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Responses of Deep Soil Carbon and Nitrogen Contents to Long-Term Retention of Alfalfa Pasture on Infertile Loess: A Synthesis Study

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Abstract: Incorporating perennial pastures into annual crop systems is an efficient means of improving soil carbon (C) sequestration and reducing the application of nitrogen (N) fertilizer on farmlands. How the soil C and N at different soil depths respond to the length of pasture duration and rainfall conditions is still being determined. In this study, we conducted a meta-analysis of data from 63 published studies to investigate the impacts of the alfalfa pasture on the incorporation of soil organic carbon (SOC), total nitrogen (STN), and available nitrogen (SAN) contents in the 0–300 cm soil profile of the Loess Plateau. An annual crop field was taken as a reference. The results showed that the average SOC content at soil depths of 0–100 and 100–200 cm in the alfalfa pasture increased by 17% and 8% ($p < 0.001$) compared to the crop field, respectively, while that at 200–300 cm decreased ($p > 0.05$). The SOC content increased with pasture age; it was the highest when the alfalfa had been planted for 5–9 years and decreased thereafter. The STN content at soil depths of 0–100 and 100–200 cm increased by 19% and 14% ($p < 0.001$), respectively; the content at depths of 200–300 cm only increased slightly ($p > 0.05$). It also increased the most when the alfalfa was 5–9 years old. The increments in the SAN content at the 0–100 and 100–200 cm soil depths were higher than those of the STN, with values of 29% and 18%, respectively, while those at depths of 200–300 cm also changed insignificantly ($p > 0.05$). The SAN content continuously increased with the age of the alfalfa, and the average increment in the 0–300 cm profile was as high as 21% when the alfalfa was ≥10 years old. The SOC and STN content increased the most under moderate rainfall conditions (350–500 mm), while the SAN content maintained the highest increment under high rainfall (500–650 mm) conditions. Therefore, ley farming with the alfalfa pasture contributed substantially to the soil C and N at depths of 200 cm in deep loess. Alfalfa should be removed in its middle ages to increase C sequestration while utilizing soil N efficiently.

Keywords: crop–pasture rotation; SOC; soil nitrogen accumulation; deep soil; carbon neutral

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1. Introduction

Promoting carbon (C) sequestration in the soil to reduce increased levels of carbon dioxide in the atmosphere is a practical approach to combating climate warming [1] (Lal, 2004). Soil organic carbon (SOC) is a common indicator of soil health and has received considerable attention from farmers, researchers, and policymakers [2–4]. Throughout human history, 133 Gt of SOC has been lost due to agricultural land use, and an essential cause of C loss has been the degradation of grasslands [5,6]. Management strategies such as crop–pasture rotation, mixed planting, and conservation tillage in agricultural systems have great potential to improve the C sequestration in farmland soil [7,8].

The Loess Plateau of China belongs to the monsoon climate region, which is characterized by uneven rainfall distribution and frequent droughts [9]. Under the influence of climate and excessive human activities, it has long become one of the most vulnerable regions in China [4,10]. Farmland, such as that for growing maize and winter wheat, which require large amounts of chemical input, places tremendous pressure on the agroecosystems' overall resilience and sustainability [11]. The introduction of perennial pasture into the traditional cropping system has tremendous potential to improve land productivity, enhance soil structure, and increase soil C and nitrogen (N) sequestration [12–16]. As a high-yield pasture, Alfalfa (*Medicago sativa* L.) is widely planted in the Loess Plateau. Planting age is a key factor determining soil C and N contents in alfalfa pasture. Soil organic matter usually accumulates in the early ages, but this accumulation might not be sustainable as biomass production decreases and decomposition increases in the later ages [17]. For example, Wan et al. [18] found that soil organic matter in the 0–200 cm soil layer was the highest for a 4-year-old alfalfa pasture and that it rarely increased from 10 to 26 years of planting. The selection of the length of the pasture phase is, therefore, essential for soil C and N management. In addition, rainfall availability largely affects soil C and N dynamics in alfalfa pastures. Soil moisture changes caused by rainfall alter the soils' physical and chemical properties and microbial activities, affecting the soil C and N content [19,20]. Some studies indicated that the SOC content was higher when rainfall was abundant; however, the soil available nitrogen content (SAN) showed an opposite trend [21]. Indeed, there has not yet been a synthetic analysis that has specifically focused on soil C and N in alfalfa pastures. A systematic synthesis is needed to enhance our understanding of how pasture incorporation could affect farmland soil C and N dynamics on a regional scale and to better describe how changes in soil C and N are linked to pasture phase management and climate conditions.

Historically, the research on changes in the soil C has been strongly inclined toward surface sampling (in 0–30 cm), where the highest SOC concentrations occur [22]. Only a few studies have been conducted on deep SOC at depths of 40–100 cm, and few studies have researched SOC in layers below 100 cm [23,24]. This could significantly underestimate soil C sequestration potential as studies have shown that deep soil below 30 cm holds approximately 30–75% of total soil C stocks [25,26]. Deep soil organic matter predominately exists in organic–mineral complexes, and deep soil is also not subjected to tillage disturbance, which contributes to the long-term stabilization of soil organic C and N [8,27]. Alfalfa planted on the loess has a deep rooting system, and there is an urgent need to identify the deep soil contribution to C and N increments in alfalfa pasture on a regional scale.

Meta-analysis uses a quantitative synthesis method to draw general conclusions on a regional scale by synthesizing multiple research results; a comprehensive statistical analysis of these studies can therefore be performed [28,29]. There is currently no up-to-date synthesis study on the effects of pasture incorporation on soil C and N increments in farmland, especially in the deep soil profile. We expected that alfalfa pasture would increase the soil organic carbon (SOC), soil total nitrogen (STN), and soil available nitrogen (SAN) in both shallow and deep soils on a regional scale and that deep soil would contribute substantially to the C and N increments. We also expected that soil C and N could increase and then decrease with alfalfa age and that C and N increments would respond differently under different rainfall conditions. To test our hypothesis, the following meta-analysis was designed: (a) to determine the magnitude of changes in SOC, STN, and SAN in response to alfalfa incorporation practices and (b) to evaluate the effects of the alfalfa planting age and rainfall conditions on the changes in soil C and N. The results provide insights into the sustainable management of the alfalfa pasture on the Loess Plateau.

2. Materials and Methods

2.1. Study Area

We conducted a meta-analysis based on previous experimental studies conducted on the Loess Plateau of China (Figure 1). The Loess Plateau is located in northwest China and has altitudes ranging between 1000 and 1600 m above sea level. The rainfall spatial distribution is uneven, increasing from 250 mm in the northwest to 800 mm in the southeast, with a mean level of 421.8 mm; approximately, 60% of the rainfall occurs during the summer and autumn months. The mean regional annual temperature is 9.0 °C. The climate in the north is arid, while that in the south is semiarid or subhumid. Alfalfa is planted as a pasture crop throughout the north to the south of the region, with a pasture length of ten or more years in the cropping systems [30].

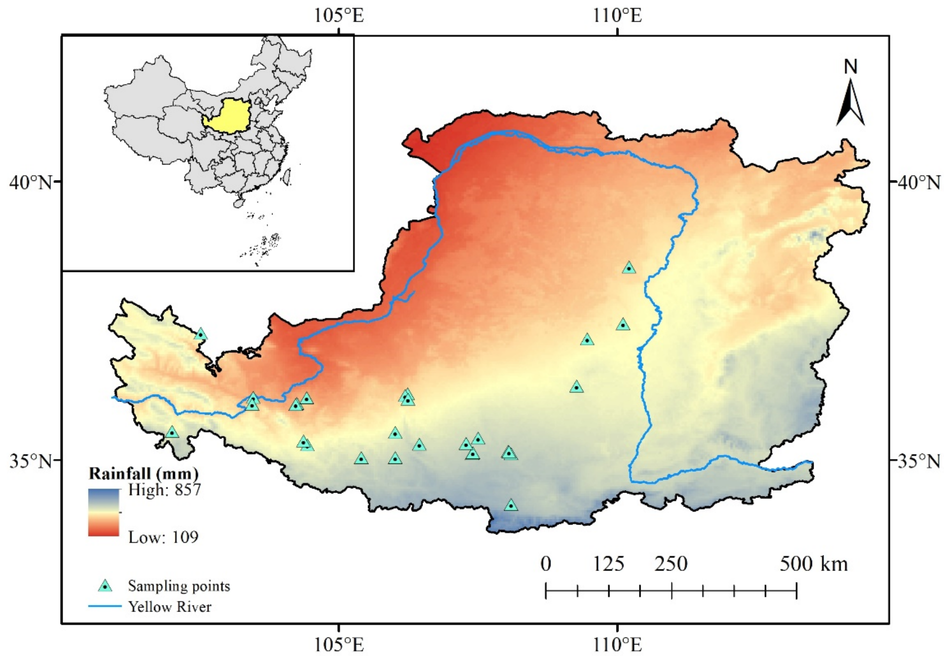

Figure 1. The geographic location of the Loess Plateau in China and the distribution of rainfall and sampling sites across the study area.

