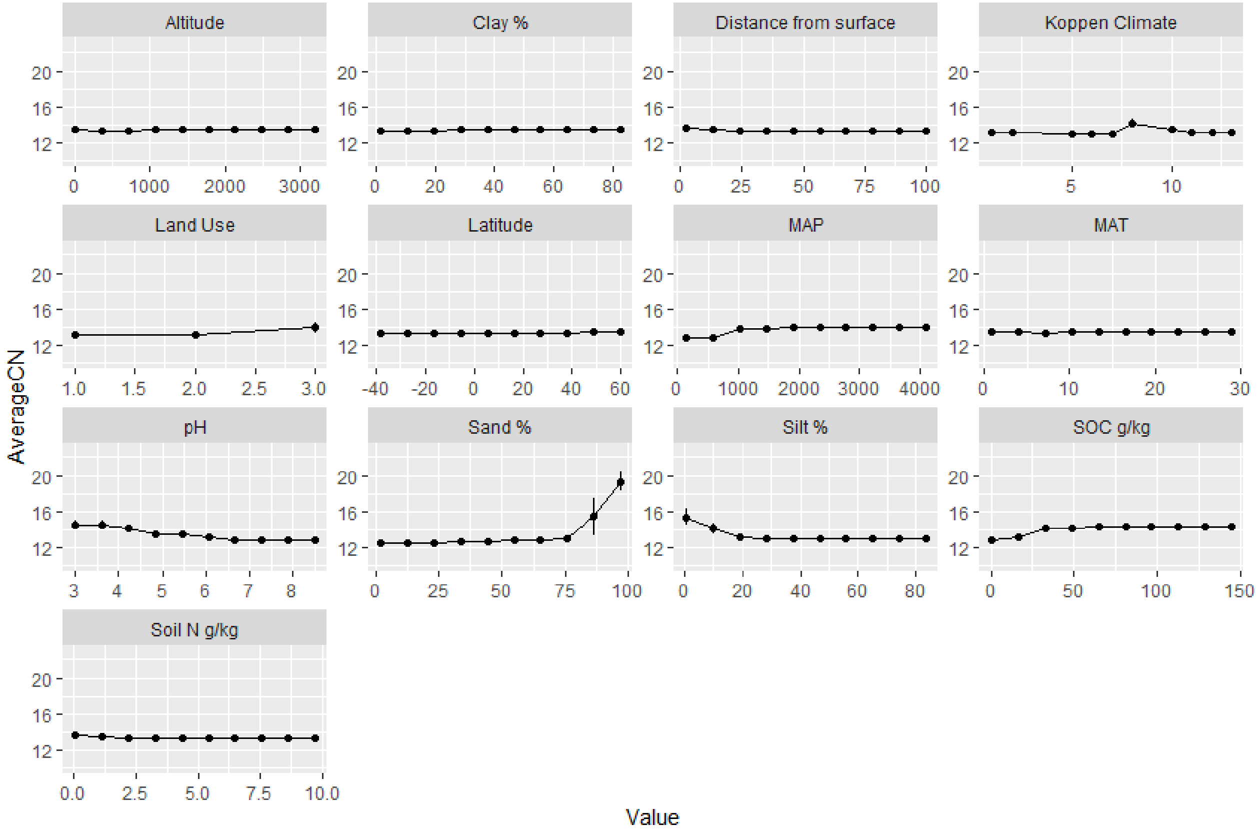

Fig. S1 Partial Dependent Plots (PDPs) of soil C:N ratios based on soil, climate, and geography variables. Koppen climate types: (1) Af; (2) Am; (5) BSk; (6) Cfa; (7) Cfb; (8) Csa; (10) Cwa; (11) Cwb; (12) Dfa (13) Dfb. Land use: (1) Grassland; (2) Cropland; (3) Forest.

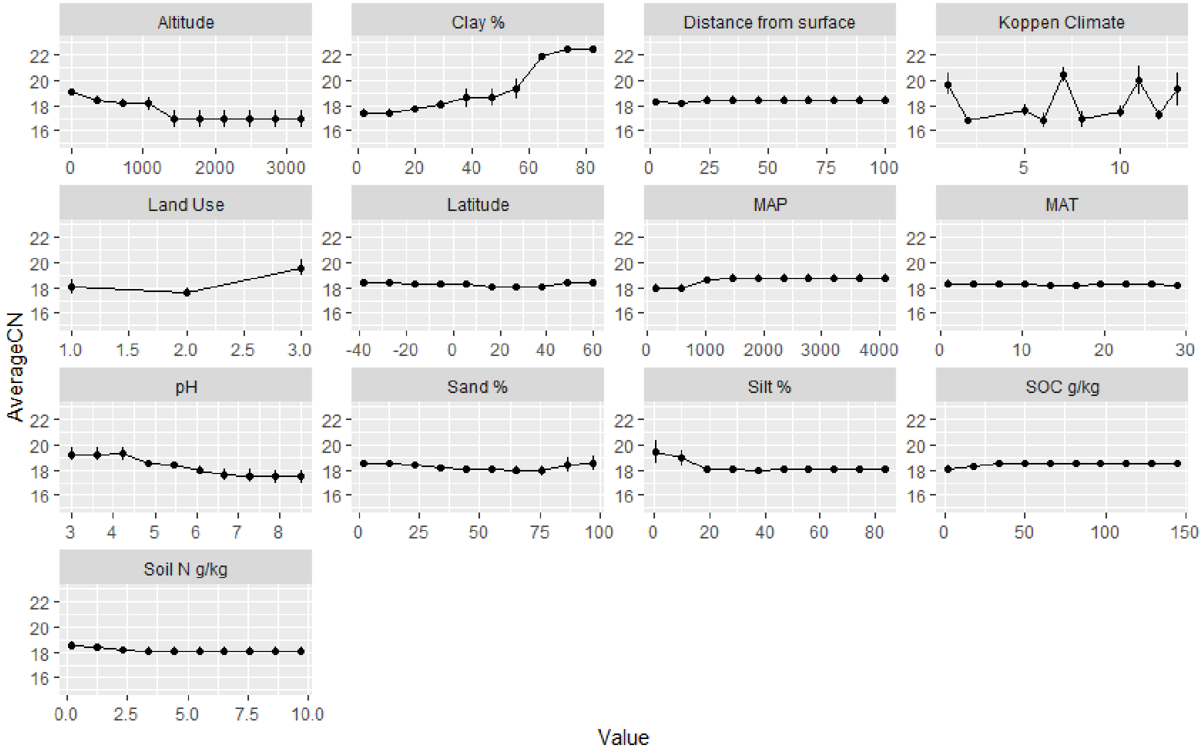

Fig. S2 Partial Dependence Plots (PDPs) of sand C:N ratios based on soil, climate, and geography variables. Koppen climate types: (1) Af; (2) Am; (5) BSk; (6) Cfa; (7) Cfb; (8) Csa; (10) Cwa; (11) Cwb; (12) Dfa; (13) Dfb. Land use: (1) Grassland; (2) Cropland; (3) Forest.

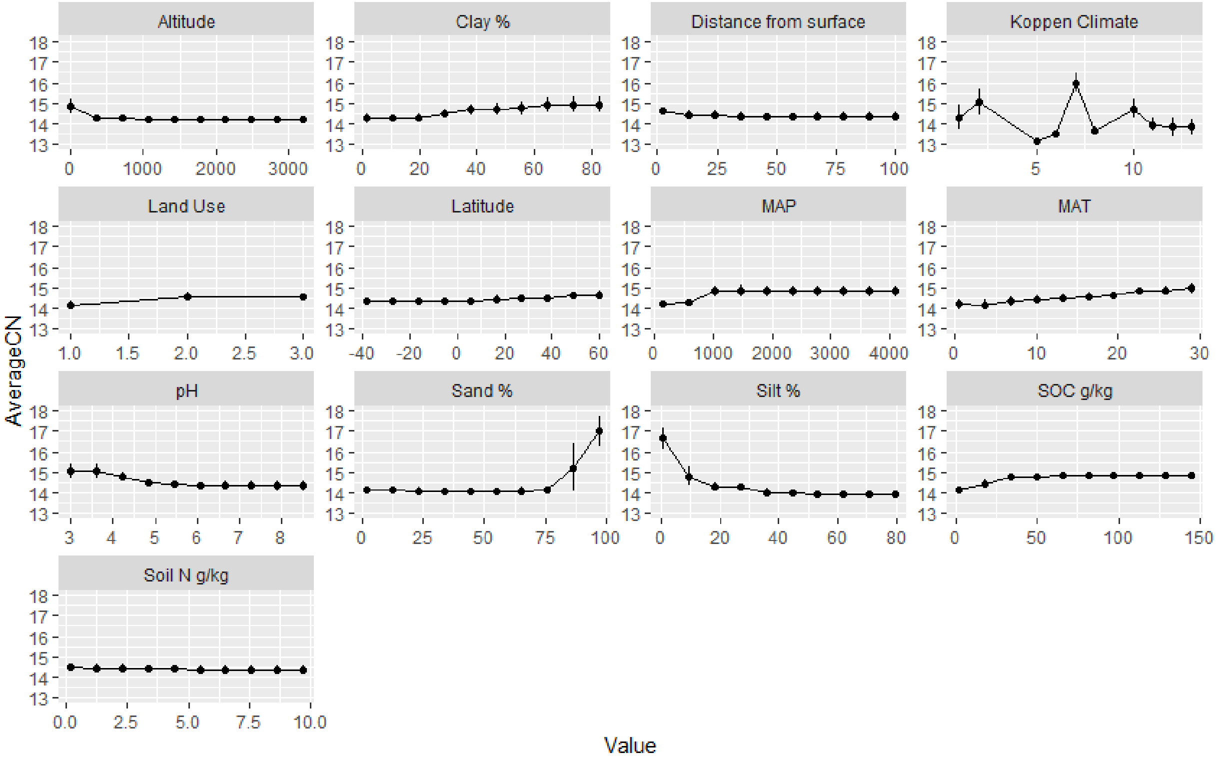

Fig. S3 Partial Dependent Plots (PDPs) of silt C:N ratios based on soil, climate, and geography variables. Koppen climate types: (1) Af; (2) Am; (5) BSk; (6) Cfa; (7) Cfb; (8) Csa; (10) Cwa; (11) Cwb; (12) Dfa; (13) Dfb. Land use: (1) Grassland; (2) Cropland; (3) Forest.

