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Soil Clay Drives Variations in Carbon and Nitrogen Stocks in Topsoil and Subsoil with Grassland Cultivation Duration: A 50-Year Chronosequence

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Abstract

Knowledge of the influence of grassland-to-cropland conversion on subsoil carbon (C) and nitrogen (N) stocks and the effect of soil properties on subsoil C and N stocks after grassland cultivation remain poorly understood. Furthermore, soil C and N changes retain a biological coupling under global changes; however, whether the coupling between soil C and N stocks can be disrupted by long-term grassland cultivation remains largely unknown. Here, we explored changes in soil C and N stocks at three soil depths (0–10, 10–30, and 30–50 cm) across a grassland cultivation chronosequence of 50 years in a semiarid agro-pastoral ecotone in northern China. Our results showed that soil C and N stocks in the 0–10 cm soil layer exhibited a valley-shaped pattern with increasing cultivation duration, reaching the lowest value at 20 years of grassland cultivation. For the 10–30 cm and 30–50 cm soil layers, soil C and N stocks within 10 years of grassland cultivation were higher than those in native grassland. Soil C and N stocks bottomed out at 20 years of grassland cultivation and then increased with the duration of grassland cultivation. Cultivation duration had no direct effect on soil C and N stocks across soil depths but exerted an indirect influence by affecting soil properties. Soil properties mainly drove variations in C and N stocks in soil, which strengthened with soil depth. Soil C and N stocks in the 0–10 cm soil layer showed a positive correlation with soil pH and soil clay and silt contents and were negatively related to soil sand content. Soil C and N stocks in the 10–30 and 30–50 cm soil layers were only positively correlated with soil clay content. These indicate that soil clay plays a vital role in regulating variations in C and N stocks in topsoil and subsoil with the duration of grassland cultivation. Moreover, soil C stocks in the three soil layers exhibited a significant correlation with soil N stocks. This reveals that the coupled correlation between soil C and N stocks still remains under long-term grassland cultivation.

Keywords: land-use change; grassland transformation; nutrient stock; soil texture; agro-pastoral ecotone

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1. Introduction

Grassland, covering over 40% of the global surface area, accounts for more than one-third of the global terrestrial carbon (C) stock and serves an essential function in maintaining biodiversity and food production [1,2]. With the rapid growth in the population, urbanization and food demand, an extensive portion of grassland has been converted to

cropland worldwide [3,4]. In China, nearly 18% of existing cropland has been converted from grassland, and between 2000 and 2015, a total of 25,579 square kilometers of grassland in China was reclaimed for cropland [5–7]. Soil C and nitrogen (N) stocks, reflecting the sustainability of soil quality, play a vital role in grassland ecosystem functions [8,9]. As the largest C reservoir in terrestrial ecosystems, even small fractional changes in soil C stocks can have disproportionate effects on atmospheric carbon dioxide (CO_2) concentrations [10,11]. N acts as a limiting nutrient for both plant production and microbial activity [12]. Grassland cultivation generally removes the vegetation cover on surface soil and causes soil erosion, leading to significant changes in soil properties [13,14]. This exerts a profound impact on soil C and N dynamics [15,16]. Thus, clarifying the response of soil C and N stocks to the conversion of grassland to cropland has implications for land-use management.

Several studies have examined how soil C and N stocks respond to grassland cultivation (i.e., grassland clearance for subsistence agriculture) [4,16–24]. However, their findings indicated that the influence of grassland cultivation on soil C and N stocks was mediated by the cultivation duration [19]. For example, Li et al. [20] analyzed 92 studies worldwide and found that soil C stocks did not vary within 5 years of grassland cultivation, and then it decreased with an increase in the duration of grassland cultivation. Soil N stocks were first reduced and then remained stable after 10–25 years of grassland cultivation. By conducting a global meta-analysis of 1110 published data sets, Kim et al. [16] reported that soil C and N stocks decreased within 10 years of grassland cultivation, and then they generally became constant. Apart from the effect of cultivation year, soil depth was reported to affect soil C and N responses to grassland cultivation [19,20,23]. For example, by contrasting soil C and N stocks between cropland and temperate grassland in Inner Mongolia, China, Zhu et al. [23] reported that soil C and N stocks at 0–10 cm soil depth in grassland were significantly higher than those in cropland, but no significant difference in soil C and N stocks between cropland and grassland was observed in the 10–20, 20–30, 30–40 and 40–50 cm soil layers. Furthermore, previous investigations examining the influence of grassland cultivation on soil C and N stocks have primarily concentrated on the topsoil (0–30 cm) layer, and how subsoil (>30 cm) C and N stocks changed with cultivation duration remained poorly understood.

Variations in soil C and N stocks can be affected by soil properties, including soil pH and soil texture [25,26]. Grassland cultivation has been reported to induce significant changes in soil properties [19,27], which may explain the underlying mechanisms of soil C and N variations after grassland cultivation. Soil pH may regulate soil C and N changes through the indirect pathway of pH-mediated microbial activity, as soil pH can influence soil microbial activity, thereby affecting soil C and N stocks [28,29]. The results from the global meta-analysis by Tang et al. [19] and Li et al. [20] showed that soil C stocks showed a negative correlation with soil pH, whereas soil N stocks showed no significant relationship with soil pH after the conversion of grassland to cropland. Soil clay and silt may regulate soil C and N changes more directly through physical protection, as soil clay and silt can absorb C and N and further protect them against microbial utilization [19,26,30]. De Rosa et al. [24] compared the difference in soil C stocks between croplands and grasslands in Europe and observed that high soil clay content positively affected the change in topsoil (0–20 cm) C stocks that resulted from land-use change between croplands and grasslands. However, knowledge of how soil properties regulate subsoil C and N stocks following grassland cultivation remains limited. Here we hypothesize that soil properties may exert a strong effect on subsoil C and N stocks after grassland cultivation.

The strict stoichiometric relationship between C and N in living organisms makes soil C and N changes biologically coupled [31,32]. Owing to the resistance of biological

processes to global change, the coupling of soil C and N cycles has the ability to remain under global change [32,33]. For example, Li et al. [9] carried out soil sampling after implementing grazing exclusion in temperate grasslands on the Loess Plateau, China, and explored the effect of grazing exclusion on soil C and N stocks. Their findings demonstrated that long-term grazing exclusion substantially enhanced soil C and N stocks through the sufficient inputs of plant residue, resulting in the coupled associations of soil C and N stocks under grazing exclusion. Grassland cultivation can cause changes in aboveground vegetation cover and soil microbial community, thereby influencing biological processes such as soil organic matter accumulation and decomposition [34–36]. However, whether the coupling between soil C and N stocks can be disrupted by grassland cultivation remains largely unknown. Here we hypothesize that grassland cultivation cannot decouple soil C and N stocks.

To fill these knowledge gaps, this study analyzed changes in soil C and N stocks and soil properties at soil depths of 0–10, 10–30, and 30–50 cm along a 50-year chronosequence of grassland cultivation in an agro-pastoral ecotone in northern China. The 0–10 and 10–30 cm soil layers were defined as topsoil, and the 30–50 cm soil layer was defined as subsoil. The present study aimed to (1) clarify variations in C and N stocks and soil properties in topsoil and subsoil with cultivation duration, (2) reveal the effects of soil properties on C and N stocks in topsoil and subsoil after grassland cultivation, and (3) examine whether the conversion of grassland to cropland could break the coupling of soil C and N stocks.

2. Materials and Methods

2.1. Site Description

Soil sampling was conducted near Guyuan National Grassland Ecosystem Field Station (41°46′ N, 115°40′ E, 1460 m a.s.l.), located in Guyuan County, Hebei Province, China. The study area experiences a typical semiarid continental monsoon climate, with a mean annual temperature of 1.4 °C. The lowest temperature of −18.6 °C occurs in January, while the highest temperature of 17.6 °C occurs in July. The mean annual precipitation is 430 mm, most of which falls between July and September. According to the FAO/UNESCO soil classification system, the soil in this region is classified as Calcisol. To explore the effects of grassland cultivation on soil C and N stocks, the croplands reclaimed for 5, 10, 20, 30, and 50 years (Y5, Y10, Y20, Y30, and Y50) were selected for soil sampling, and the native grassland was selected as the control treatment (Y0) (Figure 1). All these sampling sites were flat and more than 1 hm^2. The distance among the sampling sites was less than 20 km to avoid the interference of the effects of climate factors, soil properties and original plant community composition before grassland cultivation. The native grassland in our sampling area is temperate typical grassland, in which the dominant plant species are *Leymus chinensis* and *Stipa krylovii* [5]. The cropland converted from grassland was mainly planted with naked oat, no irrigation was adopted, rotary tillage was adopted before seeding, and seeds were sown in late April at a fertilization rate of 150 kg ha^{-1} $year^{-1}$ (diammonium phosphate), with crops harvested annually at the end of August.