2.2. Data Sources

Searches of the literature were performed using the Web of Science and CNKI (China National Knowledge Internet) for papers published between 2000 and 2021 with the keywords ("alfalfa" OR "lucerne") AND ("Loess Plateau") AND ("soil organic carbon" OR "soil organic matter" OR "SOC") AND ("soil total nitrogen" OR "soil N" OR "available N"). Some criteria were selected to avoid publication bias: (a) only experiments on the Loess Plateau in China were selected; (b) only field studies were selected, and laboratory studies were not included; (c) data on locations, annual mean temperatures, and annual mean rainfall levels were recorded; (d) the experiments had to have a matched control group (long-term consecutive annual crop fields or 1-year-old alfalfa if annual crop field was unavailable) for comparison; (e) the age of all the alfalfa was specified, and we considered them to be distinct observations if the study included a different alfalfa age for the same site; and (f) the detailed soil sampling depth was provided.

After passing the literature screening criteria, 63 studies on soil C and N dynamics after the continuous planting of alfalfa were obtained. At the same time, we compiled the most complete information from the literature about the study site and experimental design that was possible. We obtained 890 SOC, 834 soil total nitrogen (STN), and 480 available nitrogen (SAN) data points using Get-Data Graph Digitizer software (version 2.24, Russia). Data analyses were conducted using SPSS software (version 27, Armonk, NY, USA). A one-way analysis of variance (ANOVA) was used for the analysis and comparison of all the variables. Significant differences were compared by the Duncan test at $p < 0.05$. The Origin 2023 was used to draw all the graphs (version Origin Lab Origin Pro 2023, Northampton, MA, USA).

SOC was determined by the Walkley–Black method [31] and total N by the Kjeldahl method [32]. Soil available nitrogen was extracted with KCl and determined using an elemental analyzer. The SOC and STN contents were normalized in g·kg^{-1}, and SAN was standardized in mg·kg^{-1}. To clarify the dynamic trends of the C and N pools of different factors, the growth stages of alfalfa were divided into three periods: early (<5 years), middle (5–9 years), and late growth (≥10 years). In terms of the mean amounts of annual rainfall, the sampled sites were divided into low-rainfall (200–350 mm), medium-rainfall (350–500 mm), and high-rainfall (500–650 mm) sites.

2.3. Meta-Analysis

Considering that part of the collected literature only recorded the mean value without standard deviations or standard errors, we used the unweighted meta-analysis approach to maximize the number of observations included in the present analysis [33,34]. The mean response size, *RS*, was defined as:

$$RS = \frac{X_e}{X_c} - 1 \quad (1)$$

where X_e is the average of the SOC, STN, or SAN content in each soil layer of the alfalfa pasture, and X_c represents the values of the SOC, STN, or SAN content in the control. The 95% confidence interval (*CI*) of the mean for the SOC, STN, and SAN content was determined as [35,36]:

$$SE_R = \sqrt{\frac{V_R}{N}} \quad (2)$$

$$95\%\ CI = 1.96SE_R \quad (3)$$

where SE_R is the standard error of the response size for SOC, STN, and SAN; *N* is the number of samples; and V_R is the variance in the response size. In total, 95% *CI* was used to reflect the response of the SOC, STN, or SAN content to continuous alfalfa planting; if the 95% *CI* overlapped with zero, no significant response was indicated. The SOC could be calculated from the relationship between the soil organic matter (*SOM*) and *SOC*, as follows [37]:

$$SOC = SOM/1.724 \quad (4)$$

3. Results

3.1. SOC Content

The alfalfa pasture cultivation had a significantly increasing effect on the SOC content ($p < 0.001$); the average SOC content at a 0–300 cm soil depth increased by 14% compared to the reference cropland across the Loess Plateau (Figure 2a). The RS of the SOC in the 0–100 cm and 100–200 cm layers was significantly positive ($p < 0.001$), and that in the 0–100 cm was as high as 0.17. However, in the 200–300 cm layer, the RS was negative (–0.008, $p = 0.830$). After the further subdivision of the soil layer within the 0–200 cm layer, we found that the magnitude of the RS in the different layers was positive, increasing from 20 cm to 80 cm and then decreasing, with the highest value (0.22) in the 60–80 cm soil layer.

Averaged over a 0–300 cm depth, the response size was significantly positive for every alfalfa planting age group ($p < 0.001$, Figure 3). The value was 0.13 for the <5-year-old alfalfa pasture before it increased to 0.16 in the 5–9-year-old alfalfa pasture and then decreased again to 0.14 in the ≥10-year-old alfalfa pasture. For the <5 year age group, the increase in the SOC content mainly occurred in the 0–100 cm layer, and the layers below 100 cm did not increase significantly. For the 5–9 year age group, the SOC increment occurred for the whole 0–300 cm profile. For the ≥10 year age group, the RS in the 0–20 cm surface layer was as high as 0.48; however, those below the 100 cm soil layers were all lower than that of the 5–9 year age group.

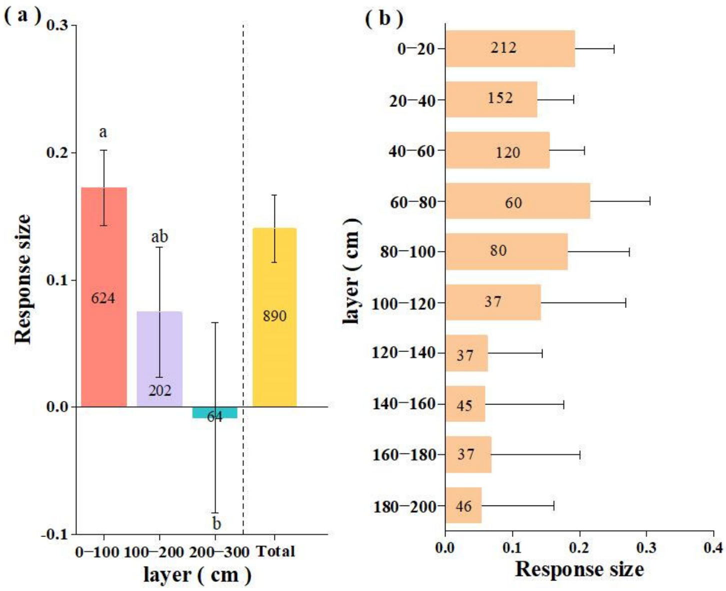

Figure 2. The response size of soil organic carbon (SOC) content in 0–100, 100–200, and 200–300 cm soil layers (**a**) and within 0–200 cm soil depth (**b**) to continuous alfalfa pasture on the Loess Plateau. The pink, purple, blue, yellow, orange bars indicate the response size of SOC content in 0–100, 100–200, 200–300, total, and 0–200 cm soil layers. Error bars represent 95% confidence intervals, and the numbers in the boxes represent the sample sizes. Different lowercase letters near the error bars in the panel indicate significant differences among different soil layers. The dashed lines are used to separate the response values of the total soil layer from the subdivided soil layer.

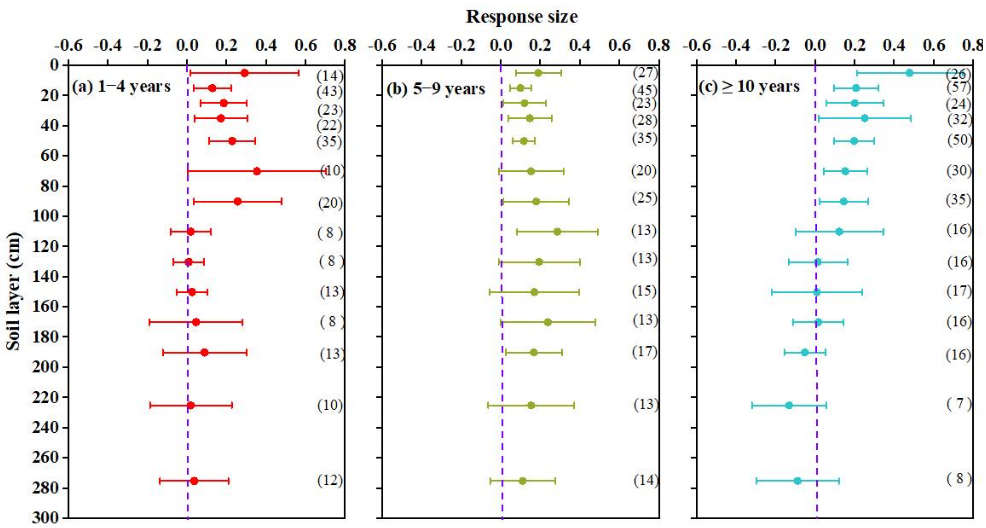

Figure 3. Response size of SOC content in the 0–300 cm soil profile to alfalfa planting on the Loess Plateau. Panels (**a**–**c**) represent <5, 5–9, and ≥10 year age groups, respectively; numbers in the brackets are the sampled size for every depth.