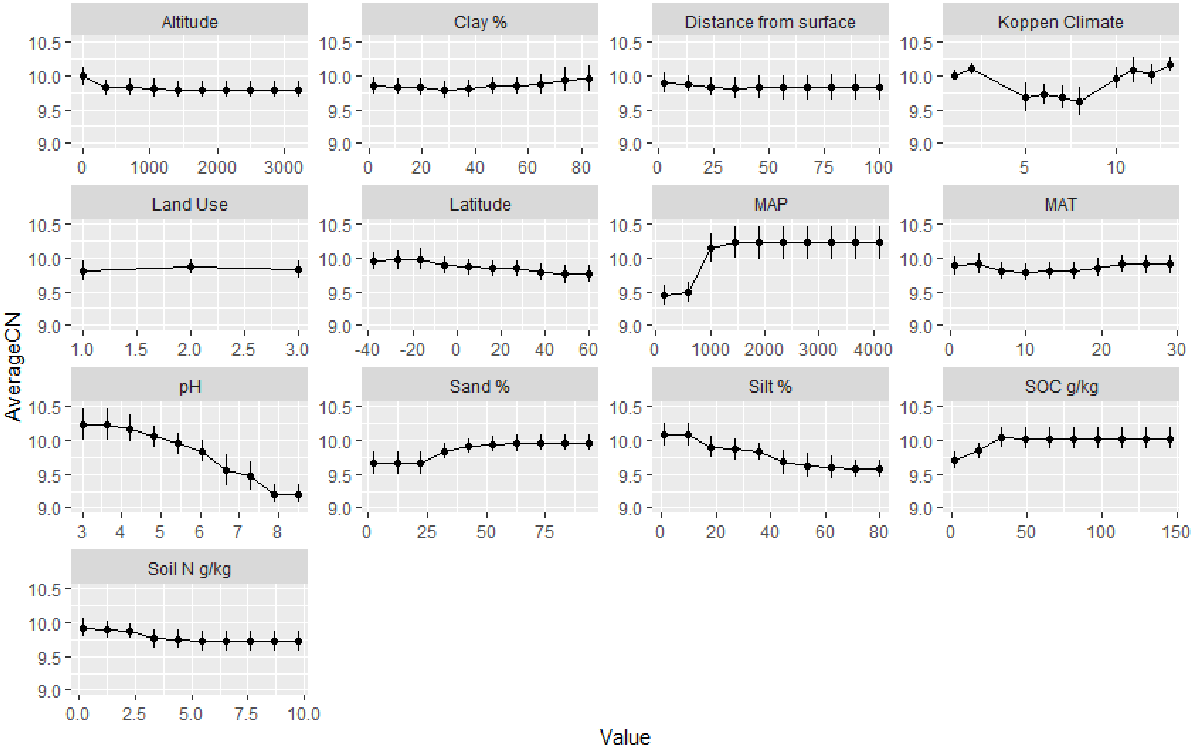

Fig. S4 Partial Dependent Plots (PDPs) of clay C:N ratios based on soil, climate, and geography variables. Koppen climate types: (1) Af; (2) Am; (5) BSk; (6) Cfa; (7) Cfb; (8) Csa; (10) Cwa; (11) Cwb (12) Dfa; (13) Dfb. Land use: (1) Grassland; (2) Cropland; (3) Forest.

CHAPTER 4

ARTICLE 2 - C:N ratios in soils and particle-size fractions as affected by soil texture and fertility in Brazilian highlands

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Highlights

C:N ratios in soils and particle-size fractions were assessed in tropical highlands

C:N ratios decrease with silt and Mn oxides contents and exchangeable bases

C:N ratios increase with clay, exchangeable Al^{3+}, and H+Al contents

N contents control the relationship between C:N ratios and soil fertility

Abstract

The effects of soil texture and fertility on C:N ratios in soils and in particle-size fractions in the humid tropics are unknown. Such knowledge is highly needed to understand the soil organic matter (SOM) dynamics and evaluate the potential of nutrient supply in these environments. Here, we assessed the effects of soil texture and fertility on C:N ratios in soils and in particle-size fractions in soils of tropical highlands in Lavras, Brazil. Soil samples were collected in soils formed on eight varying parent materials: quartzite, sericite-schist, gabbro, itabirite, serpentinite, meta-limestone, gneiss, and phyllite, at the 0-5, 30-40, and 90-100 cm soil depths. Then, soil physical and chemical characterization was performed. C:N ratios were not affected by depth (except for sand fractions), decreased with increasing silt contents, exchangeable bases, and Mn oxide contents, and increased with clay, exchangeable Al^{3+}, and H +Al contents. Notably, C:N ratios in clay fractions were highly variable and were generally lower where clay activity is higher. The inverse relationship between C:N ratios and soil fertility indicators in the studied soils is likely driven by higher retention of ammonium-N forms by clays where soil bases are higher and exchangeable Al^{3+} is low, particularly in the soil on itabirite, which was the most fertile. Although further research is needed on this relationship between C:N ratios of SOM and nutrient status and clay mineralogy, we can infer that it enhances microbial and plant activity, and thus also nutrient cyclng.

Keywords

Particle-size fractionation, soil organic matter, soil parent material, nitrogen, base cations

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1. Introduction

The mass proportion between soil organic carbon (SOC) and N contents – soil C:N ratios – is a traditional proxy for soil organic matter (SOM) quality and dynamics, and directly linked to nutrient availability or immobilization in agroecosystems. In fresh organic residues, high C:N ratios are commonly associated with slower turnover as microbial decomposition requires substantial amounts of N. However, for SOM, which already underwent complex decomposition and alteration, the interpretation of C:N ratios can be far more complex: sand-sized (2000-50 µm) SOM, which commonly comprises plant tissues only partly decomposed, typically has higher C:N ratios but is more decomposable than SOM bound to clay (<2 µm) particles, which has lower C:N ratios but is often more stable (Christensen, 2001; 1992). Because each particle-size fraction has varying mechanisms of SOM stabilization, investigating the factors that control their C:N ratios is essential to understand the potential SOC sequestration and nutrient supply in tropical ecosystems.

The literature lists a variety of environmental factors controlling SOM accumulation in tropical soils, although their effects on C:N ratios in soils and particle-size fractions are most unclear or, oftentimes, poorly assessed and discussed . Soil texture and mineralogy, which are strongly influenced by parent material, are relevant controls due to the physical protection and chemical stabilization of SOM (Angst et al., 2021; Singh et al., 2018; Six et al., 2002). In Brazilian savannas, contents of clay + silt and extractable Fe- and Al-(hydr)oxides have a positive impact on SOC retention in soils by increasing the clay-sized SOC pool (Zinn et al., 2007). However, it is not known if and how such edaphical controls affect SOM chemical composition and C:N ratios, since C and N cycles are coupled in soils, but their proportions are not necessarily fixed (Mullen, 2011). Thus, further investigation on how soil texture, mineralogy and other factors affect N cycling and availability and, therefore, C:N ratios of SOM on bulk soils and particle-size fractions, is needed.

Soil parent material exerts a strong control on soil texture, mineralogy and also fertility, which affects primary production and, thus, SOM accumulation. In turn, SOM plays a primary role in sustaining soil fertility, particularly in tropical, weathered soils (Tiessen et al., 1994), owing to its associated cation exchange capacity (CEC) and nutrient retention. However, despite these known associations, it is not well known how soil texture, mineralogy and other properties affect C:N ratios in SOM of bulk soils and on the different particle-size fractions, which hinders our understanding of soil fertility and management in these environments.

This study aimed to investigate the variations in C:N ratios in soils and particle-size fractions across a range of parent materials in tropical highlands under native forests near Lavras, Minas Gerais. We tested the following hypotheses: i) soil texture, clay mineralogy, and general fertility affect C:N ratios in bulk soils(H1), and ii) such effects vary across soil particle-size fractions (H2), as they differ in SOM stabilization mechanisms. To achieve this goal, we evaluated the physical and chemical properties of soils formed on eight contrasting parent materials, and performed correlation and regression analyses to investigate the relationships between C:N ratios in soil and in particle-size fractions and other soil properties.

2. Materials and Methods

2.1 Site description

Eight soils developed from eight different parent materials (quartzite, sericite-schist, gabbro, itabirite, serpentinite, meta-limestone, gneiss, and phyllite) were selected and sampled in triplicate for this study. All sites were located on midslopes on a moderately hilly landscape along a 20-km radius near Lavras, Minas Gerais, Brazil, and were under native tropical semideciduous forests. In this sampling we aimed to sample a large natural variability of soil parent materials while keeping other factors of soil formation (namely organisms, relief and climate) as similar as possible. Mean annual temperature and precipitation are respectively 19.3 °C and 1,530 mm, with wet summers and dry winters. According to the Köppen classification (Peel et al., 2007), the regional climate is temperate highland tropical with dry winters (Cwb). Altitude varied between 889 m (meta-limestone) and 1064 m a.s.l. (quartzite and sericite-schist). Tables 1 and 2 summarize the classification and characterization of each soil. Details on site location, lithology, soil composition and morphology can be found on (Araujo et al., 2014; Zinn et al., 2020).

Table 1. Classification, altitude, and clay mineralogy of the soils collected near Lavras, Minas Gerais, Brazil, on varying parent materials.

Parent Material	**Soil**	**Altitude (m)**	**Clay Mineralogy**
Quartzite	Humic Dystrudept	1.064	K, I, Vh, Gi, Go
Sericite-schist	Typic Haplohumult	1.064	K, I, Vh, Gi, Go
Gabbro	Rhodic Humic Acrudox	944	
Itabirite	Typic Kanhapludalf	920	K, I, Vh, Gi, Go, Hm
Serpentinite	Petroferric Acrudox	909	Hm, Gi, Go
Meta-limestone	Anionic Acrudox	889	Gi, K, Go, Hm
Gneiss	Typic Haplohumult	891	K, Gi, Go
Phyllite	Haplohumult	910	K, I, Gi, Go, Hm

K, kaolinite; I, illite; Vh, hydroxyl-interlayered vermiculite; Gi, gibbsite; Go, goethite; Hm, hematite.

2.2 Soil sampling and analyses

Three soil pits of approximately 1.2 m and 10-30 m distant were randomly marked and excavated in each soil/parent material combination described above. Disturbed soil samples were collected at the 0-5, 5-10, 10-20, 30-40, 50-60 and 90-100 cm depth intervals in each soil pit, totaling three replications per soil, in all depths. Soil samples were air dried and sieved < 2 mm, and gravels were separated and weighed when present. Analyses of all soil properties were performed without any pretreatment to remove carbonates, Fe oxides or organic matter.

Soil particle-size distribution was determined by the pipette method (Gee and Bauder, 1986) after dispersion in 1 M NaOH and shaking for 16 h. Exchangeable Al^{3+}, Ca^{2+}, and Mg^{2+} were extracted with 1.0 M KCl and determined by atomic absorption spectroscopy and titration. Exchangeable H+Al were indirectly determined by pH change in a SMP buffer (Araujo et al., 2014). Based on the obtained values, cation exchange capacity (t), cation exchange capacity at pH 7.0 (T), clay activity, sum of exchangeable bases (SB), and base saturation (V%) were calculated according to Teixeira et al. (2017).

Soil samples < 2 mm were finely ground with ball mills to determine total C and nitrogen (N) concentrations in all sampled depths. A subsample of approximately 0.3 g was used for the dry combustion method using a Variomax CNHOS analyzer (Hanau, Germany). The soils do not contained carbonates (pH < 5.7), thus the total C analyzed corresponds to soil organic carbon (SOC). We selected the 0-5, 30-40 and 90-100 cm depths for further characterization by SOC and N analyses of particle-size fractions and selective extractions in bulk soils as described next. SOC and N were determined in the sand (2000-50 μm), silt (50-2 μm) and clay (2-0 μm) fractions obtained after dispersion in 1 M NaOH, sieving to collect the sand and successive siphonings to separate clays from the silt, as described by (Yuri L. Zinn et al., 2007a).