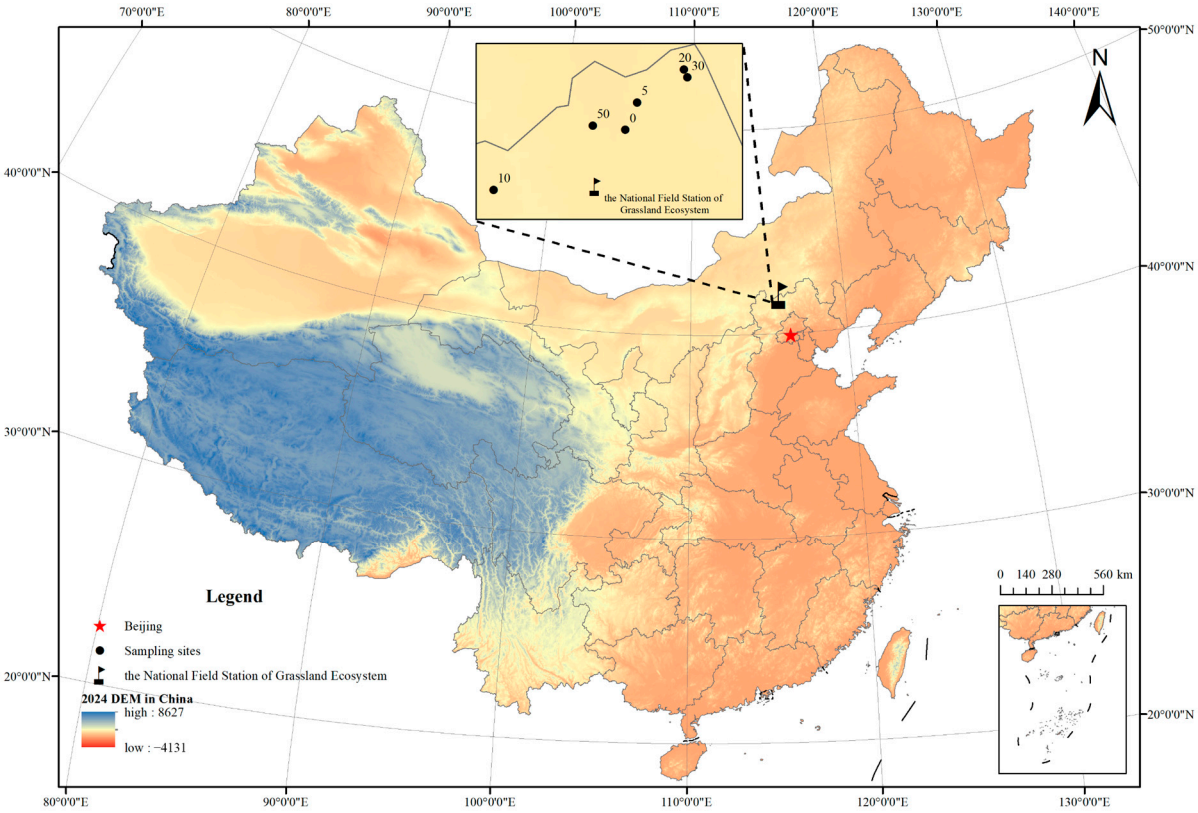


Figure 1. Location of sampling sites with different cultivation years.

2.2. Soil Sampling and Chemical Analysis

Soil sampling was conducted in September 2023. At each sampling site, 5 sampling plots (200 m^2) were set, and the distance between each plot was over 100 m. A total of 8 quadrats (0.5 × 0.5 m) were randomly set in each sampling plot. In each quadrat, 3 soil samples were randomly collected by using a soil auger, and all soil samples collected from each sampling plot were combined into a single composite sample. This study collected soil samples at soil depths of 0–10, 10–30, and 30–50 cm. This study obtained a total of 90 soil samples in this soil sampling process.

After the removal of roots and stones, soil samples were air-dried and subsequently passed through a 2 mm sieve. Soil C content was determined using the vitriol acid–potassium dichromate oxidation method [37]. Soil N content was analyzed according to the Kjeldahl method [38]. Soil bulk density at three soil depths was measured using a volumetric soil ring independently. Soil C and N stocks were calculated as the product of their respective contents, bulk density, and sampling depth. Soil pH was measured with a pH electrode at a soil-to-water ratio of 1:2.5. Soil texture, consisting of soil clay, silt, and sand contents, was measured by using a laser particle size analyzer (Malvern Masterizer, 2000, Malvern Panalytical Ltd., Malvern, Worcestershire, UK).

2.3. Statistical Analysis

To clarify the effect of grassland cultivation on soil C and N stocks and soil properties in topsoil and subsoil, a one-way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc tests was employed to compare the differences in soil C and N stocks as well as soil properties at soil depths of 0–10, 10–30, and 30–50 cm across different cultivation years. Soil properties included soil pH and soil clay, silt and sand contents. To examine whether grassland cultivation could break the coupling of C and N stocks in subsoil and topsoil, linear regression analyses were performed to explore the associations

of soil C and N stocks in the 0–10, 10–30, and 30–50 cm soil layers. Correlation analyses between soil C and N stocks and soil properties at each soil depth were conducted to detect which soil variable directly influenced variations in C and N stocks in subsoil and topsoil. SPSS 20.0 was used to finish the above-mentioned statistical analyses. Variation partitioning analysis was carried out in Canoco 5 to detect how cultivation year and soil properties independently and interactively affected soil C and N stocks at soil depths of 0–10, 10–30, and 30–50 cm. A significance level of 0.05 was used for all statistical analyses.

3. Results

3.1. Changes in Soil C and N Stocks After Grassland Cultivation

Soil C stocks in the 0–10 cm soil layer showed a valley-shaped pattern with increasing cultivation years, with the lowest value occurring in the cultivation duration of 20 years (1.13 kg/m^2) (Figure 2). The variation trends in soil C stocks in the 10–30 and 30–50 cm soil layers were similar, and the stock of soil C in the 10–30 and 30–50 cm soil layers in the Y0 treatment (3.49 kg/m^2 and 1.99 kg/m^2) was significantly lower than that in the Y5 treatment (4.92 kg/m^2 and 4.93 kg/m^2) (Figure 2). Additionally, the stocks of soil C at soil depths of both 10–30 and 30–50 cm first reduced and then increased with increasing cultivation years, and they also reached the lowest value in the cultivation duration of 20 years (1.93 kg/m^2 and 1.63 kg/m^2) (Figure 2). Soil N stocks at a soil depth of 0–10 cm first decreased and then increased with increasing cultivation years, and the lowest value of soil N stocks occurred in the Y20 (0.16 kg/m^2) and Y30 treatments (0.18 kg/m^2) (Figure 2). Soil N stocks in the 10–30 and 30–50 cm soil layers in the Y0 treatment (0.39 kg/m^2 and 0.19 kg/m^2) were both significantly lower than those in the Y5 treatment (0.57 kg/m^2 and 0.52 kg/m^2) (Figure 2). Soil N stocks in the 10–30 and 30–50 cm soil layers both decreased from the Y5 treatment to the Y20 treatment (0.25 kg/m^2 and 0.21 kg/m^2), and then the stocks of soil N in the 10–30 and 30–50 cm soil layers both increased from the Y20 treatment to the Y50 treatment (0.44 kg/m^2 and 0.34 kg/m^2) (Figure 2). The ratio of soil C stocks to soil N stocks in the 0–10 cm soil layer first increased and then decreased from the Y0 treatment to the Y20 treatment and then remained stable among the Y20, Y30 and Y50 treatments. The ratio of soil C stocks to soil N stocks in the 10–30 and 30–50 cm soil layers generally decreased from the Y0 treatment to the Y10 treatment and then remained stable among the Y10, Y20, Y30 and Y50 treatments (Figure S1). Soil C stocks in the Y0 treatment showed a decreasing trend with an increase in soil depth. The stocks of soil C in the Y5 treatment showed an increasing trend with an increase in soil depth. The variation trends in soil C stocks in the Y10, Y20, Y50 and Y50 treatments were similar, and the stocks of soil C in these treatments showed the trend of first increasing then decreasing across three soil depths (Figure 2). Soil N stocks in the Y0 treatment showed a decreasing trend with an increase in soil depth. The variation trends in soil N stocks in the Y5, Y10, Y20, Y30, and Y50 treatments were similar, and soil N stocks in these treatments first increased and then decreased with an increase in soil depth (Figure 2).