A significant increase in the SOC content was observed in the alfalfa pastures under different rainfall conditions ($p < 0.001$, Figure 4), with RS values of 0.15, 0.16, and 0.08 under 200–350, 350–500, and 500–650 mm, respectively. In the 0–100 cm layer, the SOC content increased significantly under all rainfall conditions ($p < 0.001$). In the 100–200 cm layer, the RS under 350–500 mm was significantly positive (0.12, $p < 0.001$), whereas the RS in the other two groups was insignificantly positive (0.05 under 200–350 mm and 0.01 under 500–650 mm). In the 200–300 cm layer, the RS was negative under 500–650 mm (−0.008), whereas no data were collected for the 200–350 and 350–500 mm conditions.

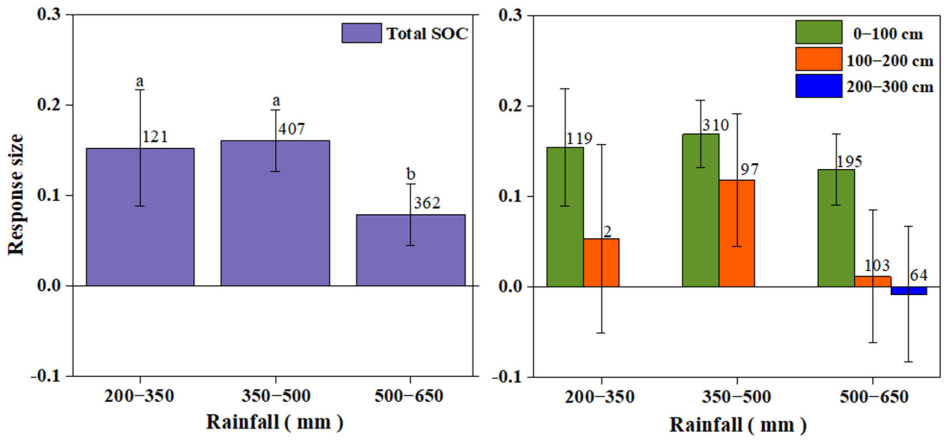

Figure 4. The response size of the SOC content in 0–300 cm profile to alfalfa planting under rainfall conditions of 200–350 mm, 350–500 mm, and 500–650 mm on the Loess Plateau. Different lowercase letters near the error bars indicate significant differences between rainfall conditions.

3.2. STN Content

A significant increase in the STN content occurred after the planting of alfalfa, as indicated by the mean RS of 0.17 at the 0–300 cm soil depth ($p < 0.001$, Figure 5). Specifically, the STN increment mainly contributed to changes in the 0–100 cm and 100–200 soil layers, with values of 0.19 and 0.14 ($p < 0.001$). Although the RS of STN in the 200–300 cm soil layer was also positive, this increase was insignificant (0.03, $p = 0.431$). Within the soil layer of 0–200 cm, the RS values were all significantly positive at each subdivided depth, and the RS in the 100–120 cm layer was significantly higher in the deep layer compared to that of 180–200 cm (Figure 5b).

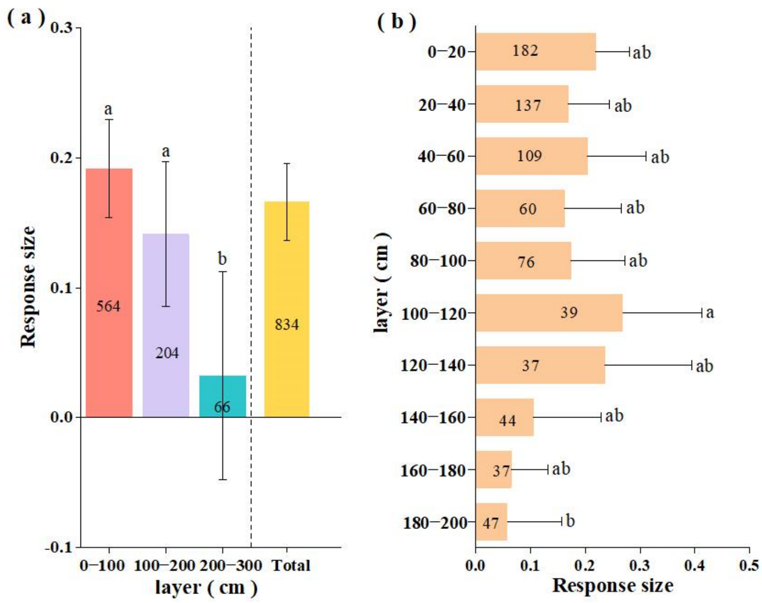

Figure 5. The response size of the soil total nitrogen (STN) content in 0–100, 100–200, and 200–300 cm soil layers (**a**) and within the 0–200 cm soil depth (**b**) to alfalfa planting on the Loess Plateau. The pink, purple, blue, yellow, orange bars indicate the response size of STN content in 0–100, 100–200, 200–300, total, and 0–200 cm soil layers. Error bars represent 95% confidence intervals, and the numbers in the boxes represent the sample size. Different lowercase letters near the error bars indicate significant differences between different layers. The dashed lines are used to separate the response values of the total soil layer from the subdivided soil layer.

Alfalfa planting had a significantly positive effect on the STN content for every age group, with a mean RS of 0.12, 0.22, and 0.18 in the <5-, 5–9-, and ≥10-year-old alfalfa pastures ($p < 0.001$, Figure 6). For the <5-year-old alfalfa, the RS in the 0–300 cm layer was positive, but this was not significant in the deep layers (140–300 cm). For the 5–9-year-old alfalfa, there was also a positive RS in the 0–300 cm soil layer; however, the 200–300 cm layer did not increase significantly. For the ≥10 year age group, an STN increment occurred in the 0–300 cm layer, and the RS in the 200–300 cm soil layer was 158–155% higher than that of the other two age groups.

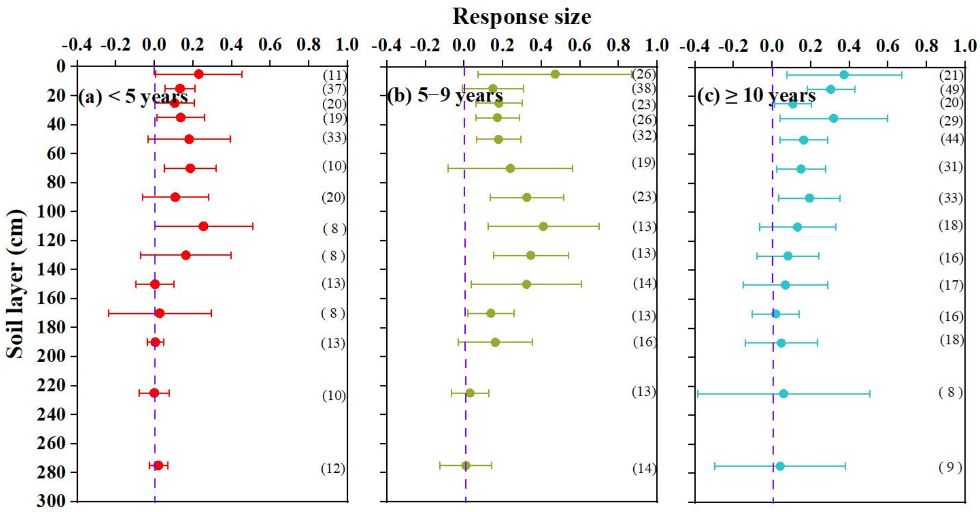

Figure 6. Response size of STN content in the 0–300 cm soil profile to alfalfa planting on the Loess Plateau. Panels (**a**–**c**) represent <5, 5–9, and ≥10 year age groups, respectively; numbers in the brackets are the sampled size for every depth.

The average STN content in the 0–300 cm layer was significantly increased by 18%, 23%, and 9% under rainfalls of 200–350, 350–500, and 500–650 mm, respectively (Figure 7). In the 0–100 cm soil layer, the RS of the STN under all rainfall conditions was significantly positive ($p < 0.001$, 0.18 under 200–350 mm, 0.27 under 350–500 mm, and 0.16 under 500–650 mm). In the 100–200 cm layer, the RS under 350–500 mm of rainfall was significantly positive (0.12, $p < 0.001$), whereas that under 500–650 mm was insignificantly positive (0.04, $p = 0.149$). In the 200–300 cm soil layer, a positive response of the STN content occurred under 350–500 mm (0.07); however, this was negative under 350–500 mm (−0.03). In study sites with a rainfall of 200 to 350 mm, we did not find STN data below 100 cm.