In bulk soil samples, the acid ammonium oxalate extraction for soil amorphous Fe, Al, and Mn oxides was carried out using 0.2 M ammonium oxalate adjusted to pH = 3.0 with 0.2 M oxalic acid (Kämpf & Schwertmann, 1982). Total Fe-, Al-, and Mn-oxides were repeatedly extracted from the soil samples using a buffer of citrate and sodium bicarbonate added with sodium dithionite (Na_2S_2O4) in a water bath at 80 °C. The suspension was later centrifuged at 1,800 rpm for five minutes. The extraction was repeated until the color of the soil sample changed to light gray (Mehra and Jackson, 2013). In the extracts, Fe, Al, and Mn were determined by atomic absorption using a Varian SpectrAA spectrophotometer. The specific surface area (SSA) of bulk soils, without any pretreatments to remove SOC, was determined using the BET method (Brunauer et al., 1938) by N2 sorption with a Flowsorb 2300 (Micromeritics, Norcross, GA, USA).

2.3 Statistical analyses

Pearson's correlation coefficients were calculated between bulk soil and particle-size C:N ratios and selected soil physical and chemical properties in each studied depth (0-5, 30-40, and 90-100 cm depth). Then, the soil properties that obtained significant correlation coefficients ($p < 0.05$) with C:N ratios were adjusted to linear and non-linear regression models to better assess the relationships between soil and particle-size C:N ratios and other soil properties. The best regression models were selected based on the Akaike's and Bayesian Information Criteria (AIC and BIC, respectively), and on the R^2 value when the models did not differ ($p > 0.05$). All statistical analyses were performed using the R Software (R Core Team, 2020).

3. Results

3.1 Soils characterization

Particle-size distribution was highly variable within the eight selected soils and across soil depths (Table 2). Sand contents varied between 76.6 g kg^{-1} in the soil on limestone and 497.4 g kg^{-1} in the soil on quartzite. Silt ranged from 95.5 to 500.1 g kg^{-1} in the soils on gabbro and itabirite, respectively. Clay contents were the lowest in the soil on quartzite (157.6 g kg^{-1}), but reached 826.9 g kg^{-1} in the soil on limestone. Overall, soils had variable but generally low fertility, except for the soil on itabirite, which is a banded iron formation often explored as an iron ore. This soil unexpectedly had very high Ca and Mg contents at the 0-5 cm depth (14.9 and 4.5 cmol$_c$ kg^{-1}, respectively), and also at the 30-40 and 90-100 cm soil depths (Table 2). Consequently, the soil on itabirite had much higher sum of base cations, cation exchange capacity at pH 7.0 and percent base saturation in all studied depths compared to the other soils. Moreover, the soil on itabirite had the highest clay activity, calculated as cation exchange capacity / clay content, at the 0-5 cm soil depth. Exchangeable Al^{+3} and H+Al were the highest in the soil on schist at the 0-5 and 30-40 cm depths. At the 90-100 cm depth, the soil on quartzite had the highest Al^{+3} content (1.4 cmol$_c$ kg^{-1}), and the soil on gabbro had the highest H+Al (5.5 cmol$_c$ kg^{-1}).

Table 2. Selected physical and chemical properties of studied soils.

Parent Material	**Sand**	**Silt**	**Clay**	**Ca**	**Mg**	**Al**	**H + Al**	**SB**	**t**	**Clay t**	**T**	**V**
		g kg^{-1}				cmolc kg^{-1}			cmolc kg^{-1}			%
0-5 cm												
Quartzite	497.4	329.9	172.7	0.1	0.1	3.3	20.6	0.4	3.7	21.3	21.0	2.0
Sericite-schist	234.4	278.7	486.9	0.1	0.1	5.4	29.0	0.6	6.0	12.2	29.6	2.0
Gabbro	157.7	95.5	746.8	0.1	0.1	4.1	26.9	0.5	4.5	6.1	27.4	1.6
Itabirite	212.9	484.0	303.1	14.9	4.5	0.0	2.8	19.8	19.9	65.6	22.7	86.8
Serpentinite	461.1	283.0	255.9	1.0	3.5	0.2	4.6	4.8	5.0	19.5	9.4	50.1
Meta-limestone	110.4	111.7	777.9	2.6	0.3	1.4	13.4	3.2	4.7	6.0	16.6	20.1
Gneiss	408.1	199.4	392.5	1.6	0.9	1.4	11.4	3.0	4.3	11.0	14.4	21.9
Phyllite	128.9	433.4	437.8	3.3	0.5	1.2	8.1	4.3	5.4	12.4	12.4	33.9
30-40 cm												
Quartzite	483.3	358.7	158.0	0.1	0.1	1.6	5.9	0.3	1.8	11.5	6.1	4.3
Sericite-schist	244.1	384.8	371.2	0.1	0.1	3.0	13.3	0.4	3.4	9.0	13.6	2.6
Gabbro	135.9	148.3	715.8	0.1	0.1	1.2	9.9	0.3	1.5	2.0	10.2	2.6
Itabirite	178.4	500.1	321.5	2.3	1.2	0.3	4.6	3.5	3.8	11.8	8.1	36.9
Serpentinite	405.1	358.0	236.9	0.1	0.5	0.0	2.5	0.7	0.7	2.9	3.1	20.9
Meta-limestone	76.6	131.8	791.6	0.1	0.1	0.6	6.3	0.3	0.9	1.2	6.6	5.0
Gneiss	374.5	257.9	367.7	0.1	0.1	1.2	3.9	0.3	1.5	4.1	4.3	8.1
Phyllite	168.5	307.8	523.8	0.9	0.1	0.7	3.6	1.0	1.8	3.4	4.7	22.0
90-100 cm												
Quartzite	497.4	345.0	157.6	0.1	0.1	1.4	4.3	0.2	1.6	10.3	4.6	5.3
Sericite-schist	328.3	370.0	301.7	0.1	0.1	1.0	4.0	0.3	1.2	4.0	4.3	6.2
Gabbro	123.5	139.5	737.0	0.1	0.1	0.4	5.5	0.3	0.6	0.8	5.8	4.4
Itabirite	230.8	458.4	310.8	1.6	0.5	0.2	2.7	2.1	2.2	7.2	4.8	33.0
Serpentinite	394.5	333.2	272.3	0.1	0.1	0.0	1.2	0.2	0.3	0.9	1.5	15.1
Meta-limestone	77.2	96.0	826.9	0.1	0.1	0.3	4.6	0.4	0.6	0.8	4.9	7.8
Gneiss	378.6	395.2	226.2	0.1	0.1	1.2	3.1	0.3	1.4	6.4	3.3	8.1
Phyllite	125.3	238.3	636.4	0.1	0.1	0.2	2.4	0.3	0.5	0.7	2.7	10.2

SB, sum of exchangeable bases; t, cation exchange capacity; Clay t, clay cation exchange capacity (t/clay (%))*100; T, cation exchange capacity at pH 7.0; V, base saturation.

Total Fe oxide content (determined via DCB) ranged from 25.1 g kg^{-1} at the 0-5 cm depth in the soil on quartzite, to 385.5 g kg^{-1} at the 90-100 cm depth in the soil on itabirite (Table 3). The soils on itabirite and serpentinite had the highest content of amorphous Fe oxides (determined via oxalate) in all soil depths, which were relatively low compared to total Fe oxides, varying between 0.46 and 11.62 g kg^{-1}. The soil on schist had the highest content of amorphous Al oxides at the 0-5 cm soil depth (9.7 g kg^{-1}), whereas the soil on limestone showed the highest content of amorphous Al at the 30-40 and 90-100 cm soil depths (9.45 and 9.5 g kg^{-1}, approximately). The soil on itabirite had much higher content of amorphous and total Mn oxides in all soil depths compared to the other soils. Lastly, SSA ranged from 4.24 m^2 g^{-1} at the 0-5 cm depth in the soil on quartzite, to 43.3 m^2 g^{-1} at the 90-100 cm depth in the soil on limestone, which had the highest SSA in all soil depths, due to its high clay contents.

Table 3. Fe_2O_3, Al_2O_3, and MnO_2 contents (g kg^{-1}) determined via oxalate and dithionite-citrate-bicarbonate (DCB), and specific surface area (SSA; m^2 g^{-1}).

Parent Material	**Fe_2O_3 ox.**	**Al_2O_3 ox.**	**MnO_2 ox.**	**Fe_2O_3 DCB**	**MnO_2 DCB**	**SSA**
			0-5 cm			
Quartzite	2.14	2.50	0.02	25.11	0.07	4.24
Sericite-schist	5.74	9.70	0.03	102.43	0.10	17.42
Gabbro	8.56	6.91	0.00	175.15	0.53	26.26
Itabirite	8.58	4.36	12.79	305.81	21.96	11.35
Serpentinite	11.39	4.46	2.31	190.80	4.51	14.78
Meta-limestone	3.86	8.83	0.00	67.33	0.08	37.03
Gneiss	2.31	3.23	0.11	33.49	0.07	12.78
Phyllite	3.31	2.33	0.33	109.12	0.48	17.70
			30-40 cm			
Quartzite	2.09	2.43	0.06	27.19	0.17	7.03
Sericite-schist	4.66	8.39	0.10	110.43	0.23	23.68
Gabbro	6.79	7.92	0.04	187.46	0.39	35.50
Itabirite	10.71	3.59	10.86	306.60	18.84	16.01
Serpentinite	9.85	4.23	1.90	215.39	4.97	21.70
Meta-limestone	2.80	9.45	0.00	71.51	0.00	43.21
Gneiss	1.56	3.20	0.42	34.90	0.72	15.91
Phyllite	3.42	3.00	0.12	128.85	0.41	25.19
			90-100 cm			
Quartzite	1.92	2.42	0.04	26.70	0.15	7.57
Sericite-schist	3.98	7.10	0.14	123.50	0.35	23.42
Gabbro	6.07	8.08	0.00	189.31	0.65	36.01
Itabirite	11.32	2.84	7.23	385.95	17.01	17.69
Serpentinite	11.62	3.72	2.38	205.03	4.34	23.63
Meta-limestone	2.70	9.51	0.00	69.95	0.00	43.30
Gneiss	0.46	1.68	0.02	26.50	0.00	11.06
Phyllite	3.38	2.71	0.14	148.48	0.40	32.39

3.2 SOC, N and C:N ratios in the whole soil and in particle-size fractions

Soil organic C varied between 3.09 and 6.94% at the 0-5 cm soil depth, with a mean value of 4.49% (Table 4). At 30-40 cm depth, SOC ranged from 0.89 and 3.66%, with a mean value of 1.86%, and at 90-100 cm depth, it had the narrowest variation (0.47-2.04%) and, as expected, the lowest mean value (0.93). Average soil N also decreased with increasing depth: from 0.35% at the 0-5 cm depth to 0.06% at the 90-100 cm depth. In contrast, bulk soil C:N ratios were little affected by increasing depth. The highest soil C:N ratio (18.7) occurred in the soil on gabbro at the 90-100 cm depth, probably reflecting the very high SOC content in that layer (2.04%). The lowest C:N ratio (9.5) occurred in the soil on itabirite at the 0-5 cm depth, where N contents are high (0.58%).

Mean SOC in the sand fraction was 2.6% at the 0-5 cm soil depth, varying between 0.46% in the coarse-textured soil on quartzite and 4.41% in the clayey soil on limestone (Table 4). At the 30-40 cm depth, mean sand C content was 0.56%, ranging from 0.11% in the soil on quartzite to 1.62% in the soil on schist. Accordingly, the lowest sand C content in all soil depths was observed in the soil on quartzite. At the 0-5 cm soil depth, mean sand C:N ratio was 17.9, and varied between 14.1 in the soil on itabirite and 22.6 in the soil on gabbro. However, sand C:N ratios were much higher in lower depths: at 30-40 cm, mean sand C:N was 30.7, varying between 13.4 in the soil on quartzite and 45.4 in the soil on gabbro, which also had the highest sand C:N ratio at the 90-100 cm depth (58.6). At the 90-100 cm depth, mean sand C:N ratio was 22.3. Sand C:N ratios had increased variability at the 90-100 cm compared to the other soil depths, ranging from 10.2 to 58.6.