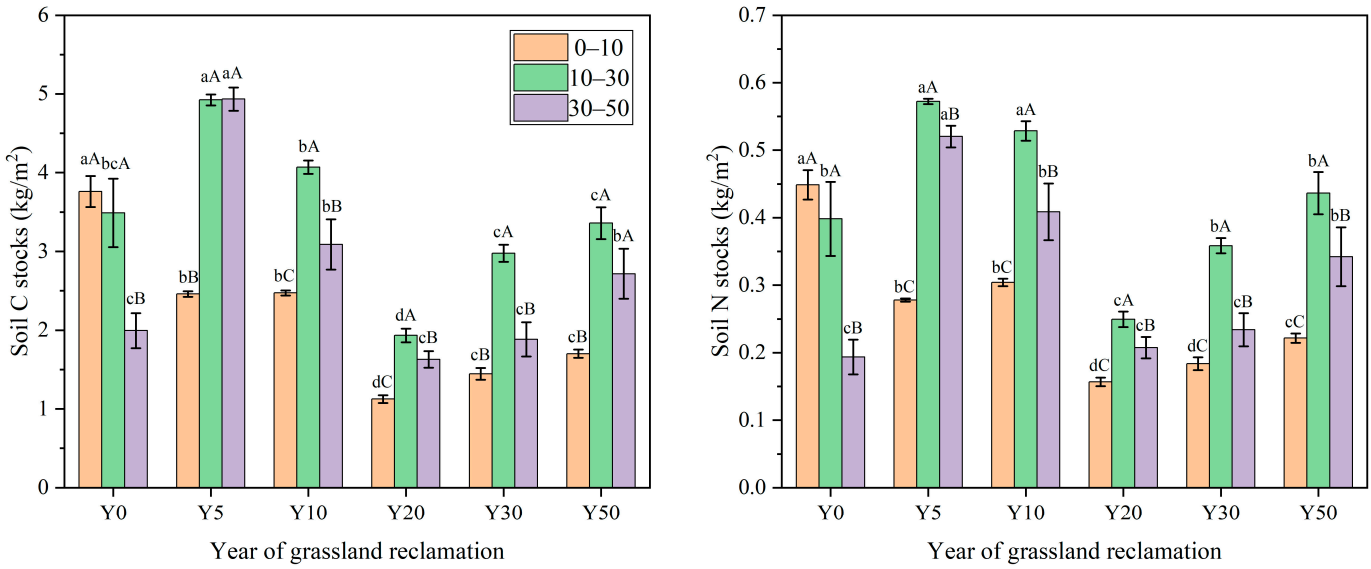


Figure 2. Changes in soil C and N stocks under different cultivation durations. Different lowercase letters indicate significant differences in soil C and N stocks within the same soil layer among different cultivation durations. Different uppercase letters indicate significant differences in soil C and N stocks within the same cultivation duration among different soil layers.

3.2. Variations in Soil Properties After Grassland Cultivation

Soil pH at soil depths of 0–10, 10–30, and 30–50 cm in the Y0 treatment was significantly higher than that in other treatments. Soil pH at soil depths of 0–10, 10–30, and 30–50 cm in the Y30 treatment reached the lowest values. Soil pH at soil depths of 0–10, 10–30 and 30–50 cm showed no significant differences among the Y5, Y10, Y20, and Y50 treatments (Figure 3). Soil clay content at a soil depth of 0–10 cm decreased from the Y0 treatment to the Y20 treatment, and those in the Y20, Y30, and Y50 treatments did not differ significantly from one another. The Y5 treatment in the 10–30 cm soil layer had significantly higher soil clay content compared to other treatments. The lowest soil clay content at a soil depth of 10–30 cm occurred in the Y30 treatment. Soil clay content at a soil depth of 10–30 cm showed no significant differences among the Y0, Y10, Y20, and Y50 treatments. For variation in soil clay content at a soil depth of 30–50 cm, the Y5 treatment held the highest value, the Y0 treatment held the second highest value, and there was no significant difference among the Y10, Y20, Y30, and Y50 treatments (Figure 3). The variation trends in soil sand content at soil depths of 0–10, 10–30, and 30–50 cm were also similar; the Y0 treatment held the lowest value, the Y5 treatment held the second lowest value, and there was no significant difference among the Y10, Y20, Y30, and Y50 treatments (Figure 3). Soil bulk density at a soil depth of 0–10 cm increased from the minimum in the Y0 treatment to the maximum in the Y30 treatment and then decreased in the Y50 treatment. Soil bulk density at a soil depth of 10–30 cm remained stable from the Y0 treatment to the Y5 treatment, declined to the minimum in the Y10 treatment, and then increased to the maximum in the Y30 treatment before decreasing in the Y50 treatment. Soil bulk density at a soil depth of 30–50 cm decreased from the Y0 treatment to the minimum in the Y10 treatment, rose to the maximum in the Y20 treatment, then decreased in the Y30 treatment and increased in the Y50 treatment (Figure S2). Soil pH in the Y0 treatment showed a decreasing trend with an increase in soil depth. Soil pH in the Y5, Y10, Y20, Y30, and Y50 treatments showed no significant change across the three soil depths (Figure 3). Soil clay content in the Y0 treatment showed the trend of first decreasing then increasing, and soil clay content in the Y20 treatment showed a decreasing trend with an increase in soil depth. Soil clay content in the Y5, Y10, Y30, and Y50 treatments showed no significant variation with an increase

in soil depth (Figure 3). The variation trends in soil silt content and soil sand content in all different cultivation years were similar, and they all showed no significant difference across the three soil depths (Figure 3).