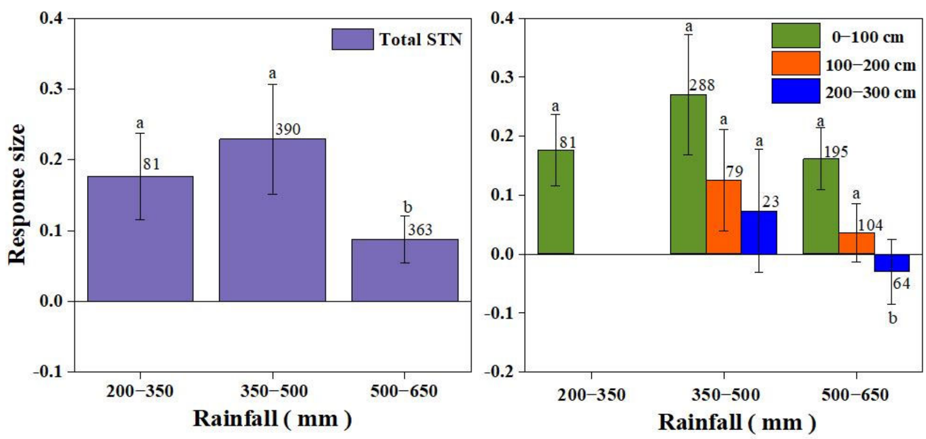

Figure 7. Response size of STN content in the 0–300 cm profile to alfalfa planting under a rainfall of 200–350 mm, 350–500 mm, and 500–650 mm on the Loess Plateau. Different lowercase letters near the error bars indicate significant differences between rainfall conditions.

3.3. SAN Content

The growing alfalfa significantly increased the SAN content in the 0–300 cm soil layer, with an average RS of 0.23 ($p < 0.001$, Figure 8). There was a noticeable difference in the RS values of the different soil layers, as the RS values were significantly positive for the SAN content in the 0–200 cm soil layer (0.29 in 0–100 cm and 0.18 in 100–200 cm); however, the increase in the 200–300 cm soil layers was not significant (0.05, $p = 0.402$). In addition, in the 0–200 cm range, the RS of the SAN in the 60–100 cm layer exceeded 0.30. The RS in the 60–80 cm layer was significantly higher in the deep layer than that of 120–160 cm.

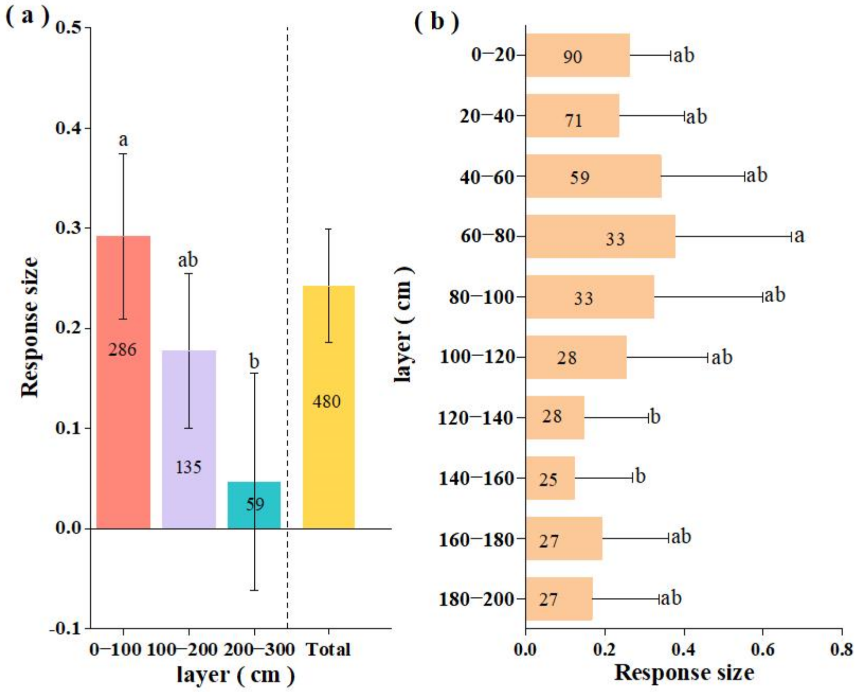

Figure 8. The response size of the soil available nitrogen (SAN) content in the 0–300 cm soil profile (**a**) and within 0–200 cm of soil depth (**b**) to alfalfa continuous planting on the Loess Plateau. The pink, purple, blue, yellow, orange bars indicate the response size of SAN content in 0–100, 100–200, 200–300, total, and 0–200 cm soil layers. Error bars represent 95% confidence intervals, and the numbers in the boxes represent the sample size. Different lowercase letters near the error bars indicate significant differences between different layers. The dashed lines are used to separate the response values of the total soil layer from the subdivided soil layer.

We found significant positive effects of alfalfa planting on the SAN content of all the age groups ($p < 0.001$, Figure 9). This value was 0.19 in the <5-year-old alfalfa pasture before it decreased to 0.17 in the 5–9-year-old alfalfa pasture and then increased again to 0.21 in the ≥10-year-old alfalfa pasture. For the <5-year-old alfalfa pasture, the RS in the 0–300 cm layer increased; however, this increase was not significant in the range below 140 cm. The RS value in the 0–300 cm soil layer for the 5–9-year-old alfalfa showed a similar tendency to that of the <5-year-old alfalfa, with reduced positive values in the deep layer. For the ≥10-year-old alfalfa, there was also an increase in RS in the 0–300 cm soil layers, especially in the 0–100 cm soil layer, where the RS was 28–53% higher than that of the other two age groups.

Overall, rainfall significantly increased the SAN content ($p < 0.001$, Figure 10). The average RS values of the SAN in the 0–300 cm layer were 0.10, 0.09, and 0.34 under rainfalls of 200–350, 350–500, and 500–650 mm, respectively. In the 0–100 cm soil layer, the average SAN content significantly increased by 10%, 12%, and 53% under rainfalls of 200–350, 350–500, and 500–650 mm, respectively. In the 100–200 cm layer, the RS of the SAN content under 500–650 mm was significantly positive (0.187, $p < 0.001$), whereas this increased insignificantly under 350–500 mm (0.037, $p = 0.091$). In the 200–300 cm layer, the RS was positive under 500–650 mm (0.047, $p = 0.402$). The samples under 200–350 mm and 350–500 mm did not record SAN data in the deeper soil.

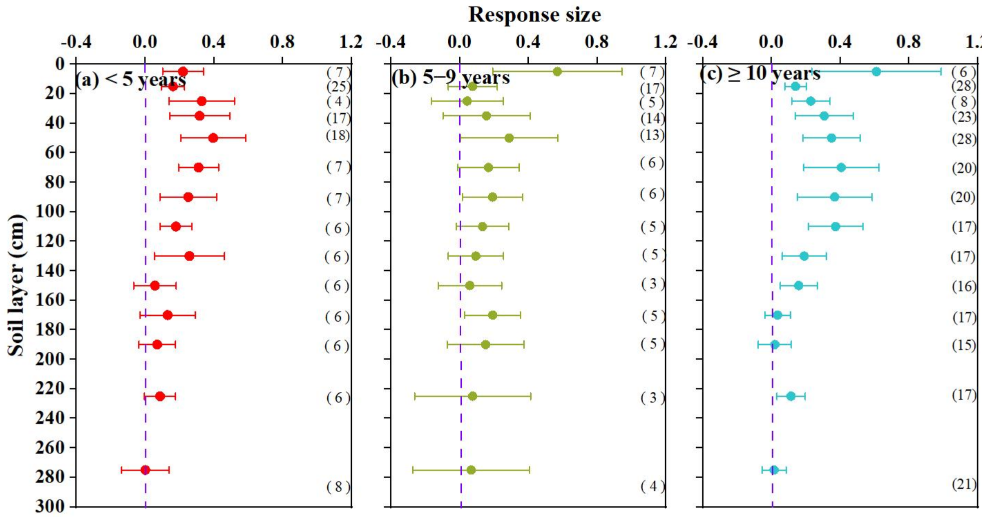

Figure 9. Response size of SAN in the 0–300 cm soil profile to alfalfa planting on the Loess Plateau. Panels (**a**–**c**) represent the <5, 5–9, and ≥10 year age groups, respectively; numbers in the brackets are the sampled size for every depth.

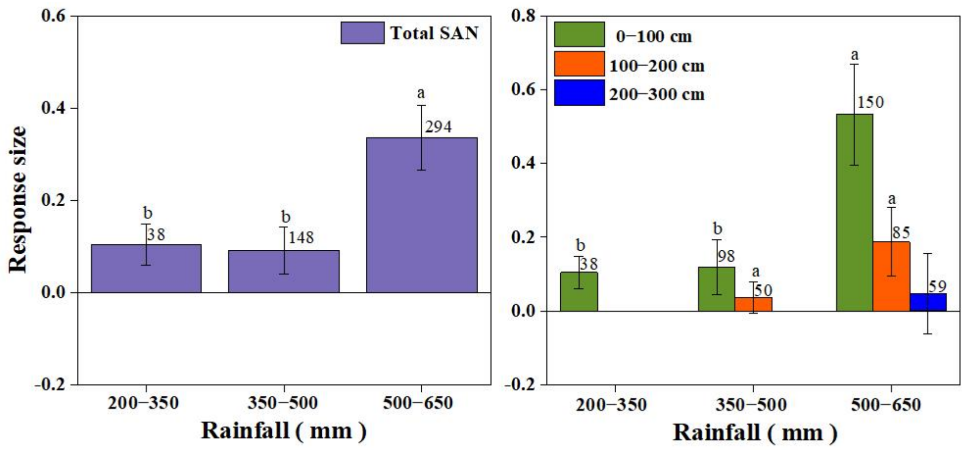

Figure 10. The response size of SAN content in the 0–300 cm profile to alfalfa planting under rainfalls of 200–350 mm, 350–500 mm, and 500–650 mm on the Loess Plateau. Different lowercase letters near the error bars indicate significant differences between rainfall conditions.