Table 4. C and N contents (%) and C:N ratios of the whole soil and particle-size fractions (*n* = 3).

Parent Material	**Soil**			**Sand**			**Silt**			**Clay**		
	C	**N**	**C:N**	**C**	**N**	**C:N**	**C**	**N**	**C:N**	**C**	**N**	**C:N**
0-5 cm												
Quartzite	3.14	0.23	13.8	0.46	0.03	16.4	1.28	0.08	16.9	9.05	0.65	13.9
Sericite-schist	6.23	0.44	14.3	3.39	0.17	19.9	3.05	0.19	15.7	5.80	0.47	12.5
Gabbro	6.94	0.45	15.6	3.76	0.17	22.6	7.72	0.42	18.7	5.18	0.46	11.4
Itabirite	5.54	0.58	9.5	3.07	0.22	14.1	2.92	0.29	10.2	6.90	1.02	6.8
Serpentinite	3.54	0.26	13.8	1.15	0.06	17.9	2.44	0.16	14.9	7.38	0.63	11.8
Meta-limestone	4.13	0.28	14.8	4.41	0.20	21.6	5.17	0.32	15.9	2.95	0.23	13.1
Gneiss	3.33	0.27	12.5	0.63	0.04	16.0	1.73	0.13	13.6	3.44	0.46	7.6
Phyllite	3.09	0.25	12.3	3.92	0.25	14.3	1.48	0.11	14.1	3.50	0.43	8.6
Mean	4.49	0.35	13.3	2.60	0.14	17.9	3.22	0.21	15.00	5.52	0.54	10.70
MSE	0.54	0.05	0.67	0.56	0.03	1.14	0.78	0.04	0.89	0.77	0.08	0.95
30-40 cm												
Quartzite	0.89	0.06	13.7	0.11	0.01	13.4	0.39	0.02	17.7	3.45	0.25	14.0
Sericite-schist	3.07	0.22	13.9	1.62	0.05	30.9	1.42	0.09	15.4	3.68	0.29	12.7
Gabbro	3.66	0.23	16.2	0.87	0.02	45.4	2.49	0.12	20.4	2.16	0.18	12.2
Itabirite	1.54	0.15	10.4	0.70	0.02	36.7	0.74	0.05	15.4	2.23	0.38	5.8
Serpentinite	1.51	0.10	14.5	0.26	0.03	35.4	0.94	0.06	16.4	3.48	0.28	12.3
Meta-limestone	1.57	0.10	16.2	0.46	0.01	45.2	1.44	0.08	17.1	1.45	0.11	13.0
Gneiss	1.42	0.13	11.5	0.15	0.01	14.9	0.56	0.04	15.2	1.42	0.21	6.9
Phyllite	1.23	0.10	12.6	0.31	0.01	23.6	0.77	0.06	13.7	1.48	0.19	7.7
Mean	1.86	0.14	13.6	0.56	0.02	30.7	1.09	0.07	16.41	2.42	0.24	10.56
MSE	0.34	0.02	0.73	0.18	0.01	4.40	0.24	0.01	0.72	0.35	0.03	1.14
90-100 cm												
Quartzite	0.63	0.04	14.8	0.13	0.01	13.3	0.24	0.02	15.3	2.68	0.16	17.6
Sericite-schist	0.67	0.05	13.2	0.28	0.02	20.5	0.26	0.02	10.6	1.32	0.08	15.9
Gabbro	2.04	0.11	18.7	0.39	0.01	58.6	1.19	0.06	21.5	1.49	0.18	8.9
Itabirite	0.63	0.06	11.0	0.25	0.01	25.9	0.30	0.02	14.8	1.14	0.20	6.0
Serpentinite	1.27	0.09	16.2	0.30	0.02	15.4	0.90	0.07	15.8	3.23	0.27	14.1
Meta-limestone	1.19	0.06	18.4	0.27	0.02	15.8	1.18	0.07	19.6	1.16	0.07	15.9
Gneiss	0.54	0.04	11.9	0.09	0.01	10.2	0.18	0.02	12.5	0.83	0.27	3.4
Phyllite	0.47	0.04	11.8	0.28	0.02	18.3	0.36	0.03	11.5	0.52	0.06	8.3
Mean	0.93	0.06	14.5	0.25	0.01	22.25	0.58	0.04	15.21	1.55	0.16	11.27
MSE	0.19	0.01	1.07	0.03	0.00	5.46	0.15	0.01	1.35	0.33	0.03	1.86

MSE, mean standard error.

In the silt fraction, mean SOC was 3.22% at the 0-5 cm depth, varying between 1.28 and 7.72% (Table 4). In all soil depths, the lowest silt C content was observed in the soil on quartzite and the highest was observed in the soil on gabbro, which had the lowest silt content. The highest silt N contents were also observed in the soil on gabbro at the 0-5 and 30-40 cm soil depths. Silt C:N ratios varied between 10.2 and 21.5 across depths, and the highest silt C:N ratio at each soil depth was found in the soil on gabbro.

In the clay fraction, SOC contents at the 0-5 cm depth varied between 2.95 and 9.05%, with a mean value of 5.52% (Table 4). At the 30-40 cm depth, clay C contents varied between 1.42 and 3.68%, with a 2.42% mean. At 90-100 cm, clay C contents varied between 0.83 and 3.23%, with the lowest mean value (1.55%). Remarkably, the clay fraction was enriched in N compared to the other particle-size fractions. Mean clay N content at the 0-5 cm depth was 0.54%, ranging between 0.23 and 1.02%. At the 30-40 cm depth, clay N contents varied between 0.11 and 0.38%, with a mean value of 0.24%. In both depths, the highest N content in clays was observed in the soil on itabirite. At the 90-100 cm depth, clay N contents varied from 0.06 and 0.27%, with a mean of 0.16%. Clay C:N ratios were little affected by increasing depth in each soil, varying between 3.4 and 17.6. The soil on quartzite showed the highest clay C:N ratios in all depths, reflecting the higher N contents in that soil.

On average, C:N ratios in particle-size fractions decreased with particle-size, in accord with increased N content in clays compared to the other size fractions (Table 4). The soil on quartzite was an exception, as their silt C:N ratios were consistently higher than the sand C:N ratios. At the 90-100 cm depth, the soils on serpentinite, limestone, and gneiss also had higher C:N ratios in the silt fraction than in the sand fraction (Figure 1).

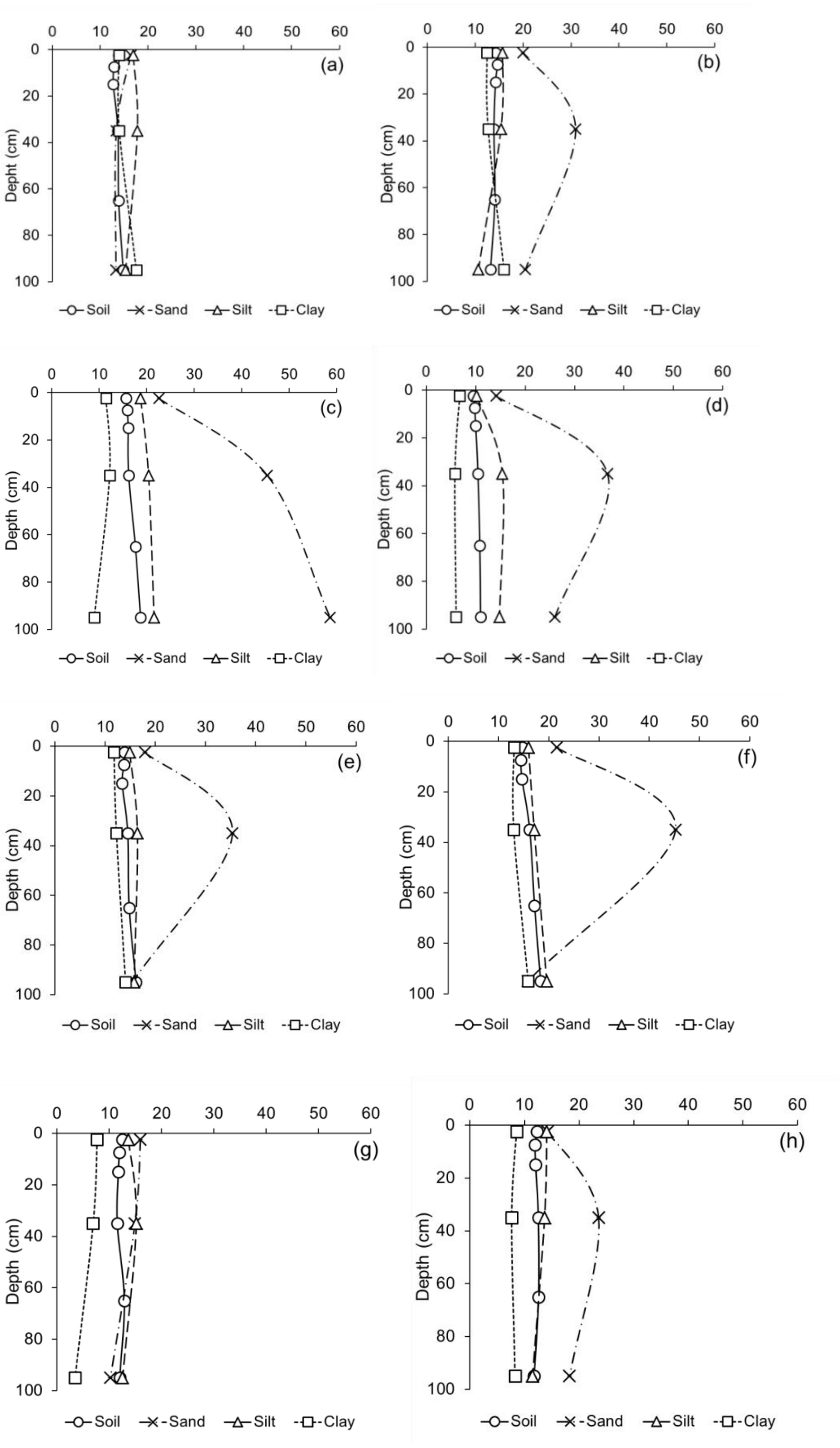

Figure 1. C:N ratios in bulk soil and sand, silt, and clay (*n* = 3) per depth in different parent materials: quartzite (a), schist (b), gabbro (c), itabirite (d), serpentinite (e), limestone (f), gneiss (g), and phyllite (h).

3.3 Relationships between C:N ratios and other soil properties

C:N ratios in bulk soils were negatively related to silt contents and positively correlated with clay contents, with no effect of sand contents (Table 5). C:N ratios in sand and silt were also correlated with silt and clay contents, especially in the 0-5 cm depth, whereas clay C:N ratios did not show consistent correlations with the contents of particle-size fractions. The positive correlations between soil C:N ratios and SSA reflect the strong positive effect of clay contents on SSA (Zinn et al., 2017). SOC and N were positively related to sand and silt C:N ratios, particularly at the 90-100 cm, but did not show any significant relationship with clay C:N ratios.