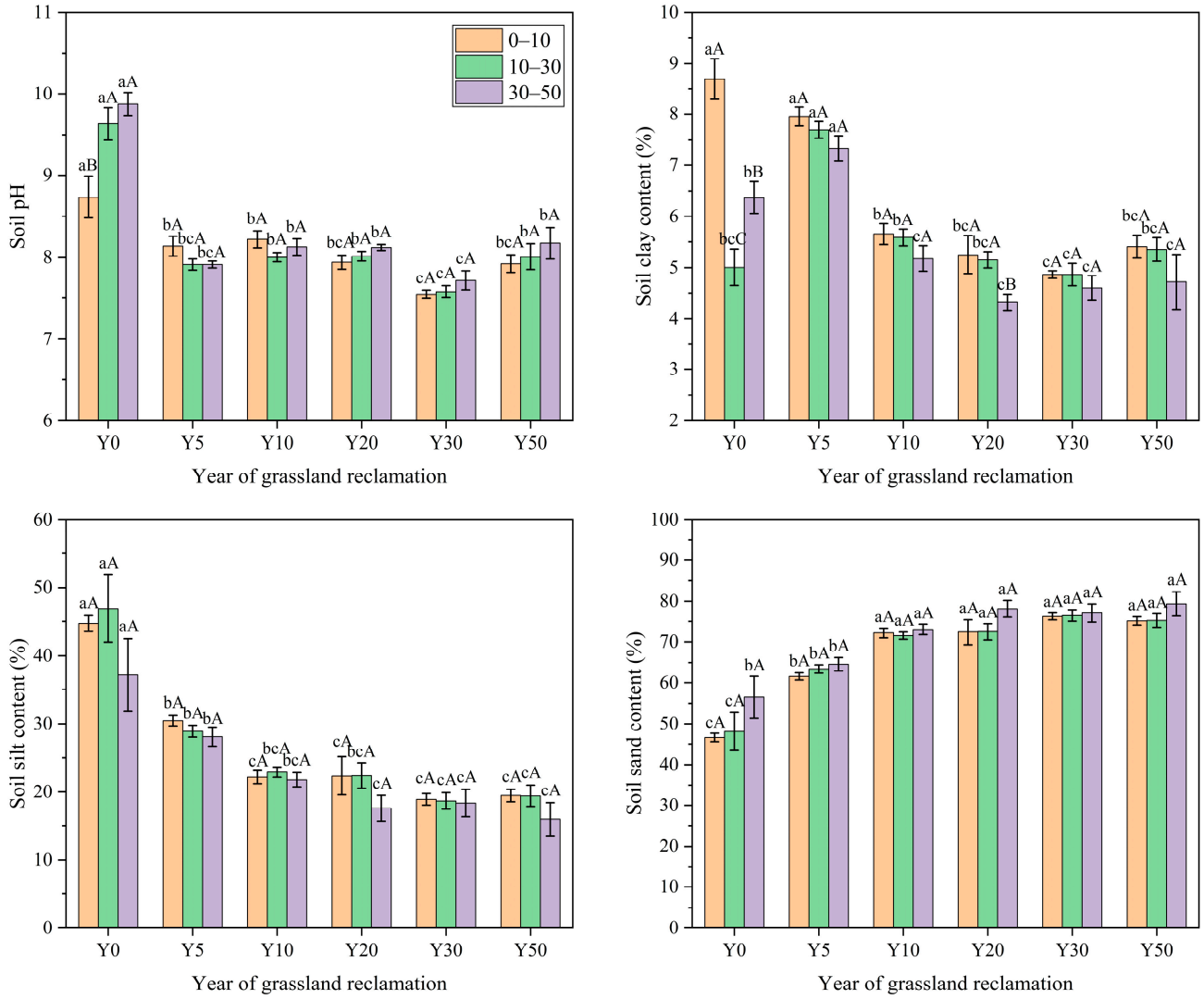


Figure 3. Variations in soil properties under different cultivation durations. Different lowercase letters indicate significant differences in soil C and N stocks within the same soil layer among different cultivation durations. Different uppercase letters indicate significant differences in soil C and N stocks within the same cultivation duration among different soil layers.

3.3. Effects of Grassland Cultivation and Soil Properties on Soil C and N Stocks

Cultivation year and soil properties together explained 72.0%, 50.4%, and 61.9% of variations in soil C and N stocks at soil depths of 0–10, 10–30, and 30–50 cm, respectively (Figure 4). However, cultivation year individually had no effect on soil C and N stocks at soil depths of 0–10, 10–30, and 30–50 cm (Figure 4). Soil properties individually accounted for 28.7%, 39.1%, and 57.5% of variations in soil C and N stocks at soil depths of 0–10, 10–30, and 30–50 cm, respectively (Figure 4). The shared contribution of cultivation year and soil properties to variations in soil C and N stocks at soil depths of 0–10, 10–30, and 30–50 cm were 43.3%, 11.3%, and 4.4%, respectively (Figure 4).

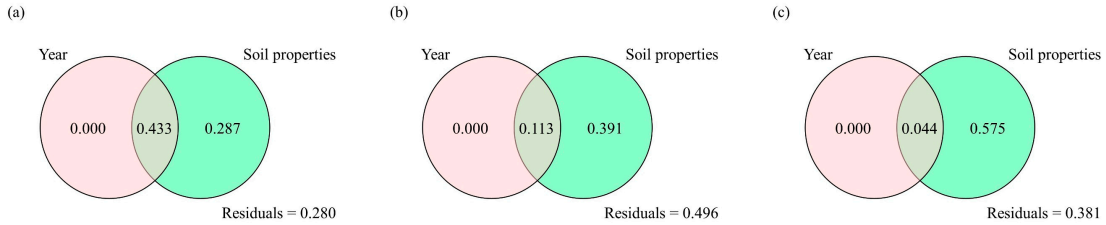


Figure 4. Variation partitioning of cultivation year and soil properties in accounting for changes in soil C and N stocks at different soil depths. Note: (**a**) 0–10 cm, (**b**) 10–30 cm, (**c**) 30–50 cm.

Soil C and N stocks at a soil depth of 0–10 cm both showed a positive correlation with soil pH, soil clay content, and soil silt content, and they had a negative association with soil sand content (Figure 5a). Soil C and N stocks in the 10–30 cm soil layer were both positively correlated with soil clay content; however, they did not correlate with soil pH, soil silt content, or soil sand content, except for the negative correlation between soil C stocks and soil sand content (Figure 5b). For the 30–50 cm soil layer, soil C and N stocks showed a positive correlation with soil clay content and had no relationships with soil pH, soil silt content, or soil sand content, except for the negative association between soil N stocks and soil pH (Figure 5c). Moreover, across the 0–10, 10–30, and 30–50 cm soil layers, soil N stocks exhibited a significant correlation with soil C stocks (Figure 6).

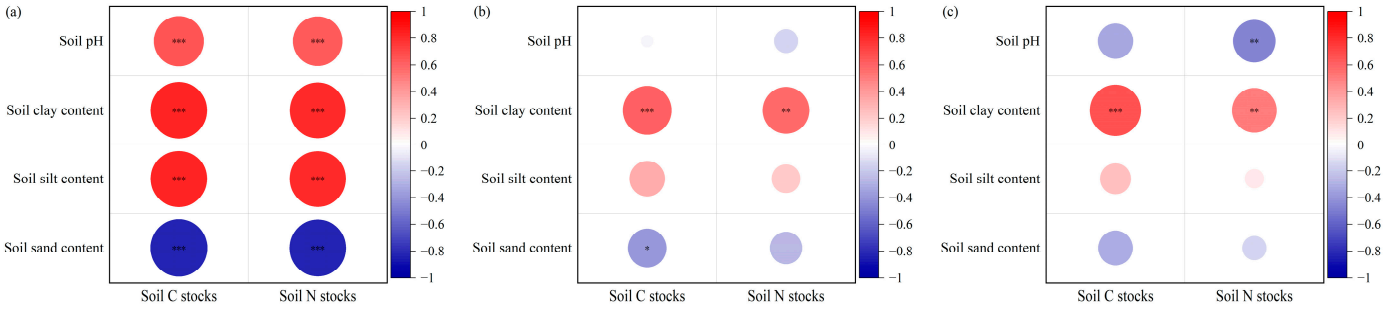


Figure 5. Relationships between soil C and N stocks and soil properties at different soil depths. Note: (**a**) 0–10 cm, (**b**) 10–30 cm, (**c**) 30–50 cm. *, ** and *** mean $p < 0.05$, $p < 0.01$ and $p < 0.001$.

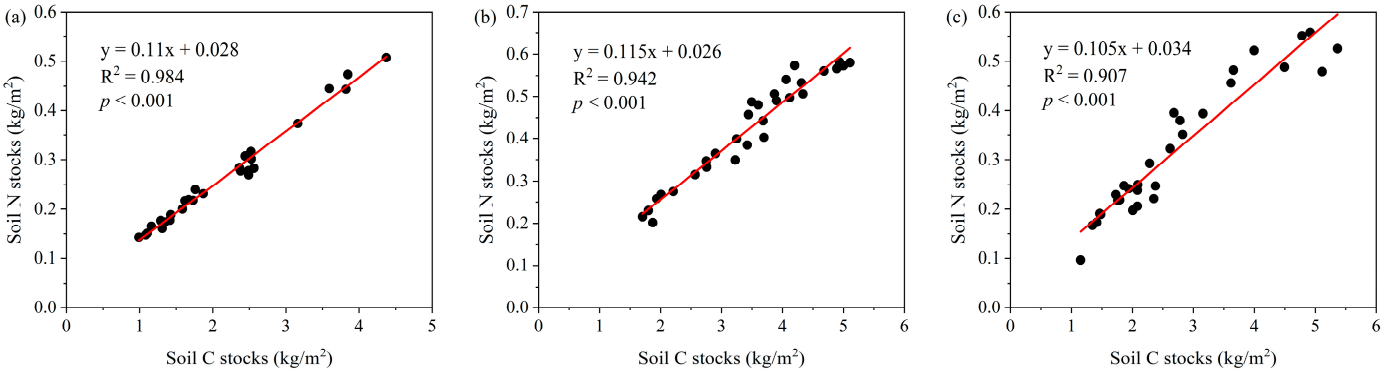


Figure 6. Correlations between soil C stocks and soil N stocks at different soil depths. Note: (**a**) 0–10 cm, (**b**) 10–30 cm, (**c**) 30–50 cm.