3.4. The Correlation of SOC with STN and SAN in Different Soil Layers

Overall, for the 0–300 cm depth, the correlations of the SOC with the STN and SAN were positive in every soil layer (Figure 11). Moreover, both positive correlations of the SOC with the STN and SAN decreased due to the deepening of the soil layer. There was a significant and positive correlation between the SOC and STN in the 0–100, 100–200, and 200–300 cm soil layers, with correlation coefficients of 0.57, 0.36, and 0.35 ($p < 0.001$). In contrast, the SOC and SAN also exhibited a significant ($p < 0.001$) positive relationship, except for that in the 200–300 cm soil layer. In addition, the correlation between the SOC and STN was more robust than that between the SOC and SAN within the same soil layer.

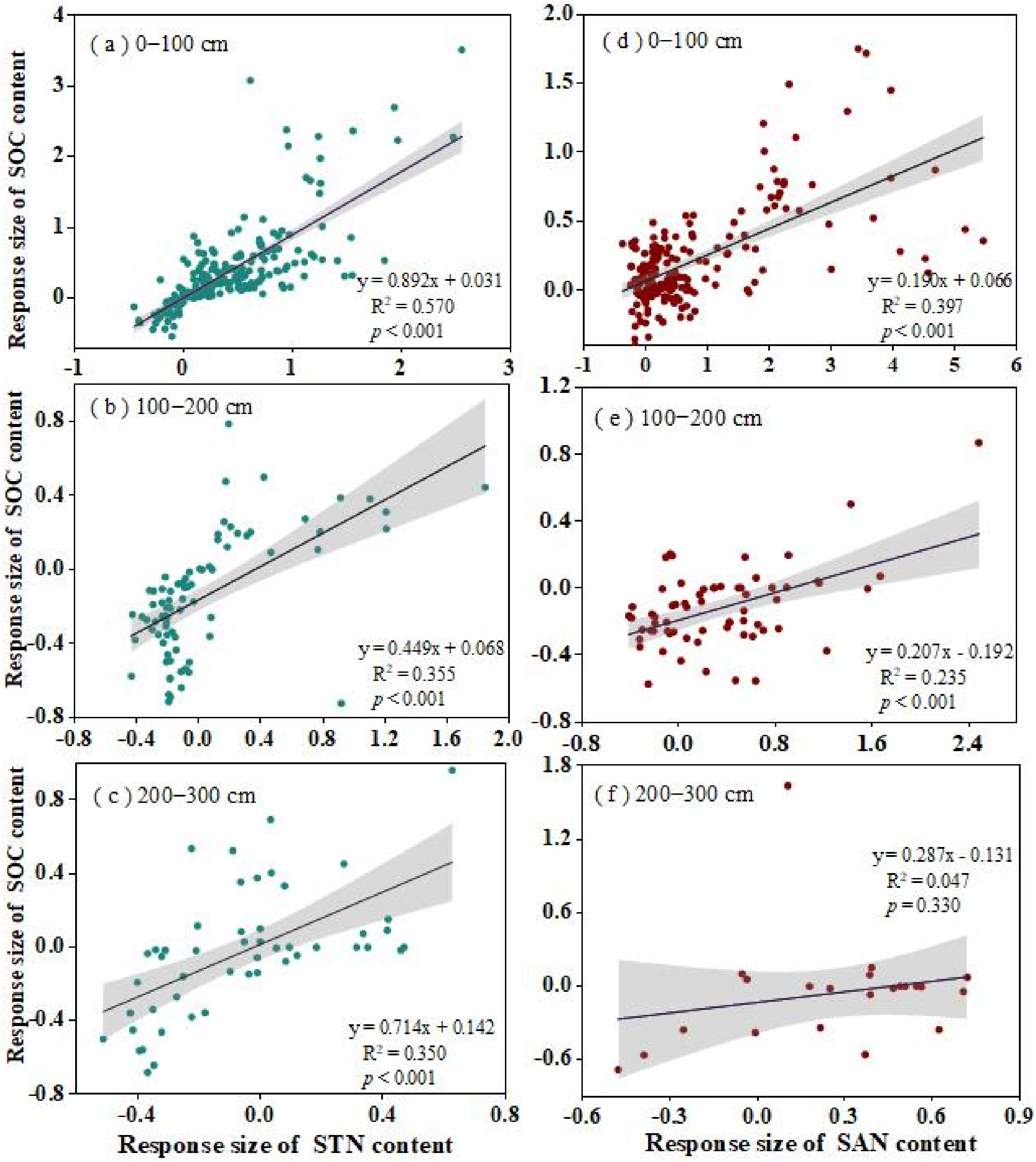

Figure 11. Linear relationship between the response size of SOC and that of STN (**a**–**c**) and SAN (**d**–**f**). Grey areas represent 95% confidence intervals. The red and green dots indicate the relationship between the SOC content and that of STN content and SAN content.

4. Discussion

4.1. Soil Carbon and Nitrogen Content in Alfalfa Pasture

Perennial legumes play an essential role in conserving and increasing soil organic matter in farmland. This study investigated the effects of alfalfa cultivation on the soil C and N content to the depth of 300 cm on the dryland Loess Plateau. We found that the SOC, STN, and SAN in the 0–100 cm soil layer significantly increased by 17–29%. The SOC, STN, and SAN in the 100–200 cm layer also moderately increased. However, the STN and SAN only increased by 3–5%, and the SOC slightly decreased by 0.8% in the 200–300 cm soil layer. The soil C and N stratification phenomenon was remarkable in the dryland cropping system, and soil depth was essential when analyzing the response of the soil C and N contents to land use changes [38]. It is commonly assumed that C in the 30 cm surface layer is the most affected by plant roots; therefore, the previous studies have mainly concentrated on the soil C in the shallow layer of 0–30 cm [39,40]. However, limited rainfall and deep loess facilitate root extension in the deep soil layers of the Loess Plateau. Wang [24] validated the fact that the alfalfa root system was distributed in the entire 0–1000 cm soil layer after 4 years of planting. Although the carbon and nitrogen concentrations decreased with the increasing depth, the total SOC and STN storage was greater in the deeper soil layers than at the surface. Therefore, we highlighted the fact that the deep soil beyond 100 cm contributed substantially to C and N accumulation in the alfalfa pasture cropping system. Similar results were reported by Schnabel et al. [41] for grazed pastures in the Great Plains of the United States, and by Ojeda et al. [42] for a perennial forage cropping system in Argentina. In addition, we concluded that C and N under 200 cm were reduced or insignificantly changed by pasture cropping, as C and N consumption could exceed production in that layer; a similar tendency was reported by Tautges et al. [8] in farmland with cover crops. Root-associated microorganism and C inputs from roots are two major processes causing deep SOC changes [43,44]. Studies found that the fresh root C input could increase microbial activity and the breakdown of SOC, leading to losses of old SOC that were greater than the production of new SOC [45,46].

General observations of the soil organic matter dynamics indicated that the significant sources of SOC were litter and humus, and an increase in the litter resulted in an increased flux of SOC into the soil [47,48]. In a deep-rooted legume pasture, root biomass decomposition and root exudates can also largely contribute to soil C and N increments [49]. Katterer et al. [50] discovered in a 15-year long-term experiment that the contribution of roots to SOC was approximately 2.3 times that of surface crop residues. Other studies have shown that the alfalfa root biomass is strongly correlated with soil C stock [42,51]. Sun [52] studied the root weight of different alfalfa varieties and found that the root weight of 0–40 cm accounted for 52–89% of the total root weight, while the value of 160–200 cm accounted for 0.28–2.56%. Therefore, the SOC in the 0–100 cm layer was more significantly enhanced than that of the deeper layers. In deep soil, the root system mainly supports the plant at a deep soil level with few branches and a small specific surface area [53]; hence, the SOC supplementation by root metabolism and exudates was also relatively low. In this study, we also found that the C content of $\geq$10 years of alfalfa in the deep soil decreased and was less than that of the value of the control farmland, which could be related to the crop's consumption of deep C and N and decomposition by microorganisms.