C:N ratios were unexpectedly and negatively correlated with the content of exchangeable basic cations in soils (Ca^{2+} and Mg^{2+}) and positively related with Al^{3+} and H+Al, which ultimately resulted in the negative correlations between soil C:N ratios and sum of exchangeable bases, CEC at soil pH, and base saturation. Such correlations were stronger at the 0-5 cm soil depth, where most of soil exchangeable cations are concentrated. Because T (CEC at pH 7.0) is the sum of exchangeable bases and potential acidity (H+Al), it did not correlate well with C:N ratios, except with sand C:N ratios at the 90-100 cm depth. Positive correlations between C:N ratios and amorphous Al content were observed, mirroring those for exchangeable Al^{3+}, and were stronger for bulk soil and sand fractions than for silt and clay fractions. C:N ratios in bulk soil and silt showed a strong, negative correlation with amorphous and total Mn oxide contents at the 0-5 cm soil depth.

Table 5. Pearson's correlation coefficients (*r*) between C:N ratios in soil and in particle-size fractions and other physical and chemical properties per soil depth (*n* = 24).

	Soil C:N			**Sand C:N**			**Silt C:N**			**Clay C:N**		
	0-5	30-40	90-100	0-5	30-40	90-100	0-5	30-40	90-100	0-5	30-40	90-100
SOC (%)	-	-	-	0.51**	0.33	0.65***	0.22	0.36	0.59**	-0.01	0.24	0.00
N (%)	-	-	-	0.08	0.22	0.49*	-0.26	0.15	0.36	-0.36	0.01	-0.12
Sand	-0.04	-0.21	-0.22	-0.28	-0.58**	-0.47*	0.00	-0.03	-0.34	0.18	0.18	0.21
Silt	-0.69***	-0.67***	-0.63***	-0.68***	-0.28	-0.34	-0.57**	-0.35	-0.54**	-0.46*	-0.32	-0.25
Clay	0.49**	0.52**	0.46*	0.66***	0.54**	0.46*	0.39	0.22	0.48*	0.19	0.06	-0.01
Ca	-0.71***	-0.42*	-0.28	-0.42*	-0.07	0.10	-0.66***	-0.24	-0.01	-0.63***	-0.39	-0.26
Mg	-0.61***	-0.40*	-0.35	-0.42*	0.09	0.09	-0.65***	-0.04	0.02	-0.49**	-0.38	-0.35
Al	0.54**	0.07	-0.12	0.51**	-0.19	-0.22	0.59**	0.01	-0.15	0.52**	0.29	0.12
H+Al	0.59**	0.38	0.37	0.59**	0.19	0.52**	0.64***	0.28	0.53**	0.56**	0.39	0.15
SB	-0.73***	-0.44*	-0.28	-0.45*	-0.03	0.10	-0.69***	-0.19	0.00	-0.63***	-0.42*	-0.27
t	-0.68***	-0.41*	-0.34	-0.36	-0.15	-0.02	-0.62***	-0.20	-0.08	-0.57**	-0.25	-0.19
Clay t	-0.72***	-0.41*	-0.35	-0.50*	-0.33	-0.19	-0.62***	-0.15	-0.23	-0.50*	-0.08	-0.11
T	0.14	0.18	0.17	0.38	0.18	0.53**	0.23	0.19	0.48*	0.18	0.21	-0.02
V (%)	-0.64***	-0.49**	-0.33	-0.44*	-0.03	-0.01	-0.66***	-0.27	-0.06	-0.63***	-0.51**	-0.30
Fe_2O_3 ox.	-0.05	-0.08	0.06	0.19	0.26	0.21	-0.09	0.06	0.16	-0.05	-0.10	-0.04
Al_2O_3 ox.	0.46*	0.65***	0.62***	0.69***	0.38	0.40*	0.29	0.29	0.47*	0.38	0.45*	0.41*
MnO_2 ox.	-0.75***	-0.56**	-0.37	-0.46*	0.08	0.05	-0.71***	-0.16	-0.03	-0.55**	-0.51**	-0.33
Fe_2O_3 DCB	-0.39	-0.12	-0.09	-0.13	0.22	0.19	-0.42*	0.11	0.09	-0.36	-0.26	-0.24
MnO_2 DCB	-0.71***	-0.52**	-0.30	-0.42*	0.08	0.02	-0.68***	-0.07	0.01	-0.53**	-0.48*	-0.32
SSA	0.47**	0.68***	0.52**	0.58**	0.58**	0.39	0.28	0.26	0.45*	0.25	0.26	0.15

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Figures S1-S4 illustrate the relationships between C:N ratios and other soil properties at the 0-5 cm depth, as well as the best regression models between C:N ratios and selected soil properties ($p < 0.05$ and those with increased R^2).

4. Discussion

4.1 Soil fertility in tropical highlands and the unusual itabirite soil

Soil fertility in most soils was low, although there was an increasing gradient towards lower C:N ratios as fertility indicators increased, which is a trend seldom reported in the literature. The correlations were also favored by the high values of the itabirite soil. Diverging from what it is commonly observed in the literature, the soil developed from itabirite had the highest soil fertility compared to the other studied soils. Overall, soils on itabirite are poor in nutrients, particularly basic cations, because the rock is commonly an aggregate of quartz, hematite and magnetite, can be strongly leached and weathered under well-drained tropical conditions (Schaefer et al., 2016). Total exchangeable bases in these soils normally range between 0.5 and 1.3 $\text{cmol}_c\ \text{kg}^{-1}$ in the topsoil (Carvalho Filho et al., 2010), which are very low values. However, Baião et al. (2021) assessed the nutrient status in a soil on dolomitic itabirite, and found high Ca^{2+} and Mg^{2+} contents (378 and 34 mg kg^{-1}, respectively) and increased CEC (15-18 $\text{cmol}_c\ \text{dm}^{-3}$), comparable with the values observed in the present study.

In our study, exchangeable bases in the itabirite soils sum up to 19.8 $\text{cmol}_c\ \text{kg}^{-1}$ in the 0-5 cm depth, and even the 30-40 and 90-100 cm layers had higher values than most reported in the literature. One potential explanation for this increased soil fertility is the presence of ferroan dolomite ($Ca(Mg,Fe,Mn)(CO_3)_2$) (Moukarika et al., 1991) associated with itabirite. The weathering of such parent material form calcite and poorly crystalized Fe-hydr(oxides), which lately form soils with increased contents of Fe-hydr(oxides) and high magnetic susceptibility, as observed by Araujo et al. (2014). It is also possible that Ca and Mg were retained on the 2:1 clay minerals, namely illite and vermiculite, and are continuously cycled within the existent plant biomass. Such fertile itabirite soils deserve further investigation as its peculiar chemical properties challenge the current views of soil formation in tropical environments.

4.2 On the SOC and N concentrations, and C:N ratios

The bulk concentrations of SOC and N in the present study, regardless of the parent materials and soil composition, are relatively high compared to most soils in Brazil. This trend was attributed to the lower temperatures resulting from the highland situation of the study sites (altitudes of ca. 1,000 m), slowing SOM decomposition and turnover (Araujo et al., 2017). Since high altitude generally involves marked effects of lower temperature and same or higher precipitations, it is regarded as a promising proxy of SOC storage in regional scales (Wiesmeier et al., 2019). The overall higher SOC and N contents at the 0-5 cm depth result from more fresh organic inputs in the topsoil, with subsequent higher SOM accumulation and nutrient cycling in that layer, compared to the subsurface layers.

SOC and N concentrations varied widely across and within particle-size fractions, and the clay fraction not always showed higher contents than the silt- and sand-sized fractions, despite its strong ability to retain SOC and N by sorption. This is caused by the so-called “SOC dilution effect” (Amelung et al., 1998; Zinn et al., 2007a), more evident at the 0-5 cm depths. This “SOC dilution effect” is the trend for SOC concentrations in a given fraction to be inversely related to the content of that fraction in the soil. For example, the soil on quartzite (497.4 g kg^{-1} sand and 172.7 g kg^{-1} clay) had the lowest SOC content in the sand fraction and the highest SOC content in the clay fraction at the 0-5 cm depth. On the other hand, the soil on limestone (110.4 g kg^{-1} sand and 777.9 g kg^{-1} clay) had the highest SOC content in the sand fraction and the lowest SOC content in the clay fraction at the same depth.

The C:N ratios in bulk soils and in particle-size fractions seem unaffected by sampling depth, except for the sand fraction. The lower sand C:N ratios at the 0-5 cm depth suggest that these partially decomposed plant residues of sand sizes can still mineralize N to plants, particularly in the soil on itabirite. With increasing depth, amount and frequency input of organic residues is likely reduced, thus limiting N cycling and availability. Besides, charcoal fragments are most likely present at the 30-40 and 90-100 cm depths (Araujo et al., 2017; Zinn et al., 2018), which contributes to high sand-sized C:N ratios, particularly in the soil on gabbro. On the contrary, charcoal is probably rare in the silt size, since its C:N ratios did not vary with depth. The low C:N ratios in the clays compared to the other size-fractions probably reflects sorption of NH_4^+ forms (Nieder et al., 2011) and perhaps other ammonium bases present in SOM compounds, such as amino-acids and amino- sugars derived from microbial activity (Bent T. Christensen, 1992; Guggenberger et al., 1994).

4.3 Relationships between C:N ratios, soil texture, and fertility

The positive correlation between clay contents and C:N ratios and the negative correlation between C:N ratios and silt contents reflects the relatively high variation in clay C:N ratios, and the limited variation in the silt size. The positive relationship between sand and silt C:N ratios and SOC at the 90-100 cm is likely due to the predominance of ancient SOM and perhaps charcoal, which is most likely devoid or poor in N.

The negative relationship between exchangeable bases, cation exchange capacity, clay activity, and base saturation with C:N ratios suggests that the retention of exchangeable bases and N (the latter promoting lower C:N ratios) in clays of the studied soils are somehow related. In other words, soils with higher retention capacity of Ca, Mg and other bases are probably more able to also retain more N, which would ultimately result in lower C:N ratios. Accordingly, when SOC and N contents are plotted vs. sum of bases at the 0-5 cm depth (Figure 2), we note that SB increases with N contents, whereas no clear trend was observed for SOC. It is worth mentioning that these findings derive from correlations and regression models; thus, they do not constitute a cause-effect relationship, that is, they do not allow us to explain the mechanisms driving such N-bases dynamics.

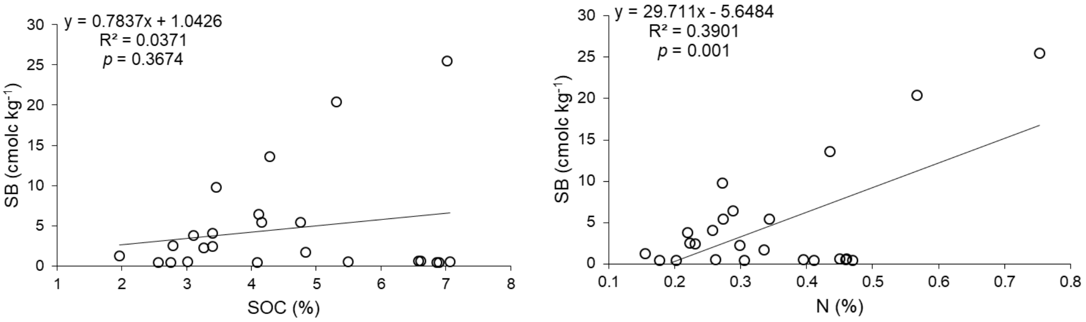

Figure 2. Linear regression models between SOC, N, and sum of exchangeable bases (SB) at the 0-5 cm soil depth.