4. Discussion

4.1. Changes in Soil C and N Stocks Resulting from the Conversion of Grassland to Cropland

This study found that over 50% of soil C and N stocks at a soil depth of 0–10 cm was lost within 20 years of grassland cultivation (Figure 2). This is consistent with most previous conclusions that the conversion of grassland to cropland significantly reduces soil C and N

stocks [4,6,16,19–22]. Notably, Yang et al. [21] performed a meta-analysis of the response of soil C changes to grassland transformation for temperate grasslands in China, and their meta-analysis results showed that soil C loss as a result of grassland conversion was lower than 20%, which was lower than the loss of soil C stocks observed in this study. The windy weather in our research area might account for this, since the windy weather promotes soil erosion, leading to more severe soil C loss. Thus, future studies monitoring wind erosion data are needed to provide quantitative support for this hypothesis. Moreover, soil C and N stocks at a soil depth of 0–10 cm increased from 20 years of grassland cultivation to 50 years of grassland cultivation (Figure 2). This suggests that the slow recovery of soil C and N conditions starts after 20 years of grassland cultivation. This pattern aligned with some previous findings [14,22,39]. For example, by exploring changes in soil fertility along a 60-year chronosequence of grassland cultivation in Northern China, Tang et al. [39] found that soil C and N variations showed a slow trend of recovery with cultivation period after 25 years of the cultivation of native grassland.

Variations in soil C and N stocks at soil depths of 10–30 and 30–50 cm differed from those in the 0–10 cm soil layer. Within the first 10 years of grassland cultivation, soil C and N stocks at soil depths of 10–30 and 30–50 cm were generally higher than those in native grassland (Figure 2). This was partly similar to the finding reported by Wang et al. [4]. By analyzing the change in soil C density in 2140 topsoil samples (0–20 cm) across China's black soil region over the past 35 years, Wang et al. [4] observed that soil C density was enriched within 10 years of grassland cultivation. Soil C and N stocks at soil depths of 10–30 and 30–50 cm reached their minimum values after 20 years of grassland cultivation. Then they were both enhanced with cultivation duration (Figure 2). These aligned with variations in soil C and N stocks in the 0–10 cm soil layer, indicating the slow recovery of C and N stocks in the whole soil profile (0–50 cm) after 20 years of grassland cultivation. The input accumulation consisting of synthetic nutrient inputs and crop residue return might account for the recovery of C and N stocks, which needed further studies. Moreover, the changing trends in soil bulk density at the three soil depths along the cultivation chronosequence generally differed from those in soil C and N stocks, indicating that changes in soil C and N stocks at the three soil depths after grassland cultivation could be more influenced by soil C and N contents than by soil bulk density. Soil C and N stocks within different cultivation years significantly varied across the three soil depths, which was consistent with previous findings that soil depth mediated variations in soil C and N stocks after grassland cultivation [19,20,23].

4.2. Variations in Soil C and N Stocks as Affected by Grassland Cultivation and Soil Properties

Cultivation year exerted no direct influence on soil C and N stocks in the 0–10 cm soil layer, whereas soil properties directly affected variations in soil C and N stocks at a soil depth of 0–10 cm (Figure 4a). The shared influences of cultivation year and soil properties on changes in soil C and N stocks at a soil depth of 0–10 cm were 43.3% (Figure 4a). These findings indicates that soil properties play a significant role in driving changes in soil C and N stocks at a soil depth of 0–10 cm, and grassland cultivation may have an indirect influence on soil C and N stocks through the effect of soil properties. Soil pH was positively correlated with variations in soil C and N stocks (Figure 5a). This differed from previous findings that soil pH was negatively related to soil C stocks and soil pH exerted no correlation with soil N stocks [19,20]. This inconsistency could be associated with the initial soil pH at our study site. Soil pH in the native grassland in this study was 8.7, which was higher than that in other study sites [6,39,40]. Soil alkalinity tended to be relieved after grassland cultivation. The reduced soil pH could enhance microbial activity and further promote the turnover of soil C and N, thereby reducing soil C and N stocks [41]. In addition

to pH, soil texture was also significantly correlated with soil C and N stocks (Figure 5). Soil clay and silt contents were positively related to variations in soil C and N stocks at a soil depth of 0–10 cm (Figure 5a). This aligned strongly with the positive correlation between soil clay and silt contents and soil C and N stocks reported in previous studies [19,20,24]. Clay and silt particles can stabilize organic C and N through sorption, aggregation, and the formation of mineral–organic complexes, thus limiting their accessibility to microbial decomposers [42,43]. Soil clay and silt contents in the 0–10 cm soil layer generally decreased with cultivation year (Figure 3). This might be because grassland cultivation broke the protection of vegetation cover on soil fine particles, leading to the losses of soil clay and silt during wind erosion. Thus, the decline in clay and silt contents in the topsoil after grassland cultivation appeared to weaken the protective capacity of soil nutrients, contributing to the C and N losses in topsoil (Figure 2). By contrast, the significant negative correlation between soil C and N stocks and soil sand content existed in the topsoil (Figure 5a), in agreement with previous findings [19,20]. This indicates that coarse-textured soils have a weak retention and stabilization capacity of nutrients. Compared with clay and silt, sand particles have a larger size and lower specific surface area, lacking the ability to effectively adsorb nutrients [42,44].

For the 10–30 cm soil layer, soil properties exerted a significant influence on soil C and N stocks, whereas cultivation year showed no effect on soil C and N stocks (Figure 4b). This also indicates that soil properties have a strong effect on soil C and N stocks and that grassland cultivation indirectly affects soil C and N stocks through the influence of soil properties. In contrast, soil pH, soil silt content, and soil sand content showed no significant correlation with soil C and N stocks (Figure 5b). This may be attributable to the inconsistent variation tendency between soil pH, soil silt and sand contents and soil C and N stocks with cultivation year. Only soil clay content exhibited a significant correlation with soil C and N stocks, as soil clay content and soil C and N stocks changed synchronously with cultivation year (Figure 5b). Soil clay content at a soil depth of 10–30 cm first increased and then decreased with cultivation year (Figure 3). The reason for the increase in soil clay content within 5 years of grassland cultivation could be that in the early stage of grassland cultivation, deep tillage could translocate a portion of soil clay from the 0–10 cm soil layer to the 10–30 cm soil layer. For the 30–50 cm soil layer, soil pH and soil silt and sand contents still showed no significant relationship with soil C and N stocks, and soil clay content uniquely accounted for variations in soil C and N stocks (Figure 5c). These results taken together suggest that the factors influencing soil C and N stock changes shift across soil depths. Moreover, the individual contribution of soil properties to variations in soil C and N stocks increased, and the shared contribution of cultivation year and soil properties to variations in soil C and N stocks decreased with soil depth (Figure 4), indicating that soil properties played a stronger role in driving variations in soil C and N stocks when soil depth increased. Thus, this confirmed our first hypothesis that soil properties could exert a strong effect on subsoil C and N stocks after grassland cultivation. Considering the positive relationships of soil C and N stocks and soil clay content in the 0–10, 10–30, and 30–50 cm soil layers, this study suggests that the alterations in soil C and N stocks across soil depths after grassland cultivation can be increasingly governed by soil properties, particularly soil clay content. Mao et al. [45] quantified the relative importance of different influencing factors regulating variation in soil C stocks in the ecologically vulnerable region (Sanjiang Plain) of China, and they reported that soil clay content was the most dominant controlling factor and explained the largest proportion (21.2%) of the variance in soil C stocks in topsoil. Our research, conducted in another ecologically vulnerable region (agro-pastoral ecotone in northern China), yielded similar findings that soil clay content was the major driver of variations in C and N stocks in topsoil and subsoil. Hence, we emphasized that assessments

of grassland-to-cropland conversion impacts on soil nutrients should consider the spatial heterogeneity in soil texture, especially soil clay content, to accurately predict and manage soil C and N dynamics across different soil depths.