Changes in SOC directly affect the form and N availability and thus determine the soil N content [54,55]. Our research validated that the SOC and STN had a similar changing trend and that there were positive correlations between the SOC, STN, and SAN across the entire 0–300 cm soil profile. Additionally, we revealed that this correlation gradually decreased as the soil layer deepened. Li [28] et al. studied the relationship between STN and SOC and found a positive correlation coefficient of 0.799. Similarly, Heng et al. [56] also indicated that there was a positive relationship between STN and SOC and found that the correlation coefficient decreased with the soil depth. By contrast, the correlation between SOC and SAN was not strong enough, as the SAN was also affected by other factors, such as biological N_2 fixation and N leaching loss.

4.2. Factors Affecting Soil SOC, STN, and SAN

Rainfall is one of the main climate factors that affect soil C and N contents in dryland conditions. On one hand, climatic conditions affect vegetation productivity, thereby determining the amount of C and N input in the soil. On the other hand, climate can affect the decomposition and transformation of organic matter by microorganisms through changes in the soil moisture and temperature, thereby determining the nutrient output [57]. Different rainfall patterns differently affected the soil C sequestration in the deep loess. Liu et al. [29] reported that SOC was weakly related to rainfall, while Sun et al. [58] showed that the SOC content increased linearly with an increase in the mean annual rainfall. Through the synthesis of the experimental data of 63 experiments with variable rainfall, we clarified that the response sizes of the SOC content at the depth of 0–100 cm were all positive under different rainfall conditions; however, below a depth of 100 cm, the SOC content was significantly increased by 12% under medium-rainfall (350–500 mm) conditions, while it only increased by 5% and 2% under 200–350 and >500 mm of rainfall. A similar tendency was observed for STN. In arid to subhumid conditions, precipitation limits plant production and decomposition, with plant production responding more strongly than decomposition [19]. The lack of water leads to weed invasion and the death of large areas of alfalfa fibrous roots, leading to a low accumulation of organic matter. However, the decomposition of soil organic carbon (SOC) was sensitive to changes in the soil temperature and moisture. An increase in rainfall may increase the mineralization rate of soil organic matter by increasing the number of soil microorganisms [59]. Previous studies have reported that the production and breakdown of organic matter both rise with temperature in humid climates; however, the relative increases in decomposition were greater [60,61]. As a result, the deep soil C and N content in the 500–650 mm rainfall areas was lower than that in the 350–500 mm rainfall areas.

As a perennial crop, the planting age of alfalfa largely affected its production potential, root distribution, and soil moisture conditions [30]. This study found that the soil C and N content continued to change; the soil SOC and STN content peaked at 5–9 planting years, and the SAN content peaked after 10 years. The alfalfa planting age affected its growth and N_2 fixation capacity [62], thereby changing the soil C and N content. Studies revealed that the 5–6-year-old alfalfa root biomass was generally higher than that of other ages, and its biomass in the profile above 25 cm was twice that of the 4-year-old alfalfa [63,64]. Yang et al. [65] found that the root nodules of 5-year-old alfalfa in different soil layers were 1.43–2.81 times that of 2-year-old alfalfa. In addition, some studies have shown that alfalfa mainly absorbs nutrients from the soil in its early stages of development, thus negatively affecting organic matter accumulation [4,66]. Therefore, alfalfa pastures were shown to have a higher soil organic matter content in the middle ages (5–9 years) compared to the early growth ages (<5 years). However, the soil water content was significantly reduced as the alfalfa plants matured. According to Ali et al. [30], the soil water content decreased by 35.6% after the alfalfa had been planted for ten years; this resulted in reduced alfalfa N_2 fixation capacity and plant growth. This study showed that the SAN content increased significantly after growing for more than ten years. This agreed with the results of Bai [67], who stated that a higher SAN content was found in middle- and late-aged alfalfa pastures, especially in the upper 60 cm range. With an increase in the number of alfalfa growth years, the soil bulk density of each alfalfa pasture soil layer decreased as the total porosity increased [68,69]. At the same time, it was found that the soil C/N ratio decreased after planting alfalfa over 10 years; this enhanced the mineralization and organic matter decomposition of soil microorganisms [69]. The productivity and water use efficiency of alfalfa decreased, and the root death increased during late growth, reducing the amount of organic matter injected into the soil [9]. The reduction in the soil's available carbon sources, which provided energy for biological nitrogen fixation processes, further affected the nitrogen fixation of microorganisms [70]. The combined action of these processes could have resulted in a higher content of available nitrogen in the alfalfa that was older than 10 years.

4.3. Implications for Soil and Vegetation Management

Roots are critical for driving changes in soil C stocks [71,72]. Changes in land use, such as the conversion of cropland from shallow-rooted annual crops to deep-rooted species, affect the vertical distribution of C in soils through the effects associated with different vegetation root systems [19,73]. The SOC content in the alfalfa pasture exhibited "surface gathering" and was significantly higher in the shallow soil layer than in the deeper layers. Therefore, after alfalfa was planted for a while and the topsoil C and N contents were improved, the conditions were conducive to the development of the following shallow-rooted annual crops. Consequently, with regard to the economic benefits, C storage, and sustainable alfalfa development, we recommend that alfalfa be rotated with annual crops to promote soil C and N utilization. At the same time, it was found that changes in rainfall greatly affected the accumulation and consumption of C and N in deep soil. Therefore, in areas with scarce rainfall, the soil moisture could be increased through reasonable irrigation measures to achieve a positive effect on the accumulation of C and N in the soil below 100 cm.

Our study area was concentrated on the Loess Plateau of China. Such a small area made our study more targeted, as we expected; however, at the same time, this limited the number of samples. Therefore, the limited sample size may have weakened the ability of this meta-analysis to explain the effects of alfalfa planting on the soil C and N content. Studies have found that alfalfa yield and water-use efficiency continuously decreased with planting age after the optimum growth age [74]. Simultaneously, the soil C and N contents increased significantly in the early ages but declined in the late ages. As the water cycle was closely related to the C and N cycles, further research should comprehensively consider this to obtain optimal planting measures for alfalfa and annual crop rotation.

5. Conclusions

This study performed a meta-analysis of SOC, STN, and SAN content data in 0–300 cm soil profiles in an alfalfa pasture on infertile loess soil. After successive alfalfa planting, the SOC in the 0–100 cm soil layer increased by 17%, and the STN and SAN increased by 19% and 29%, respectively; all the indicators also increased accordingly in the 100–200 cm layer. The STN and SAN contents below 200 cm increased by 3% and 5%, while the SOC decreased by 0.8%. The SOC and STN contents in the middle-aged alfalfa pastures increased the most, with increments of 16% and 22%, respectively, whereas the SAN content in the alfalfa that was over ten years old increased the most, with an increment of 21%. The results of this study highlight the contribution of alfalfa pasture to C sequestration soil fertility improvement in dryland farmlands. We suggest constructing alfalfa and shallow-rooted crop rotation systems to improve SOC sequestration and soil fertility and reducing N fertilizer use in the study area.

Author Contributions: Z.W. designed the study; G.A. and L.W. collected data and conducted statistical analyses; G.A. drafted the manuscript; Z.W. edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Lal, R. Soil carbon sequestration to mitigate climate change. *Geoderma* **2004**, *123*, 1–22. [CrossRef]
2. Houghton, J.T.; Jenkins, G.J.; Ephraums, J.J. *Climate Change: The IPCC Intergovernmental Panel on Climate Change Scientific Assessment*; Cambridge University Press: Cambridge, UK, 1990.
3. Schmidt, M.W.I.; Torn, M.S.; Abiven, S.; Dittmar, T.; Guggenberger, G.; Janssens, I.A.; Kleber, M.; Koegel, K.I.; Lehmann, J.; Manning, D.A.C.; et al. Persistence of soil organic matter as an ecosystem property. *Nature* **2011**, *478*, 49–56. [CrossRef] [PubMed]
4. Wang, W.; Cheng, Z.G.; Li, M.Y.; Wang, B.Z.; Li, J.Y.; Su, Y.Z.; Batool, A.; Xiong, Y.C. Increasing periods after seeding under twice-annually harvested alfalfa reduces soil carbon and nitrogen stocks in a semiarid environment. *Land. Degrad. Dev.* **2020**, *31*, 2872–2882. [CrossRef]
5. Sanderman, J.; Hengl, T.; Fiske, G.J. Soil carbon debt of 12,000 years of human land use. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 9575–9580. [CrossRef] [PubMed]
6. Rumpel, C.; Amiraslani, F.; Koutika, L.S.; Smith, P.; Whitehead, D.; Wollenberg, E. Put more carbon in soils to meet Paris climate pledges. *Nature* **2018**, *564*, 32–34. [CrossRef]