Baião et al. (2021) argues that soil fertility in soils derived from some iron formations in Minas Gerais is linked with increased SOM contents, plant biodiversity, and biological N fixation. It is possible that the microbial alteration of SOM and retention of NH_4^+ and similar groups onto clay minerals contributed to the high N content in the soil on itabirite and other more fertile soils, which also had high contents of Ca^{2+} and Mg^{2+}. Such increased natural fertility favors plant productivity and nutrient cycling, which contributes to cycle and maintain N and other nutrients in the plant-soil system, thus lowering the C:N ratios in soils and in particle-size fractions. Future research should focus on microbial and plant diversity to clarify the underlying processes of soil fertility in such soils, and in specific mechanisms through which different clay minerals retain N and N groups in SOM.

From a practical viewpoint, our findings on low C:N ratios with increased SB allows us to propose that soil best management practices, such as liming or crop rotation including legumes, can positively affect soil fertility in the soils of the studied region not only through increase of exchangeable bases but also to increase SOM production (Castro et al., 2015), nutrient cycling (Vazquez et al., 2019), and N mineralization (Adams & Martin, 2015), with subsequent adsorption into clays. Including legumes in crop rotations increases the N inputs in the soil-plant system via biological fixation, which may improve primary production, SOM accumulation, and contribute to maintenance of natural soil fertility (Abdalla et al., 2019; Liebman et al., 2018). Such practices must be carried out carefully to prevent the priming effect of SOM (Kuzyakov, 2010), in which fresh OM inputs stimulate the degradation of the native SOM, thus having negative impacts on long-term SOC sequestration.

The positive relationship between C:N ratios and Al^{3+} and H+Al suggests that SOC retention in these soils is mostly driven by Al^{3+} at the 0-5 cm depth, and that carboxylic groups in SOM are a main source of acidity in these soils. In fact, in soils under tropical forests, SOC had a strong, positive correlation with oxalate-extractable Al (Rasmussen et al., 2018), evidencing the relevance of Al-organic complexes in the SOM stabilization. Poorly crystalline Fe- and Al-hydr(oxides) have increased capacity to form covalent bonds with SOM via hydroxyl groups, providing chemical protection to SOM against microbial degradation and, thus, reducing SOC mineralization (Singh et al., 2018). Here, however, total and amorphous Fe-hydr(oxides) did not correlate with C:N ratios. Accordingly, Araujo et al. (2017) did not identify any significant correlations between SOC and Fe_2O_3 contents in these soils, demonstrating that Fe-hydr(oxides) played a minor role on SOC retention in tropical highlands.

Lastly, the negative relationship between C:N ratios and total and amorphous Mn-(hydr)oxides is difficult to interpret since such data is not commonly determined. Again, such relationship is driven by N contents, as no clear trend was observed between SOC and Mn contents (Tables 3 and 4). It is well established that increased contents of exchangeable Mn in soils can reduce nodulation and N fixation in legumes (Döbereiner, 1966). Thus, we would not expect increased levels of N in such soils, but that is precisely what our results show: the highest Mn contents were also found in the fertile soil on itabirite. It is likely that the Mn in that soil is derived from the parent material (itabirite associated with ferroan dolomite), considering that high Mn contents are also observed at the 90-100 cm depth, whereas the high N contents, particularly at the 0-5 cm depth, are linked to SOM accumulation.

Conclusions

We investigated C:N ratios in soils in and particle-size fractions in tropical soils developed from contrasting parent materials. C:N ratios were unaffected by depth (except for sand fractions), decreased with increasing sum of exchangeable bases and Mn oxide contents, and increased with exchangeable Al^{3+} and H+Al contents. The inverse relationship between C:N ratios and soil fertility indicators in the studied soils can be ascribed to the increased N content, particularly in the soil on itabirite, which unexpectedly presented high natural fertility. Such knowledge can be useful to guide agronomic practices, such as liming and crop rotations, in order to improve SOM accumulation and maintain soil fertility in the studied soils. Future research should investigate the microbial and plant diversity in such soils, as well as organo-mineral interactions, as they can be relevant drivers of SOC dynamics, nutrient cycling, and availability in these environments.

Acknowledgments

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Supplemental Information

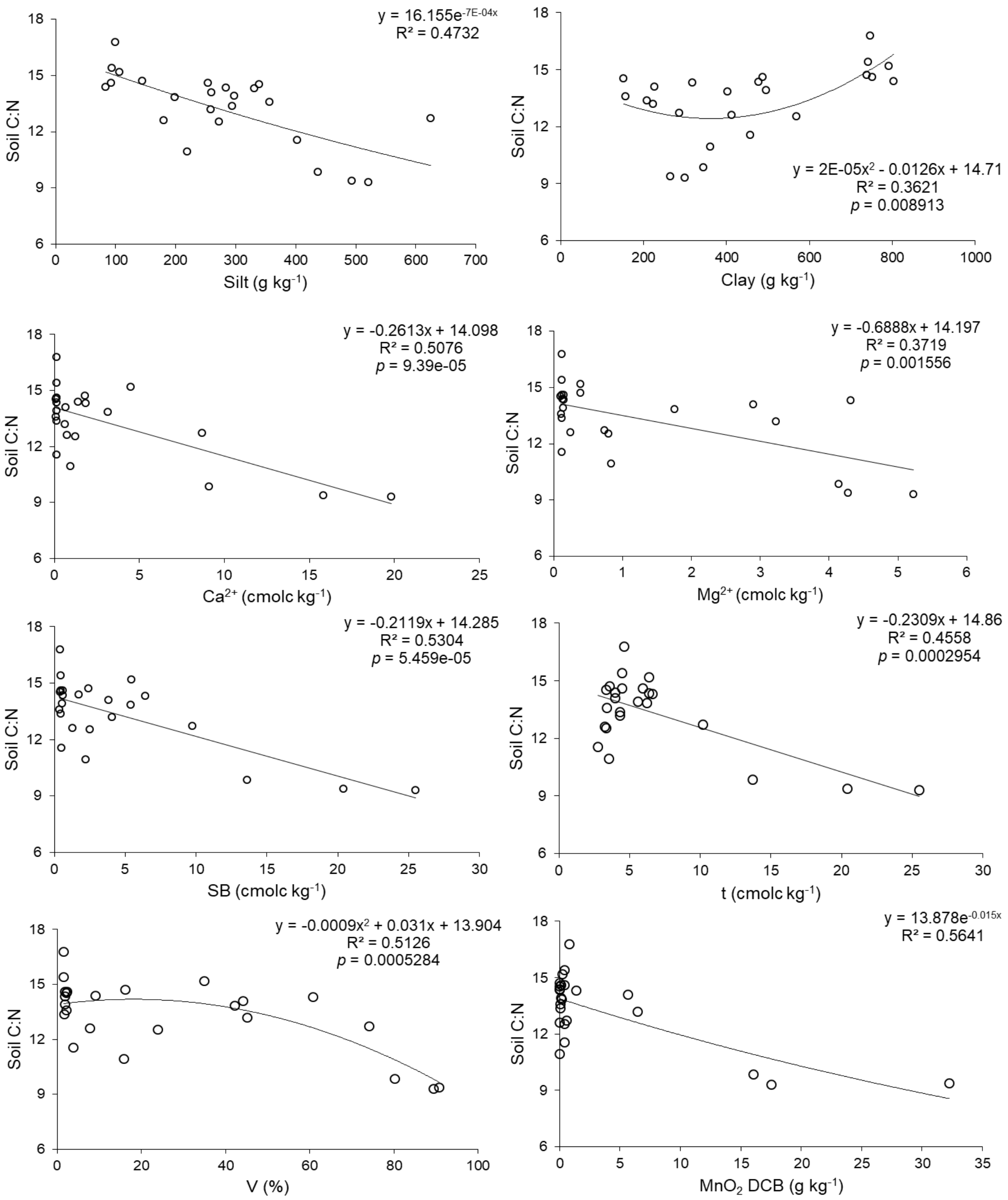

Figure S1. Regression models between soil physical and chemical properties and soil C:N ratios ($n = 24$) at the 0-5 cm depth.

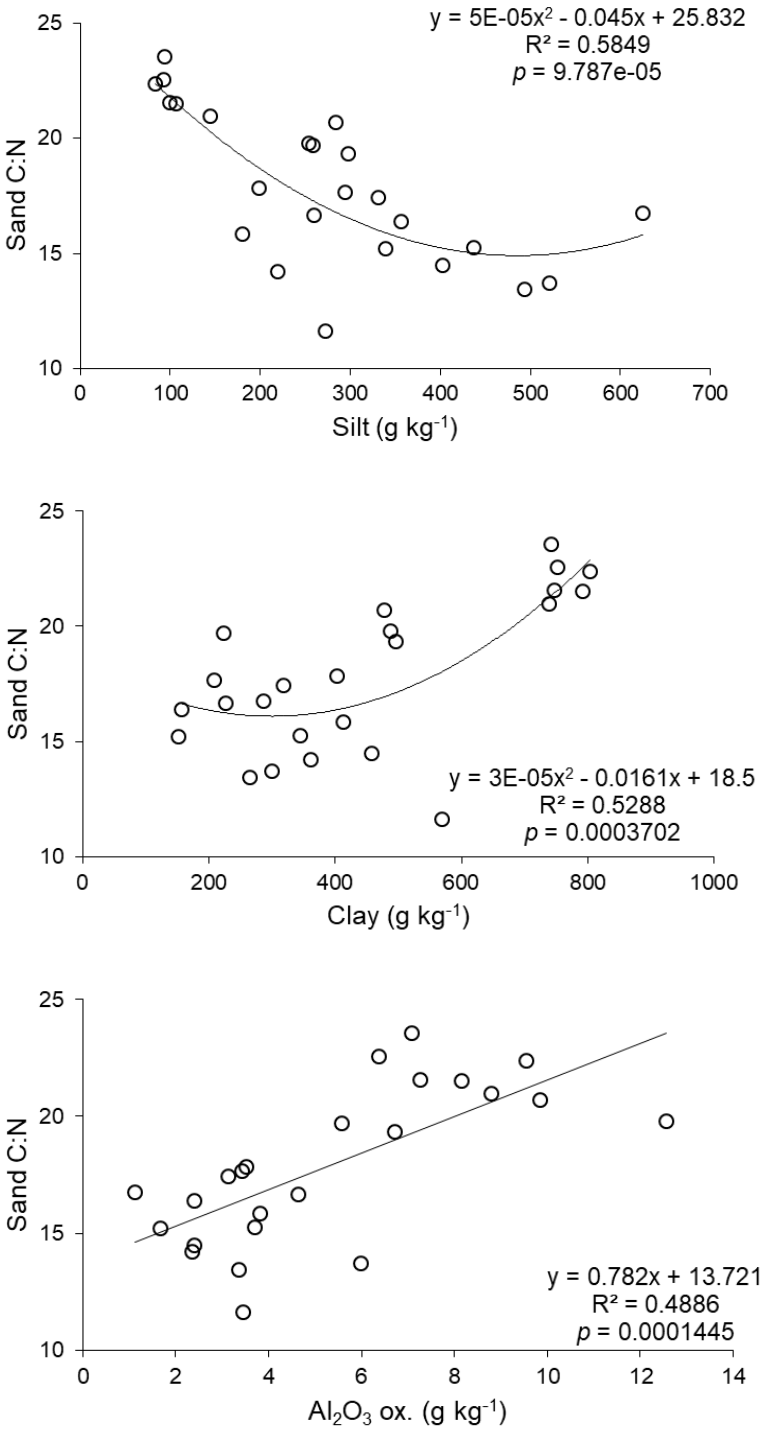

Figure S2. Regression models between soil physical and chemical properties and sand C:N ratios ($n = 24$) at the 0-5 cm depth.