4.3. The Coupling of Soil C and N Stocks After the Conversion of Grassland to Cropland

Organisms maintain a strict stoichiometric relationship for C and N variations under a changing environment [46], and this makes soil C and N dynamics remain tightly coupled under global change [47]. A recent broad-scale soil investigation in an agro-pastoral ecotone of northern China, which examined how grassland-to-cropland conversion affected soil C and N variations, reported that grassland cultivation did not disrupt C–N coupling in either topsoil or subsoil [30]. In this study, soil samples were collected along a 50-year cultivation chronosequence to detect the effect of grassland cultivation on soil C and N coupling from the view of the temporal gradient. Our results showed that soil C and N stocks exhibited synchronous variations across the 50-year chronosequence of grassland cultivation (Figure 2). This indicates that long-term grassland cultivation cannot disrupt the coupled linkage between soil C and N stocks. We further analyzed the relationships between soil C stocks and soil N stocks across three soil layers. We found that soil C stocks exhibited a significant correlation with soil N stocks in the 0–10, 10–30 and 30–50 cm soil layers (Figure 6). Meanwhile, the ratio of soil C stocks to soil N stocks at three soil depths generally remained stable after 10 years of grassland cultivation (Figure S2). Collectively, these results suggest that the long duration of grassland cultivation cannot decouple soil C and N stocks, thus supporting our second hypothesis. The sustained coupled relationship could be explained by the resistance of biological processes regulating soil C and N mineralization and stabilization to grassland cultivation [48,49]. The development of terrestrial C-N coupled models has become a research focus in recent years, driven by the recognition that the interplay of terrestrial C and N cycles is critical for predicting the long duration dynamics of C and N pools within plant–litter–soil systems and how these systems respond to global change [50–52]. Our results therefore highlight the necessity for future models of biogeochemistry and coupled terrestrial C-N dynamics, particularly those aimed at simulating land-use change influences in arid and semiarid ecosystems, to consider the tight C-N coupling under long-term grassland cultivation.

This study has several limitations that warrant further investigation. This study did not detect the responses of soil microbial properties to grassland cultivation or how these responses affected the alterations in soil C and N stocks. Soil microorganisms are key drivers of soil C and N cycling, mediating crucial processes including organic matter decomposition and biological nitrogen fixation [53,54]. Numerous studies have demonstrated that grassland cultivation substantially modifies soil microbial community structure, microbial biomass, and soil extracellular enzyme activities [55–57]. These changes in soil microbial properties can significantly affect variations in soil C and N stocks. For instance, Tang et al. [19] synthesized data from 81 studies worldwide to reveal the underlying mechanisms of soil C loss following the conversion of grassland to cropland, and they found that soil C and microbial biomass C decreased by 31.96% and 41.2% after grassland cultivation, and a positive correlation existed between microbial biomass C change and soil C variation. Similarly, Ding et al. [58] collected soil samples at a farm in Keshan, Heilongjiang Province, northeast China, and detected the influence of grassland conversion on soil C stocks and soil microbial residual C stocks. They found that grassland conversation led to significant soil C loss, and the reduction in soil microbial residual C was directly correlated with soil C loss. Notably, our results showed that soil C and N stocks started to recover slowly after 20 years of grassland cultivation, suggesting that soil microorganisms might play an increasingly vital role in regulating C and N dynamics during this recovery phase. Long-term

observations of changes in soil microbial community structure under grassland cultivation showed that bacterial and fungal community compositions in cropland gradually became more similar to those in native grassland [39]. Meanwhile, the biomass in fungi and bacteria could return to equal or even exceed the levels found in undisturbed grasslands after a long duration of grassland cultivation [36]. The restoration of microbial biomass and soil microbial community structure might contribute to the generation of microbial necromass and the formation of stable mineral-associated organic matter [59,60], thereby facilitating the recovery of soil C and N stocks after long-term grassland cultivation. Hence, future studies should further investigate changes in soil microbial properties along long-duration cultivation chronosequences and explore the underlying biological mechanisms responsible for the long-term variations in soil C and N stocks after grassland conversion.

5. Conclusions

This study elucidated changes in C and N stocks in topsoil and subsoil across a 50-year cultivation chronosequence in an agro-pastoral ecotone of northern China. Our findings demonstrated that soil C and N stocks at a soil depth of 0–10 cm first decreased and then increased compared to the initial decrease along the cultivation chronosequence, with the lowest value occurring at 20 years of grassland cultivation. In contrast, for the 10–30 cm and 30–50 cm soil layers, soil C and N stocks within 10 years of grassland cultivation were generally higher than those in native grassland. After that, soil C and N stocks reached the minimum value after 20 years of grassland cultivation and then increased with cultivation duration. Across all three soil layers, cultivation year had no direct effect on soil C and N stocks and indirectly affected soil C and N stocks through the influence of soil properties. The influence of soil properties on soil C and N stocks increased with soil depth. In the 0–10 cm soil layer, soil C and N stocks showed positive correlations with soil pH, soil clay content, and soil silt content while exhibiting a negative correlation with soil sand content. However, soil C and N stocks at soil depths of 10–30 and 30–50 cm were both positively correlated with soil clay content. These results suggest that soil clay content, rather than cultivation duration, governs the long-term shifts in soil C and N stocks in the context of grassland cultivation. Furthermore, the significant relationships of soil C and N stocks across the three soil layers still existed in the context of grassland cultivation, indicating that long-term grassland cultivation could be incapable of decoupling soil C and N stocks.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture16090923/s1, Figure S1. Variation in soil C stocks to soil N stocks under different cultivation durations. Figure S2. Variation in soil bulk density under different cultivation durations.

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Abbreviations

The following abbreviations are used in this manuscript:

C	Carbon
N	Nitrogen
CO_2	Carbon dioxide
LSD	Least significant difference

References

1. Bardgett, R.D.; Bullock, J.M.; Lavorel, S.; Manning, P.; Schaffner, U.; Ostle, N.; Chomel, M.; Durigan, G.; Fry, E.L.; Johnson, D.; et al. Combatting Global Grassland Degradation. *Nat. Rev. Earth Environ.* **2021**, *2*, 720–735. [CrossRef]
2. Bai, Y.; Cotrufo, M.F. Grassland Soil Carbon Sequestration: Current Understanding, Challenges, and Solutions. *Science* **2022**, *377*, 603–608. [CrossRef] [PubMed]
3. Helfrich, M.; Nicolay, G.; Well, R.; Buchen-Tschiskale, C.; Dechow, R.; Fuß, R.; Gensior, A.; Paulsen, H.M.; Berendonk, C.; Flessa, H. Effect of Chemical and Mechanical Grassland Conversion to Cropland on Soil Mineral N Dynamics and N_2O Emission. *Agric. Ecosyst. Environ.* **2020**, *298*, 106975. [CrossRef]
4. Wang, X.; Li, S.; Wang, L.; Zheng, M.; Wang, Z.; Song, K. Effects of Cropland Reclamation on Soil Organic Carbon in China's Black Soil Region over the Past 35 Years. *Glob. Change Biol.* **2023**, *29*, 5460–5477. [CrossRef]
5. Guo, J.; Li, J.; Liu, K.; Tang, S.; Zhai, X.; Wang, K. Analysis of Soil Microbial Dynamics at a Cropland-Grassland Interface in an Agro-Pastoral Zone in a Temperate Steppe in Northern China. *CATENA* **2018**, *170*, 257–265. [CrossRef]
6. Wu, T.-H.; Hu, Y.-F.; Zhang, Y.-Y.; Shu, X.-Y.; Yang, Z.-P.; Zhou, W.; Huang, C.-Y.; Li, J.; Li, Z.; He, J.; et al. Changes in Soil Organic Carbon and Its Fractions under Grassland Reclamation in Alpine-Cold Soils, China. *Soil Water Res.* **2022**, *17*, 211–221. [CrossRef]
7. Kong, L.; Wu, T.; Xiao, Y.; Xu, W.; Zhang, X.; Daily, G.C.; Ouyang, Z. Natural Capital Investments in China Undermined by Reclamation for Cropland. *Nat. Ecol. Evol.* **2023**, *7*, 1771–1777. [CrossRef]
8. Kassa, H.; Dondeyne, S.; Poesen, J.; Frankl, A.; Nyssen, J. Impact of Deforestation on Soil Fertility, Soil Carbon and Nitrogen Stocks: The Case of the Gacheb Catchment in the White Nile Basin, Ethiopia. *Agric. Ecosyst. Environ.* **2017**, *247*, 273–282. [CrossRef]
9. Li, J.; Wang, K.; Shangguan, Z.; Deng, L. Coupling and Decoupling of Soil Carbon, Nitrogen and Phosphorus Stocks Following Grazing Exclusion in Temperate Grasslands. *CATENA* **2022**, *211*, 106003. [CrossRef]
10. Wang, Q.; Zhao, X.; Chen, L.; Yang, Q.; Chen, S.; Zhang, W. Global Synthesis of Temperature Sensitivity of Soil Organic Carbon Decomposition: Latitudinal Patterns and Mechanisms. *Funct. Ecol.* **2019**, *33*, 514–523. [CrossRef]
11. Tan, Q.; Han, W.; Li, X.; Wang, G. Clarifying the Response of Soil Organic Carbon Storage to Increasing Temperature through Minimizing the Precipitation Effect. *Geoderma* **2020**, *374*, 114398. [CrossRef]
12. Anas, M.; Liao, F.; Verma, K.K.; Sarwar, M.A.; Mahmood, A.; Chen, Z.-L.; Li, Q.; Zeng, X.-P.; Liu, Y.; Li, Y.-R. Fate of Nitrogen in Agriculture and Environment: Agronomic, Eco-Physiological and Molecular Approaches to Improve Nitrogen Use Efficiency. *Biol. Res.* **2020**, *53*, 47. [CrossRef]
13. Zhang, J.H.; Wang, Y.; Li, F.C. Soil Organic Carbon and Nitrogen Losses Due to Soil Erosion and Cropping in a Sloping Terrace Landscape. *Soil Res.* **2015**, *53*, 87–96. [CrossRef]
14. Wang, C.; Li, L.; Yan, Y.; Cai, Y.; Xu, D.; Wang, X.; Chen, J.; Xin, X. Effects of Cultivation and Agricultural Abandonment on Soil Carbon, Nitrogen and Phosphorus in a Meadow Steppe in Eastern Inner Mongolia. *Agric. Ecosyst. Environ.* **2021**, *309*, 107284. [CrossRef]
15. Guillaume, T.; Bragazza, L.; Levasseur, C.; Libohova, Z.; Sinaj, S. Long-Term Soil Organic Carbon Dynamics in Temperate Cropland-Grassland Systems. *Agric. Ecosyst. Environ.* **2021**, *305*, 107184. [CrossRef]
16. Kim, D.-G.; Kirschbaum, M.U.F.; Eichler-Löbermann, B.; Gifford, R.M.; Liáng, L.L. The Effect of Land-Use Change on Soil C, N, P, and Their Stoichiometries: A Global Synthesis. *Agric. Ecosyst. Environ.* **2023**, *348*, 108402. [CrossRef]
17. Qiao, N.; Xu, X.; Cao, G.; Ouyang, H.; Kuzyakov, Y. Land Use Change Decreases Soil Carbon Stocks in Tibetan Grasslands. *Plant Soil* **2015**, *395*, 231–241. [CrossRef]
18. Song, X.-D.; Yang, F.; Ju, B.; Li, D.-C.; Zhao, Y.-G.; Yang, J.-L.; Zhang, G.-L. The Influence of the Conversion of Grassland to Cropland on Changes in Soil Organic Carbon and Total Nitrogen Stocks in the Songnen Plain of Northeast China. *CATENA* **2018**, *171*, 588–601. [CrossRef]
19. Tang, S.; Guo, J.; Li, S.; Li, J.; Xie, S.; Zhai, X.; Wang, C.; Zhang, Y.; Wang, K. Synthesis of Soil Carbon Losses in Response to Conversion of Grassland to Agriculture Land. *Soil Tillage Res.* **2019**, *185*, 29–35. [CrossRef]
20. Li, S.; Xu, J.; Tang, S.; Zhan, Q.; Gao, Q.; Ren, L.; Shao, Q.; Chen, L.; Du, J.; Hao, B. A *Meta*-Analysis of Carbon, Nitrogen and Phosphorus Change in Response to Conversion of Grassland to Agricultural Land. *Geoderma* **2020**, *363*, 114149. [CrossRef]