7. Lal, R.; Follett, R.F.; Kimble, J.; Cole, C.V. Managing U.S. cropland to sequester carbon in soil. *J. Soil Water Conserv.* **1999**, *54*, 374–381.
8. Tautges, N.E.; Chiartas, J.L.; Amélie, C.M.G.; O'Geen, A.T.; Scow, K.M. Deep soil inventories reveal that impacts of cover crops and compost on soil carbon sequestration differ in surface and subsurface soils. *Glob. Chang. Biol.* **2019**, *25*, 3753–3766. [CrossRef]
9. Wang, L.L.; Xie, J.H.; Luo, Z.Z.; Niu, Y.N.; Coulter, J.A.; Zhang, R.Z.; Li, L.L. Forage yield, water use efficiency, and soil fertility response to alfalfa growing age in the semiarid Loess Plateau of China. *Agric. Water Manag.* **2021**, *243*. [CrossRef]
10. Jiang, J.P.; Xiong, Y.C.; Jia, Y.; Li, F.M.; Xu, J.Z.; Jiang, H.M. Soil quality dynamics under successional alfalfa field in the semi-arid Loess Plateau of northwestern China. *Arid. Land. Res. Manag.* **2007**, *21*, 287–303. [CrossRef]
11. Deng, L.; Wang, K.B.; Li, J.P.; Shangguan, Z.P.; Sweeney, S. Carbon storage dynamics in slfalfa (*Medicago sativa*) fields in the hilly-gully region of the Loess Plateau, China. *Clean-Soil Air Water* **2014**, *42*, 1253–1262. [CrossRef]
12. Wick, B.; Kuhne, R.F.; Vlek, P.L.G. Soil microbiological parameters as indicators of soil quality under improved fallow management systems in south-western Nigeria. *Plant Soil* **1998**, *202*, 97–107. [CrossRef]
13. Carlsson, G.; Huss, D.K. Nitrogen fixation in perennial forage legumes in the field. *Plant Soil* **2003**, *253*, 353–372. [CrossRef]
14. Fu, X.L.; Shao, M.A.; Wei, X.R.; Horton, R. Soil organic carbon and total nitrogen as affected by vegetation types in Northern Loess Plateau of China. *Geoderma* **2010**, *155*, 31–35. [CrossRef]
15. Liu, Y.; Tang, H.Y.; Muhammad, A.; Huang, G. Emission mechanism and reduction countermeasures of agricultural greenhouse gases—A review. *Greenh. Gases Sci. Technol.* **2019**, *9*, 160–174. [CrossRef]
16. Shamseldin, A. Future outlook of transferring biological nitrogen fixation (BNF) to cereals and challenges to retard achieving this dream. *Curr. Microbiol.* **2022**, *79*, 171. [CrossRef] [PubMed]
17. Ji, B.; Wang, Z.J.; Pan, Z.B.; Xu, H.; Xie, Y.Z. Soil carbon storage characteristics of alfalfa (*Medicago sativa*) artificial grasslands in the semi-arid hilly gully region of the Loess Plateau, China. *Russ. J. Ecol.* **2020**, *51*, 466–476.
18. Wan, S.M.; Han, Q.F.; Hu, S.L.; Jia, Z.K.; Yang, B.P. Soil nitrogen consumption characteristics of alfalfa grassland in the semi-humid areas of the Loess Plateau. *Plant Nutr. Fertil. Sci.* **2008**, *14*, 84–89. (In Chinese with English Abstract)
19. Jobbagy, E.G.; Jackson, R.B. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol. Appl.* **2000**, *10*, 423–436. [CrossRef]
20. Bronson, K.F.; Zobeck, T.M.; Chua, T.T.; Acosta, M.V.; van Pelt, R.S.; Booker, J.D.; Bronson, K.F.; Zobeck, T.M.; Chua, T.T.; Acosta, M.V.; et al. Carbon and nitrogen pools of southern high plains cropland and grassland soils. *Soil Sci. Soc. Am. J.* **2004**, *68*, 1695–1704. [CrossRef]
21. Ding, J. The Crop Growth of Different Year in Alfalfa Rotation Field and the Soil Environmental Research. Master's Thesis, Northwest A&F University, Xianyang, China, 2013. (In Chinese with English abstract).
22. Burke, I.C.; Yonker, C.M.; Parton, W.J.; Cole, C.V.; Flach, K.; Schimel, D.S. Texture, climate, and cultivation effects on soil organic matter content in US grassland soils. *Soil Sci. Soc. Am. J.* **1989**, *53*, 800–805. [CrossRef]
23. Wang, D.; Liu, Y.; Wu, G.L.; Ding, L.M.; Yang, Z.; Hao, H.M. Effect of rest-grazing management on soil water and carbon storage in an arid grassland (China). *J. Hydrol.* **2015**, *527*, 754–760.
24. Wang, L.; Ali, G.; Wang, Z.K. Deep soil water depletion and soil organic carbon and total nitrogen accumulation in a long-term alfalfa pasture. *Land Degrad. Dev.* **2023**, *34*, 2164–2176. [CrossRef]
25. Olson, K.R.; AlKaisi, M.M. The importance of soil sampling depth for accurate account of soil organic carbon sequestration, storage, retention and loss. *Catena* **2015**, *125*, 33–37. [CrossRef]
26. VandenBygaart, A.J.; Bremer, E.; McConkey, B.G.; Ellert, B.H.; Janzen, H.H.; Angers, D.A.; Carter, M.R.; Drury, C.F.; Lafond, G.P.; McKenzie, R.H. Impact of sampling depth on differences in soil carbon stocks in long-term agroecosystem experiments. *Soil Sci. Soc. Am. J.* **2011**, *75*, 226–234. [CrossRef]
27. Koegel, K.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]
28. Li, D.; Wang, Z.F.; Zheng, J.B. Contents of soil organic matter, nitrogen, phosphorus and potassium under different land-use patterns in purple Hill Area. *J. Soil Sci.* **2009**, *40*, 310–314. (In Chinese with English Abstract)
29. Liu, C.A.; Li, F.R.; Zhou, L.M.; Zhang, R.H.; Yu, J.; Lin, S.L.; Wang, L.J.; Siddique, K.H.M.; Li, F.M. Effect of organic manure and fertilizer on soil water and crop yields in newly-built terraces with loess soils in a semi-arid environment (EI). *Agric. Water Manag.* **2013**, *117*, 123–132. [CrossRef]
30. Ali, G.; Wang, Z.; Li, X.; Jin, N.; Chu, H.; Jing, L. Deep soil water deficit and recovery in alfalfa fields of the Loess Plateau of China. *Field Crop Res.* **2021**, *260*, 107990. [CrossRef]
31. Page, A.L.; Keeney, D.R. Methods of soil analysis. Part 2. Chemical and microbiological properties. In *Total Carbon, Organic Carbon and Organic Matter*; Nelson, D.W., Sommers, L.E., Eds.; ASA, SSSA: Madison, WI, USA, 1982; pp. 539–594.
32. Page, A.L.; Keeney, D.R. Methods of soil analysis. Part 2. Chemical and microbio-logical properties. In *Nitrogen-Total*; Bremner, J.M., Mulvaney, C.S., Eds.; Soil Science Society of America: Madison, WI, USA, 1982; pp. 595–624.
33. Powers, J.S.; Corre, M.D.; Twine, T.E.; Veldkamp, E. Geographic bias of field observations of soil carbon stocks with tropical land-use changes precludes spatial extrapolation. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6318–6322. [CrossRef]
34. Su, B.Q.; Shangguan, Z.P. Decline in soil moisture due to vegetation restoration on the Loess Plateau of China. *Land Degrad. Dev.* **2019**, *30*, 290–299. [CrossRef]