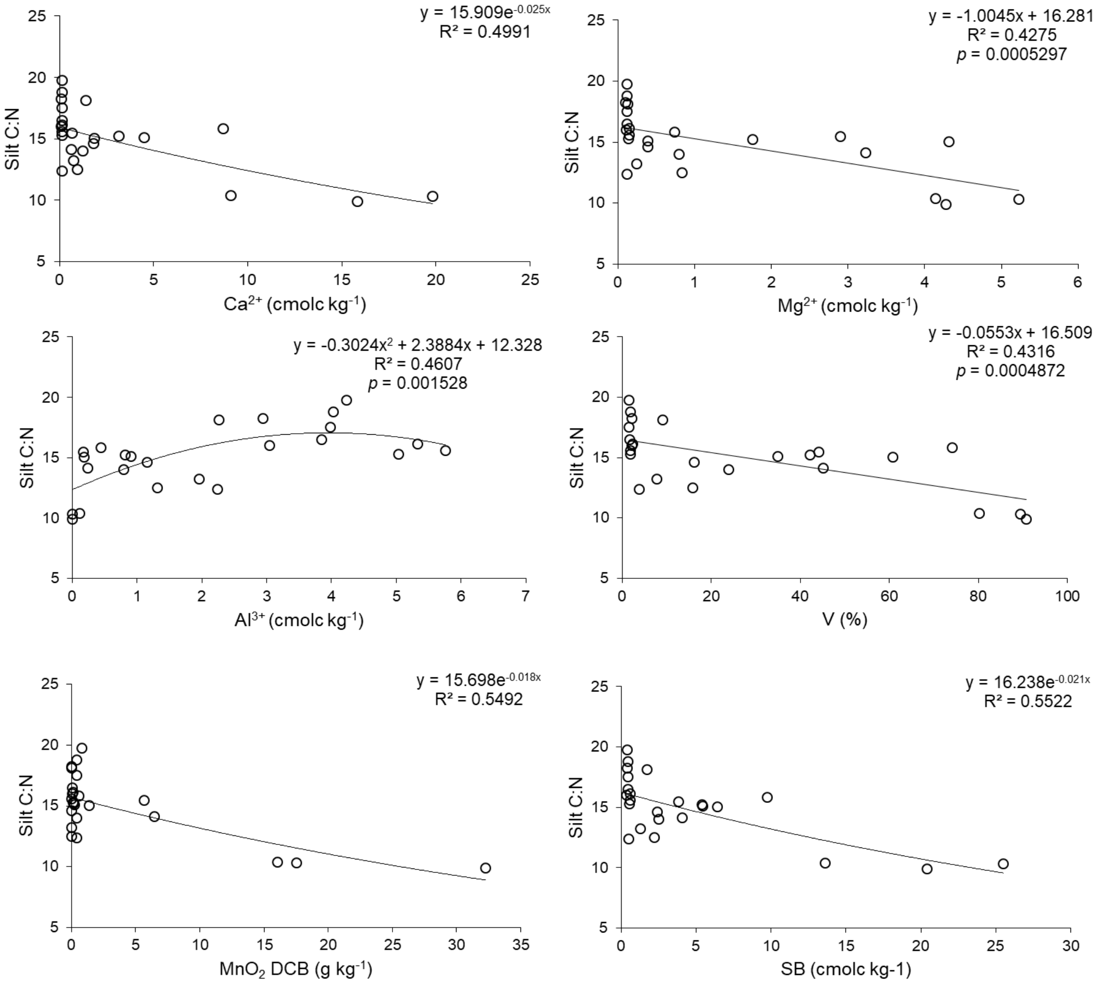

Figure S3. Regression models between soil physical and chemical properties and silt C:N ratios (n = 24) at the 0-5 cm depth.

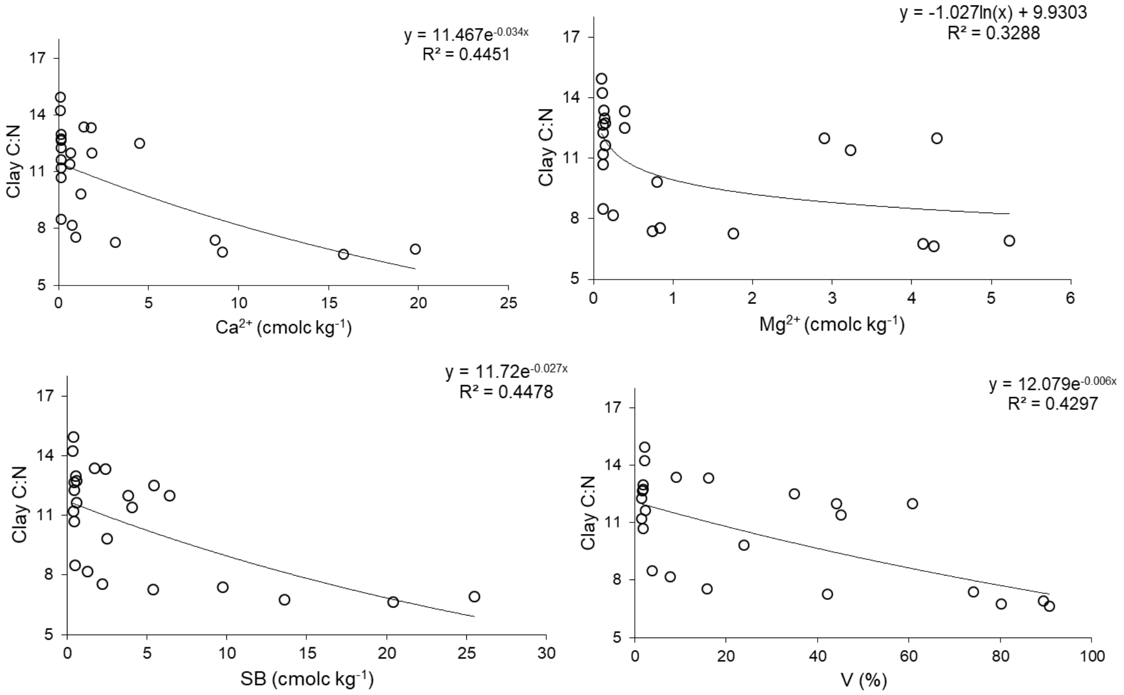

Figure S4. Regression models between soil physical and chemical properties and clay C:N ratios (*n* = 24) at the 0-5 cm depth.

APPENDIX

Appendix 1. Global database compiled in chapter 3 to model C:N ratios in soils and in particle-size fractions.