21. Yang, W.-S.; Liu, Y.; Zhao, J.; Chang, X.; Wiesmeier, M.; Sun, J.; López-Vicente, M.; García-Ruiz, R.; Gómez, J.A.; Zhou, H.; et al. SOC Changes Were More Sensitive in Alpine Grasslands than in Temperate Grasslands during Grassland Transformation in China: A Meta-Analysis. *J. Clean. Prod.* **2021**, *308*, 127430. [CrossRef]
22. Wang, Y.; Luo, G.; Li, C.; Ye, H.; Shi, H.; Fan, B.; Zhang, W.; Zhang, C.; Xie, M.; Zhang, Y. Effects of Land Clearing for Agriculture on Soil Organic Carbon Stocks in Drylands: A Meta-Analysis. *Glob. Change Biol.* **2023**, *29*, 547–562. [CrossRef]
23. Zhu, N.; Yan, Y.; Bai, K.; Zhang, J.; Wang, C.; Wang, X.; Xu, D.; Liu, J.; Xin, X.; Chen, J. Conversion of Croplands to Shrublands Does Not Improve Soil Organic Carbon and Nitrogen but Reduces Soil Phosphorus in a Temperate Grassland of Northern China. *Geoderma* **2023**, *432*, 116407. [CrossRef]
24. De Rosa, D.; Ballabio, C.; Lugato, E.; Fasiolo, M.; Jones, A.; Panagos, P. Soil Organic Carbon Stocks in European Croplands and Grasslands: How Much Have We Lost in the Past Decade? *Glob. Change Biol.* **2024**, *30*, e16992. [CrossRef] [PubMed]
25. Luo, Z.; Feng, W.; Luo, Y.; Baldock, J.; Wang, E. Soil Organic Carbon Dynamics Jointly Controlled by Climate, Carbon Inputs, Soil Properties and Soil Carbon Fractions. *Glob. Change Biol.* **2017**, *23*, 4430–4439. [CrossRef] [PubMed]
26. Tan, Q.; Wang, G.; Smith, M.D.; Chen, Y.; Yu, Q. Temperature Patterns of Soil Carbon: Nitrogen: Phosphorus Stoichiometry along the 400 Mm Isohyet in China. *CATENA* **2021**, *203*, 105338. [CrossRef]
27. Bai, Z.; Zheng, L.; Bai, Z.; Jia, A.; Wang, M. Long-Term Cultivation Alter Soil Bacterial Community in a Forest-Grassland Transition Zone. *Front. Microbiol.* **2022**, *13*, 1001781. [CrossRef]
28. Kemmitt, S.J.; Wright, D.; Goulding, K.W.T.; Jones, D.L. pH Regulation of Carbon and Nitrogen Dynamics in Two Agricultural Soils. *Soil Biol. Biochem.* **2006**, *38*, 898–911. [CrossRef]
29. Tan, Q.; Wang, G.; Liu, X.; Hao, T.; Tan, W. Responses of Soil Organic Carbon Turnover to Nitrogen Deposition Are Associated with Nitrogen Input Rates: Derived from Soil ^{14}C Evidences. *Environ. Pollut.* **2018**, *238*, 500–507. [CrossRef]
30. Tan, Q.; Liu, L.; Dou, P.; Wang, K. Grassland Conversion To Cropland Leads To Greater Influences of Climate Change on Variations of Carbon and Nitrogen in Subsoil than Topsoil. *J. Soil Sci. Plant Nutr.* **2026**, *26*, 783–796. [CrossRef]
31. Finzi, A.C.; Austin, A.T.; Cleland, E.E.; Frey, S.D.; Houlton, B.Z.; Wallenstein, M.D. Responses and Feedbacks of Coupled Biogeochemical Cycles to Climate Change: Examples from Terrestrial Ecosystems. *Front. Ecol. Environ.* **2011**, *9*, 61–67. [CrossRef]
32. Delgado-Baquerizo, M.; Maestre, F.T.; Gallardo, A.; Bowker, M.A.; Wallenstein, M.D.; Quero, J.L.; Ochoa, V.; Gozalo, B.; García-Gómez, M.; Soliveres, S.; et al. Decoupling of Soil Nutrient Cycles as a Function of Aridity in Global Drylands. *Nature* **2013**, *502*, 672–676. [CrossRef] [PubMed]
33. Yu, Z.; Wang, M.; Huang, Z.; Lin, T.-C.; Vadeboncoeur, M.A.; Searle, E.B.; Chen, H.Y.H. Temporal Changes in Soil C-N-P Stoichiometry over the Past 60 Years across Subtropical China. *Glob. Change Biol.* **2018**, *24*, 1308–1320. [CrossRef] [PubMed]
34. French, K.E.; Tkacz, A.; Turnbull, L.A. Conversion of Grassland to Arable Decreases Microbial Diversity and Alters Community Composition. *Appl. Soil Ecol.* **2017**, *110*, 43–52. [CrossRef]
35. Graham, C.; Ramos-Pezzotti, M.; Lehman, M. Short-Term Impacts to the Soil Microbial Population during Grassland Conversion to Cropland. *Soil Tillage Res.* **2021**, *206*, 104839. [CrossRef]
36. Ren, C.; Liu, K.; Dou, P.; Li, J.; Wang, K. The Changes in Soil Microorganisms and Soil Chemical Properties Affect the Heterogeneity and Stability of Soil Aggregates before and after Grassland Conversion. *Agriculture* **2022**, *12*, 307. [CrossRef]
37. Lu, R.K. *Chemical Analysis Method of Agricultural Soil*; Agricultural Technology Press: Beijing, China, 2000.
38. Nelson, D.W.; Sommers, L.E. Total Carbon, Organic Carbon, and Organic Matter. In *Methods of Soil Analysis*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 1982; pp. 539–579.
39. Tang, J.; Yin, J.; Davy, A.J.; Pan, F.; Miao, R.; Han, X. Changes in Soil Fertility and Microbial Communities Following Cultivation of Native Grassland in Horqin Sandy Land, China: A 60-Year Chronosequence. *Ecol. Process.* **2023**, *12*, 18. [CrossRef]
40. Jiao, Y.; Xu, Z.; Zhao, J.; Yang, W. Changes in Soil Carbon Stocks and Related Soil Proper-Ties along a 50-Year Grassland-to-Cropland Conversion Chronosequence in an Agro-Pastoral Ecotone of Inner Mongolia, China: Changes in Soil Carbon Stocks and Related Soil Proper-Ties along a 50-Year Grassland-to-Cropland Conversion Chronosequence in an Agro-Pastoral Ecotone of Inner Mongolia, China. *J. Arid Land* **2012**, *4*, 420–430. [CrossRef]
41. Bai, T.; He, S.; Li, Y.; Deng, J.; Sang, F.; Zhao, Y.; Wang, H.; He, T.; Zhang, K.; Qiu, Y.; et al. Unexpected Suppressive Fungal Diversity and Stimulative Soil Carbon Loss under Soil Acidification in an Alkaline Grassland. *Funct. Ecol.* **2025**, *39*, 114–127. [CrossRef]
42. Chenu, C.; Plante, A.F. Clay-Sized Organo-Mineral Complexes in a Cultivation Chronosequence: Revisiting the Concept of the 'Primary Organo-Mineral Complex'. *Eur. J. Soil Sci.* **2006**, *57*, 596–607. [CrossRef]
43. Kögel-Knabner, I.; Guggenberger, G.; Kleber, M.; Kandeler, E.; Kalbitz, K.; Scheu, S.; Eusterhues, K.; Leinweber, P. Organo-Mineral Associations in Temperate Soils: Integrating Biology, Mineralogy, and Organic Matter Chemistry. *J. Plant Nutr. Soil Sci.* **2008**, *171*, 61–82. [CrossRef]
44. Hassink, J. The Capacity of Soils to Preserve Organic C and N by Their Association with Clay and Silt Particles. *Plant Soil* **1997**, *191*, 77–87. [CrossRef]