35. Deng, L.; Yan, W.M.; Zhang, Y.W.; Shangguan, Z.P. Severe depletion of soil moisture following land-use changes for ecological restoration: Evidence from northern China. *For. Ecol. Manag.* **2016**, *366*, 1–10. [CrossRef]
36. Luo, Y.; Zhang, H.D. Elevated CO stimulates net accumulations of carbon and nitrogen in land ecosystems: A meta-analysis. *Ecology* **2006**, *87*, 53–63. [CrossRef] [PubMed]
37. Collins, M.E.; Kuehl, R.J. Organic matter accumulation and organic soils. In *Wetland Soils, Genesis, Hydrology, Landscapes, and Classification*; Lewis Pub: Boca Raton, FL, USA, 2001; pp. 137–162.
38. Clement, C.; Sleiderink, J.; Svane, S.F.; Smith, A.G.; Diamantopoulos, E.; Desbroll, D.B.; Thorup, K.K. Comparing the deep root growth and water uptake of intermediate wheatgrass (Kernza (R)) to alfalfa. *Plant Soil* **2022**, *472*, 369–390. [CrossRef]
39. Yang, Y.H.; Jiang, P.A.; Luo, M. Effects of planting Medicago Sativa L on soil fertility. *Arid. Land Geogr.* **2005**, *28*, 248–251. (In Chinese with English Abstract)
40. Minasny, B.; Malone, B.P.; McBratney, A.B.; Angers, D.A.; Arrouays, D.; Chambers, A.; Chaplot, V.; Chen, Z.S.; Cheng, K.; Das, B.S.; et al. Soil carbon 4 per mille. *Geoderma* **2017**, *292*, 59–86. [CrossRef]
41. Schnabel, R.R.; Franzluebbers, A.J.; Stout, W.L.; Sanderson, M.A.; Stuedeman, J.A. The effects of pasture management practices. In *The Potential of US Grazing Land to Sequester Carbon and Mitigate the Greenhouse Effect*; Follett, R.F., Kimble, J.M., Lal, R., Eds.; Lewis Publishers: Boca Raton, FL, USA, 2001; pp. 291–322.
42. Ojeda, J.J.; Caviglia, P.O.; Agnusdei, G.M. Vertical distribution of root biomass and soil carbon stocks in forage cropping systems. *Plant Soil* **2018**, *423*, 175–191. [CrossRef]
43. Gross, C.D.; Harrison, R.B. The case for digging deeper: Soil organic carbon storage, dynamics, and controls in our changing world. *Soil Syst.* **2019**, *3*, 28. [CrossRef]
44. Angst, G.; Messinger, J.; Greiner, M.; Häusler, W.; Hertel, D.; Kirfel, K.; Kögel-Knabner, I.; Leuschner, C.; Rethemeyer, J.; Mueller, C.W. Soil organic carbon stocks in topsoil and subsoil controlled by parent material, carbon input in the rhizosphere, and microbial-derived compounds. *Soil Biol. Biochem.* **2018**, *122*, 19–30. [CrossRef]
45. Pausch, J.; Zhu, B.; Kuzyakov, Y.; Cheng, W.X. Plant inter-species effects on rhizosphere priming of soil organic matter decomposition. *Soil Biol. Biochem.* **2013**, *57*, 91–99. [CrossRef]
46. Dijkstra, F.A.; Cheng, W. Interactions between soil and tree roots accelerate long-term soil carbon decomposition. *Ecol. Lett.* **2007**, *10*, 1046–1053. [CrossRef]
47. Kalbitz, K.; Kaiser, K. Contribution of dissolved organic matter to carbon storage in forest mineral soils. *J. Plant Nutr. Soil Sci.* **2008**, *171*, 52–60. [CrossRef]
48. Froberg, M.; Kleja, D.B.; Bergkvist, B.; Tipping, E.; Mulder, J. Dissolved organic carbon leaching from a coniferous forest floor—A field manipulation experiment. *Biogeochemistry* **2005**, *75*, 271–287. [CrossRef]
49. Gentile, R.M.; Martino, D.L.; Entz, M.H. Root characterization of three forage species grown in southwestern Uruguay. *Canad. J. Plant Sci.* **2003**, *83*, 785–788. [CrossRef]
50. Katterer, T.; Bolinder, M.A.; Andren, O.; Kirchmann, H.; Menichetti, L. Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. *Agric. Ecosyst. Environ.* **2011**, *141*, 184–192. [CrossRef]
51. Gentile, R.M.; Martino, D.L.; Entz, M.H. Influence of perennial forages on subsoil organic carbon in a long-term rotation study in Uruguay. *Agric. Ecosyst. Environ.* **2005**, *105*, 419–423. [CrossRef]
52. Sun, Q.; Han, J.; Gui, R.; Liu, G. Root and crown trait of alfalfa in kerqin sandy land. *Acta Agrestia Sin.* **2001**, *9*, 269. (In Chinese with English Abstract)
53. Bullied, W.J.; Entz, M.H. Soil water dynamics after alfalfa as influenced by crop termination technique. *Agron. J.* **1999**, *91*, 294–305. [CrossRef]
54. Wibowo, H.; Kasno, A. Soil organic carbon and total nitrogen dynamics in paddy soils on the Java Island, Indonesia. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *648*, 012192. [CrossRef]
55. Hao, W.F.; Shan, C.J.; Liang, Z.S.; Chen, C.G. The study on the relation between soil nutrient and productivity of plantation robinia pseudoacacia forest in the Loess Plateau and gully area of northern Shaanxi. *Chin. Agric. Sci. Bull.* **2005**, *21*, 129–135. (In Chinese with English Abstract)
56. Heng, T.; Wu, J.G.; Xie, S.Y.; Wu, M.X. The responses of soil C and N, microbial biomass C or N under alpine meadow of Qinghai-Tibet Plateau to the change of temperature and precipitation. *Chin. Agric. Sci. Bull.* **2011**, *27*, 425–430. (In Chinese with English Abstract)
57. Davidson, R. The changing model of soil. *Soil Sci. Soc. Am.* **2012**, *76*, 766–778.
58. Sun, L.; Zhang, G.H.; Luan, L.L.; Li, Z.W.; Geng, R. Distribution of soil organic carbon in surface soil along a precipitation gradient in loess hilly area. *Chin. J. Appl. Ecol.* **2016**, *27*, 532–538. (In Chinese with English Abstract)
59. Xu, Z.; Yu, G.; Zhang, X.; Ge, J.; He, N.; Wang, Q.; Wang, D. The variations in soil microbial communities, enzyme activities and their relationships with soil organic matter decomposition along the northern slope of Changbai Mountain. *Appl. Soil Ecol.* **2015**, *86*, 19–29. (In Chinese with English Abstract) [CrossRef]
60. Oades, J.M. The retention of organic matter in soils. *Biogeochemistry* **1988**, *5*, 35–70. [CrossRef]
61. Schlesinger, W.H. *Biogeochemistry, an Analysis of Global Change*; Academic Press: San Diego, CA, USA, 1997.
62. Li, Y.S.; Huang, M. Pasture yield and soil water depletion of continuous growing alfalfa in the Loess Plateau of China. *Agric. Ecosyst. Environ.* **2008**, *124*, 24–32. [CrossRef]

63. Huang, Z.; Liu, Y.; Cui, Z.; Fang, Y.; He, H.H.; Liu, B.R.; Wu, G.L. Soil water storage deficit of alfalfa (*Medicago sativa*) grasslands along ages in arid area (China). *Field Crop Res.* **2018**, *221*, 1–6. [CrossRef]
64. Hakl, J.; Fuksa, P.; Santrucek, J.; Maskova, K. The development of lucerne root morphology traits under high initial stand density within a seven year period. *Plant Soil Environ.* **2011**, *57*, 81–87. [CrossRef]
65. Yang, H.S.; Zhang, Q.G.; Liu, J.; Tai, J.C.; Miao, S.T. Distribution of root and rhizosphere microorganism in alfalfa with different ages. *Pratacult. Sci.* **2007**, *24*, 38–41. (In Chinese with English Abstract)
66. Tai, J.; Yang, H.; Zhang, Q.; Song, C. Influence of Planting Years on Nitrogen-fixing Capacity of Rhizosphere and Contents of Carbon and Nitrogen in Artificial Pastures of Alfalfa. *Chin. J. Soil Sci.* **2010**, *41*, 603–607. (In Chinese with English Abstract)
67. Bai, Y. Study on Root and Soil Properties of Natural Grassland under Different Encloding Period in Loess Area. Master's Thesis, Northwest A&F University, Xianyang, China, 2014. (In Chinese with English Abstract)
68. Yang, H.S.; Cao, M.J.; Fu, F.; Zhang, Q.G.; Sun, D.Z. Effects of the number of growth years of alfalfa on the physical and chemical properties of soil. *Chin. J. Grassl.* **2006**, *28*, 29–32. (In Chinese with English Abstract)
69. Zhang, S.M. The Productivity of Alfalfa Grassland and It's Effect on Environment in Gully Region of the Loess Plateau. Master's Thesis, Northwest A&F University, Xianyang, China, 2007. (In Chinese with English Abstract)
70. Wang, X.f.L.; Zhuzhu, Z.; Li, L.L.; Niu, Y.N.; Sun, P.Z.; Hai, L.; Li, L.Z. Characteristics of structure and abundance of soil nitrogen-fixing bacterial community in alfalfa with different growing ages in the Loess Plateau. *Chin. J. Eco-Agric.* **2019**, *27*, 358–368.
71. Fornara, D.A.; Tilman, D. Plant functional composition influences rates of soil carbon and nitrogen accumulation. *J. Ecol.* **2008**, *96*, 314–322. [CrossRef]
72. Cong, W.F.; Hoffland, E.; Li, L.; Six, J.; Sun, J.H.; Bao, X.G.; Zhang, F.S.; Vander, W.W. Intercropping enhances soil carbon and nitrogen. *Glob. Chang. Biol.* **2015**, *21*, 1715–1726. [CrossRef] [PubMed]
73. Jackson, R.B.; Canadell, J.G.; Ehleringer, J.R.; Mooney, H.A.; Schulze, E.D. A global analysis of root distributions for terrestrial biomes. *Oecologia* **1996**, *108*, 389–411. [CrossRef] [PubMed]
74. Gu, Y.J.; Han, C.L.; Fan, J.-W.; Shi, X.P.; Kong, M.; Shi, X.Y.; Siddique, K.H.M.; Zhao, Y.Y.; Li, F.M. Alfalfa forage yield, soil water and P availability in response to plastic film mulch and P fertilization in a semiarid environment. *Field Crop Res.* **2018**, *215*, 94–103. [CrossRef]