Study #	Location	*n*	References
1	USA and Canada	21	Amelung, W. *et al.* Carbon, Nitrogen, and Sulfur Pools in Particle-Size Fractions as Influenced by Climate. *Soil Sci. Soc. Am. J.* **62**, 172–181 (1998).
2	Canada	2	Anderson, D. W., Saggar, S., Bettany, J. R. & Slewart, J. W. B. Particle Size Fractions and Their Use in Studies of Soil Organic Matter: The Nature and Distribution of Forms of Carbon, Nitrogen, and Sulfur. *Soil Sci. Soc. Am.* **45**, 767–772 (1981).
3	Canada	9	Angers, D. A., N'dayegamiye, A. & Côté, D. Tillage-Induced Differences in Organic Matter of Particle-Size Fractions and Microbial Biomass. *Soil Sci. Soc. Am. J.* **57**, 512–516 (1993).
4	Germany	2	Angst, G., Kögel-Knabner, I., Kirfel, K., Hertel, D. & Mueller, C. W. Spatial distribution and chemical composition of soil organic matter fractions in rhizosphere and non-rhizosphere soil under European beech (Fagus sylvatica L.). *Geoderma* **264**, 179–187 (2016).
5	Australia and China	5	Baldock, J. A. *et al.* Aspects of the chemical structure of soil organic materials as revealed by solid-state13C NMR spectroscopy. *Biogeochemistry* **16**, 1–42 (1992).
6	Ethiopia	10	Gelaw, A. M., Singh, B. R. & Lal, R. Soil quality indices for evaluating smallholder agricultural land uses in northern ethiopia. *Sustainability* **7**, 2322–2337 (2015).
7	Germany	4	Guggenberger, G., Christensen, B. T. & Zech, W. Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. *Eur. J. Soil Sci.* **45**, 449–458 (1994).
8	Thailand	12	Jindaluang, W., Kheoruenromne, I., Suddhiprakarn, A., Singh, B. P. & Singh, B. Nature of Organic Matter Associated with Particle-sized Fractions of Thai Soils. *Thai J. Agric. Sci.* **47**, 167–184 (2014).
9	Germany	3	Knicker, H., Schmidt, M. W. I. & Kögel-Knabner, I. Nature of organic nitrogen in fine particle size separates of sandy soils of highly industrialized areas as revealed by NMR spectroscopy. *Soil Biol. Biochem.* **32**, 241–252 (2000).
10	Canada	5	Baah-Acheamfour, M., Carlyle, C. N., Bork, E. W. & Chang, S. X. Trees increase soil carbon and its stability in three agroforestry systems in central Alberta, Canada. *For. Ecol. Manage.* **328**, 131–139 (2014).
11	Canada	1	Catroux, G. & Schnitzer, M. Chemical, Spectroscopic, and Biological Characteristics of the Organic Matter in Particle Size Fractions Separated from an Aquoll. *Soil Sci. Soc. Am.* **51**, 1200–1207 (1987).
12	Denmark	5	Christensen, B. T. Carbon and Nitrogen in Particle Size Fractions Isolated from Danish Arable Soils by Ultrasonic Dispersion and Gravity-Sedimentation. *Acta Agric. Scand.* **35**, 175–187 (1985).
13	Denmark	3	Christensen, B. T. Effects of animal manure and mineral fertilizer on the total carbon and nitrogen contents of soil size fractions. *Biol. Fertil. Soils* **5**, 304–307 (1988).
14	Canada	2	Tiessen, H. & Stewart, W. B. Particle-size Fractions and their Use in Studies of Soil Organic Matter: Cultivation Effects on Organic Matter Composition in Size Fractions. *Soil Sci. Soc. Am.* **47**, 509–514 (1983).
15	France	21	Jolivet, C., Arrouays, D., Lévèque, J., Andreux, F. & Chenu, C. Organic carbon dynamics in soil particle-size separates of sandy Spodosols when forest is cleared for maize cropping. *Eur. J. Soil Sci.* **54**, 257–268 (2003).
16	Canada	3	Angers, D. A. & Dayegamiye, A. N. Effects of manure application on carbon, nitrogen, and carbohydrate contents of a silt loam and its particle-size fractions. *Biol. Fertil. Soils* **11**, 79–82 (1991).
17	Taiwan	3	Shiau, Y., Chen, J., Chung, T., Tian, G. & Chiu, C. 13C NMR spectroscopy characterization of particle - size fractionated soil organic carbon in subalpine forest and grassland ecosystems. *Bot. Stud.* **58**, 1–7 (2017).
18	Canada	12	Diochon, A., Gregorich, E. G., Kellman, L., Morrison, M. & Ma, B.-L. Greater soil C inputs accelerate loss of C in cropping systems with low N input. *Plant Soil* **400**, 93–105 (2016).
19	Brazil	23	Araujo, M. A. de. RETENÇÃO DE CARBONO ORGÂNICO DO SOLO NO SUL DE MINAS GERAIS: EFEITO DA ROCHA DE ORIGEM? (Universidade Federal de Lavras, 2015).
20	Austria, Germany and Italy	4	Stemmer, M., Gerzabek, M. H. & Kandeler, E. Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication. *Soil Biol. Biochem.* **30**, 9–17 (1998).
21	Denmark, Germany and Italy	3	Stemmer, M. ., Lutzow, M. . VON, Kandeler, E. ., Pichlmayer, F. & Gerzabek, M. H. The effect of maize straw placement on mineralization of C and N in soil particle size fractions. *Eur. J. Soil Sci.* **50**, 73–85 (1999).
22	Spain	1	Nicolás, C., Hernández, T. & García, C. Organic amendments as strategy to increase organic matter in particle-size fractions of a semi-arid soil. *Appl. Soil Ecol.* **57**, 50–58 (2012).
23	Japan	1	Asano, M. & Wagai, R. Distinctive organic matter pools among particle- size fractions detected by solid-state C-NMR, δ C and δ N analyses only after strong dispersion in an allophanic Andisol detected by solid-state 13 C-NMR, δ 13 C and δ 15 N analyses only after. *Soil Sci. Plant Nutr.* **61**, 242–248 (2015).
24	Benin	2	Gavinelli, E. *et al.* A routine method to study soil organic matter by particle - size fractionation: Examples for tropical soils. *Commun. Soil Sci. Plant Anal.* **26**, 1749–1760 (1995).
25	Burkina Faso and Congo	4	Barthes, B. G. *et al.* Determining the distributions of soil carbon and nitrogen in particle size fractions using near-infrared reflectance spectrum of bulk soil samples. *Soil Biol. Biochem.* **40**, 1533–1537 (2008).
26	France	1	Quenea, K., Lamy, I., Winterton, P., Bermond, A. & Dumat, C. Interactions between metals and soil organic matter in various particle size fractions of soil contaminated with waste water. *Geoderma* **149**, 217–223 (2009).
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28	Burkina Faso	2	Pallo, J. P. F., Sawadogo, N., Zombre, N. P. & Sedogo, M. P. STATUT DE LA MATIERE ORGANIQUE DES CAMBISOLS ET DES LIXISOLS SOUS FORMATIONS NATURELLES DE LONGUE DUREE EN ZONE NORD-SOUDANIENNE AU BURKINA FASO. *Agron. Africaine* **21**, 215–229 (2009).
29	Mexico	8	Covaleda, S., Pajares, S., Gallardo, J. F. & Etchevers, J. D. Short-term changes in C and N distribution in soil particle size fractions induced by agricultural practices in a cultivated volcanic soil from Mexico. *Org. Geochem.* **37**, 1943–1948 (2006).
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31	Taiwan	2	Chen, J. S. & Chiu, C. Y. Characterization of soil organic matter in different particle-size fractions in humid subalpine soils by CP/MAS 13C NMR. *Geoderma* **117**, 129–141 (2003).
32	Brazil	4	Diekow, J., Mielniczuk, J., Knicker, H., Dick, D. P. & Kögel-knabner, I. Carbon and nitrogen stocks in physical fractions of a subtropical Acrisol as influenced by long-term no-till cropping systems and N fertilisation. *Plant Soil* **268**, 319–328 (2005).
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34	China	1	Liang, Q. *et al.* Effects of 15 years of manure and mineral fertilizers on enzyme activities in particle-size fractions in a North China Plain soil. *Eur. J. Soil Biol.* **60**, 112–119 (2014).
35	USA	4	Lorenz, K., Lal, R. & Shipitalo, M. J. Chemical stabilization of organic carbon pools in particle size fractions in no-till and meadow soils. *Biol. Fertil. Soils* **44**, 1043–1051 (2008).
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37	Italy, France, Denmark and Germany	4	Schöning, I., Morgenroth, G. & Kögel-Knabner, I. O/N-alkyl and alkyl C are stabilised in fine particle size fractions of forest soils. *Biogeochemistry* **73**, 475–497 (2005).
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39	Germany	1	Schmidt, M. W. I., Knicker, H., Hatcher, P. G. & Kögel-Knabner, I. Impact of brown coal dust on the organic matter in particle-size fractions of a Mollisol. *Org. Geochem.* **25**, 29–39 (1996).
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42	Germany	1	Bimüller, C. *et al.* Decoupled carbon and nitrogen mineralization in soil particle size fractions of a forest topsoil. *Soil Biol. Biochem.* **78**, 263–273 (2014).
43	China	3	Ding, F., Sun, W. & Huang, Y. Net N2O production from soil particle size fractions and its response to changing temperature. *Sci. Total Environ.* **650**, 97–104 (2019).
44	Greece	2	Eleftheriadis, A., Lafuente, F. & Turrión, M. B. Effect of land use, time since deforestation and management on organic C and N in soil textural fractions. *Soil Tillage Res.* **183**, 1–7 (2018).
45	China	14	He, N. P., Wang, R. M., Zhang, Y. H. & Chen, Q. S. Carbon and Nitrogen Storage in Inner Mongolian Grasslands: Relationships with Climate and Soil Texture. *Pedosphere* **24**, 391–398 (2014).
46	China	1	Ling, N. *et al.* Response of the bacterial diversity and soil enzyme activity in particle-size fractions of Mollisol after different fertilization in a long-term experiment. *Biol. Fertil. Soils* **50**, 901–911 (2014).
47	Tonga	6	Pochet, G., Van der Velde, M., Vanclooster, M. & Delvaux, B. Hydric properties of high charge, halloysitic clay soils from the tropical South Pacific region. *Geoderma* **138**, 96–109 (2007).
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50	Spain	4	Caravaca, F., Lax, A. & Albaladejo, J. Organic matter, nutrient contents and cation exchange capacity in fine fractions from semiarid calcareous soils. *Geoderma* **93**, 161–176 (1999).
51	Canada	5	Hinds, A. A. & Lowe, L. E. DISTRIBUTION OF CARBON, NITROGEN, SULPHUR AND PHOSPHORUS IN PARTICLE-SIZE SEPARATES FROM GLEYSOLIC SOILS. *Can. J. Soil Sci.* **60**, 783–786 (1980).
52	Germany	3	Kahle, M., Kleber, M. & Jahn, R. Carbon storage in loess derived surface soils from Central Germany: Influence of mineral phase variables. *J. Plant Nutr. Soil Sci.* **165**, 141–149 (2002).
53	USA	2	Quideau, S. A., Chadwick, O. A., Benesi, A., Graham, R. C. & Anderson, M. A. A direct link between forest vegetation type and soil organic matter composition. *Geoderma* **104**, 41–60 (2001).
54	USA	2	Quideau, S. A., Anderson, M. A., Graham, R. C., Chadwick, O. A. & Trumbore, S. E. Soil organic matter processes: Characterization by 13C NMR and 14C measurements. *For. Ecol. Manage.* **138**, 19–27 (2000).
55	Canada	1	Schulten, H.-R., Leinweber, P. & Sorge, C. Composition of organic matter in particle-size fractions of an agricultural soil. *J. Soil Sci.* **44**, 677–691 (1993).
56	France	2	Fernández-Ugalde, O. *et al.* Does phyllosilicate mineralogy explain organic matter stabilization in different particle-size fractions in a 19-year C3/C4 chronosequence in a temperate Cambisol? *Geoderma* **264**, 171–178 (2016).

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57	USA	3	Zhang, X. *et al.* Land-use effects on amino sugars in particle size fractions of an Argiudoll. *Appl. Soil Ecol.* **11**, 271–275 (1999).
58	China	1	El-Naggar, A. *et al.* Biochar influences soil carbon pools and facilitates interactions with soil: A field investigation. *L. Degrad. Dev.* **29**, 2162–2171 (2018).
59	Iran	8	Beheshti, A., Raiesi, F. & Golchin, A. Soil properties, C fractions and their dynamics in land use conversion from native forests to croplands in northern Iran. *Agric. Ecosyst. Environ.* **148**, 121–133 (2012).
60	UK	11	Shiel, R. S. Variation in amounts of carbon and nitrogen associated with particle size fractions of soils from the Palace Leas meadow hay plots. *J. Soil Sci.* **37**, 249–257 (1986).
61	Argentina	2	Desjardins, T., Folgarait, P. J., Pando-Bahuon, A., Girardin, C. & Lavelle, P. Soil organic matter dynamics along a rice chronosequence in north-eastern Argentina: Evidence from natural13C abundance and particle size fractionation. *Soil Biol. Biochem.* **38**, 2753–2761 (2006).
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63	Germany	2	Heim, A. & Schmidt, M. W. I. Lignin is preserved in the fine silt fraction of an arable Luvisol. *Org. Geochem.* **38**, 2001–2011 (2007).
64	South Africa	6	Lobe, I., Amelung, W. & Du Preez, C. C. Losses of carbon and nitrogen with prolonged arable cropping from sandy soils of the South African Highveld. *Eur. J. Soil Sci.* **52**, 93–101 (2001).
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67	Sweden and Germany	2	Pronk, G. J., Heister, K. & Kögel-Knabner, I. Iron Oxides as Major Available Interface Component in Loamy Arable Topsoils. *Soil Sci. Soc. Am. J.* **75**, 2158–2168 (2011).
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69	Belgium	1	Jegajeevagan, K., Sleutel, S., Ameloot, N., Kader, M. A. & De Neve, S. Organic matter fractions and N mineralization in vegetable-cropped sandy soils. *Soil Use Manag.* **29**, 333–343 (2013).
70	Russia	2	Kalinina, O., Goryachkin, S. V., Karavaeva, N. A., Lyuri, D. I. & Giani, L. Dynamics of carbon pools in post-agrogenic sandy soils of southern taiga of Russia. *Carbon Balance Manag.* **5**, 1–10 (2010).
71	Ethiopia	4	Solomon, D., Fritzsche, F., Lehmann, J., Tekalign, M. & Zech, W. Soil Organic Matter Dynamics in the Subhumid Agroecosystems of the Ethiopian Highlands. *Soil Sci. Soc. Am. J.* **66**, 969–978 (2002).
72	Brazil	4	Silva, C. A., Anderson, S. J. & Vale, F. R. Carbono, nitrogênio e enxofre em frações granulométricas de dois latossolos submetidos à calagem e adubação fosfatada. *Rev. Bras. Ciência do Solo* **23**, 593–602 (1999).
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74	China	1	Zhang, Q. *et al.* Fatty-Acid Profiles and Enzyme Activities in Soil Particle-Size Fractions under Long-Term Fertilization. *Soil Sci. Soc. Am. J.* **80**, 97–111 (2016).

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