45. Mao, D.H.; Wang, Z.M.; Li, L.; Miao, Z.H.; Ma, W.H.; Song, C.C.; Ren, C.Y.; Jia, M.M. Soil Organic Carbon in the Sanjiang Plain of China: Storage, Distribution and Controlling Factors. *Biogeosciences* **2015**, *12*, 1635–1645. [CrossRef]
46. Yu, Z.; Zhang, C.; Liu, X.; Lei, J.; Zhang, Q.; Yuan, Z.; Peng, C.; Koerner, S.E.; Xu, J.; Guo, L. Responses of C:N:P Stoichiometric Correlations among Plants, Soils and Microorganisms to Warming: A Meta-Analysis. *Sci. Total Environ.* **2024**, *912*, 168827. [CrossRef]
47. Zheng, S.; Xia, Y.; Hu, Y.; Chen, X.; Rui, Y.; Gunina, A.; He, X.; Ge, T.; Wu, J.; Su, Y.; et al. Stoichiometry of Carbon, Nitrogen, and Phosphorus in Soil: Effects of Agricultural Land Use and Climate at a Continental Scale. *Soil Tillage Res.* **2021**, *209*, 104903. [CrossRef]
48. Mooshammer, M.; Wanek, W.; Zechmeister-Boltenstern, S.; Richter, A.A. Stoichiometric Imbalances between Terrestrial Decomposer Communities and Their Resources: Mechanisms and Implications of Microbial Adaptations to Their Resources. *Front. Microbiol.* **2014**, *5*, 22. [CrossRef] [PubMed]
49. Wagg, C.; Schlaeppi, K.; Banerjee, S.; Kuramae, E.E.; van der Heijden, M.G.A. Fungal-Bacterial Diversity and Microbiome Complexity Predict Ecosystem Functioning. *Nat. Commun.* **2019**, *10*, 4841. [CrossRef] [PubMed]
50. Thornton, P.E.; Lamarque, J.-F.; Rosenbloom, N.A.; Mahowald, N.M. Influence of Carbon-Nitrogen Cycle Coupling on Land Model Response to CO_2 Fertilization and Climate Variability. *Glob. Biogeochem. Cycles* **2007**, *21*, GB4018. [CrossRef]
51. Zaehle, S.; Medlyn, B.E.; De Kauwe, M.G.; Walker, A.P.; Dietze, M.C.; Hickler, T.; Luo, Y.; Wang, Y.-P.; El-Masri, B.; Thornton, P.; et al. Evaluation of 11 Terrestrial Carbon–Nitrogen Cycle Models against Observations from Two Temperate Free—Air CO_2 Enrichment Studies. *New Phytol.* **2014**, *202*, 803–822. [CrossRef]
52. Du, Z.; Weng, E.; Jiang, L.; Luo, Y.; Xia, J.; Zhou, X. Carbon–Nitrogen Coupling under Three Schemes of Model Representation: A Traceability Analysis. *Geosci. Model Dev.* **2018**, *11*, 4399–4416. [CrossRef]
53. Bardgett, R.D.; van der Putten, W.H. Belowground Biodiversity and Ecosystem Functioning. *Nature* **2014**, *515*, 505–511. [CrossRef]
54. Hu, X.; Liu, J.; Liang, A.; Gu, H.; Liu, Z.; Jin, J.; Wang, G. Soil Metagenomics Reveals Reduced Tillage Improves Soil Functional Profiles of Carbon, Nitrogen, and Phosphorus Cycling in Bulk and Rhizosphere Soils. *Agric. Ecosyst. Environ.* **2025**, *379*, 109371. [CrossRef]
55. Mencel, J.; Mocek-Płóciniak, A.; Kryszak, A. Soil Microbial Community and Enzymatic Activity of Grasslands under Different Use Practices: A Review. *Agronomy* **2022**, *12*, 1136. [CrossRef]
56. Li, Y.; Sang, J.; Zou, C.; Zhang, Q.; Yang, Q.; Xu, G.; Kim, D.-G.; Denton, M.D.; Rosa Carmona, C.; Zhao, H.; et al. Impacts of Conversion of Cropland to Grassland on the C-N-P Stoichiometric Dynamics of Soil, Microorganisms, and Enzymes across China: A Synthesis. *CATENA* **2024**, *246*, 108456. [CrossRef]
57. Zhang, T.; Li, S.; Xing, T.; Liu, J.; Sun, Z.; Yao, Z.; Tang, S.; Jin, K. The Coupling of Bacterial and Fungal Diversity under Land-Use Conversion in Global Grassland Regions Is Limited by Perturbation Intensity. *Soil Tillage Res.* **2025**, *254*, 106751. [CrossRef]
58. Ding, X.; Zhang, B.; Wei, Z.; He, H.; Filley, T.R. Conversion of Grassland into Cropland Affects Microbial Residue Carbon Retention in Both Surface and Subsurface Soils of a Temperate Agroecosystem. *Biol. Fertil. Soils* **2020**, *56*, 137–143. [CrossRef]
59. Kallenbach, C.M.; Frey, S.D.; Grandy, A.S. Direct Evidence for Microbial-Derived Soil Organic Matter Formation and Its Ecophysiological Controls. *Nat. Commun.* **2016**, *7*, 13630. [CrossRef]
60. Luo, J.; Liao, G.; Banerjee, S.; Gu, S.; Liang, J.; Guo, X.; Zhao, H.; Liang, Y.; Li, T. Long-Term Organic Fertilization Promotes the Resilience of Soil Multifunctionality Driven by Bacterial Communities. *Soil Biol. Biochem.* **2023**, *177*, 108922. [CrossRef]

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