Long-Term Growth of Alfalfa Increased Soil Organic Matter Accumulation and Nutrient Mineralization in a Semi-Arid Environment

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Land use patterns and vegetation coverage in semi-arid areas of the Loess Plateau have undergone great changes due to the implementation of the "Grain for Green" project. The introduction of legume pasture species, such as alfalfa (*Medicago sativa* L.) and sweet clover (*Melilotus officinalis* L.), is one of the most efficient methods of vegetation restoration and reconstruction in this region. However, there is a need for an effective assessment of the root system distribution and its interaction with soil after long-term introduction. An experiment involving the introduction of alfalfa and sweet clover on abandoned farmlands was initiated in 2003 to assess the long-term effects. After 17 years, root and soil samples at depths of 0–20 and 20–60 cm were collected to characterize the root biomass, root carbon (C), nitrogen (N), and phosphorus (P), soil microbial biomass carbon (MBC) and nitrogen (MBN), soil organic carbon (SOC), and soil N and P. The results showed that the root biomass density of alfalfa in the 0–20 and 20–60 cm layers (63.72 and 12.27 kg m^{-3}, respectively) were significantly higher than for sweet clover (37.43 and 8.97 kg m^{-3}, respectively) and under natural abandonment (38.92 and 9.73 kg m^{-3}, respectively). The SOC, total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), nitrate-nitrogen (NO$_3^-$-N), MBC and MBN in the 0–20 and 20–60 cm layers were higher after alfalfa introduction compared with sweet clover introduction and natural abandonment, although the ammonia-nitrogen (NH$_4^+$-N) concentration in the 0–20 cm layer was lower. There were significantly positive correlations between root biomass density and both soil nutrients and microbial biomass, while there was a negative correlation between the soil NH$_4^+$-N and root biomass density. These results indicate that alfalfa root growth improved soil organic matter accumulation and nutrient mineralization. The accumulation and mineralization of soil nutrients also guaranteed root and microorganism growth. Therefore, it was concluded that alfalfa introduction will promote soil nutrients immobilization and mineralization and may enable sustainable land use in the semi-arid region of the Loess Plateau, China.

Keywords: legumes, *Medicago sativa* L., land use change, plant nutrients, root biomass, soil properties

INTRODUCTION

Soil contains the largest carbon (C) pool in the terrestrial ecosystem, being 3.3 times larger than the atmospheric pool and 4.5 times larger than the biotic storage (Lal, 2004). It plays a vital role in regulating climate warming (Melillo et al., 2002; Zhou et al., 2012). In addition, soil nitrogen (N) and phosphorus (P) are essential elements for vegetation growth (Westheimer, 1987; Azziz et al., 2012; Yamashita et al., 2020). The soil C, N, and P cycles are strongly coupled with biological processes (Elser et al., 2000; Reed et al., 2015). Land use changes due to either natural or anthropogenic activities, can induce changes in the C balance and nutrient flow, thereby altering ecosystem functions (Post and Kwon, 2000; Deng et al., 2016). Slight changes in C and N exchanges between the soil ecosystem and atmosphere could have a significant impact on global climate change (Classen et al., 2015). Therefore, it is essential to assess the effects of land use on soil biogeochemical cycles in the future under global climate change.
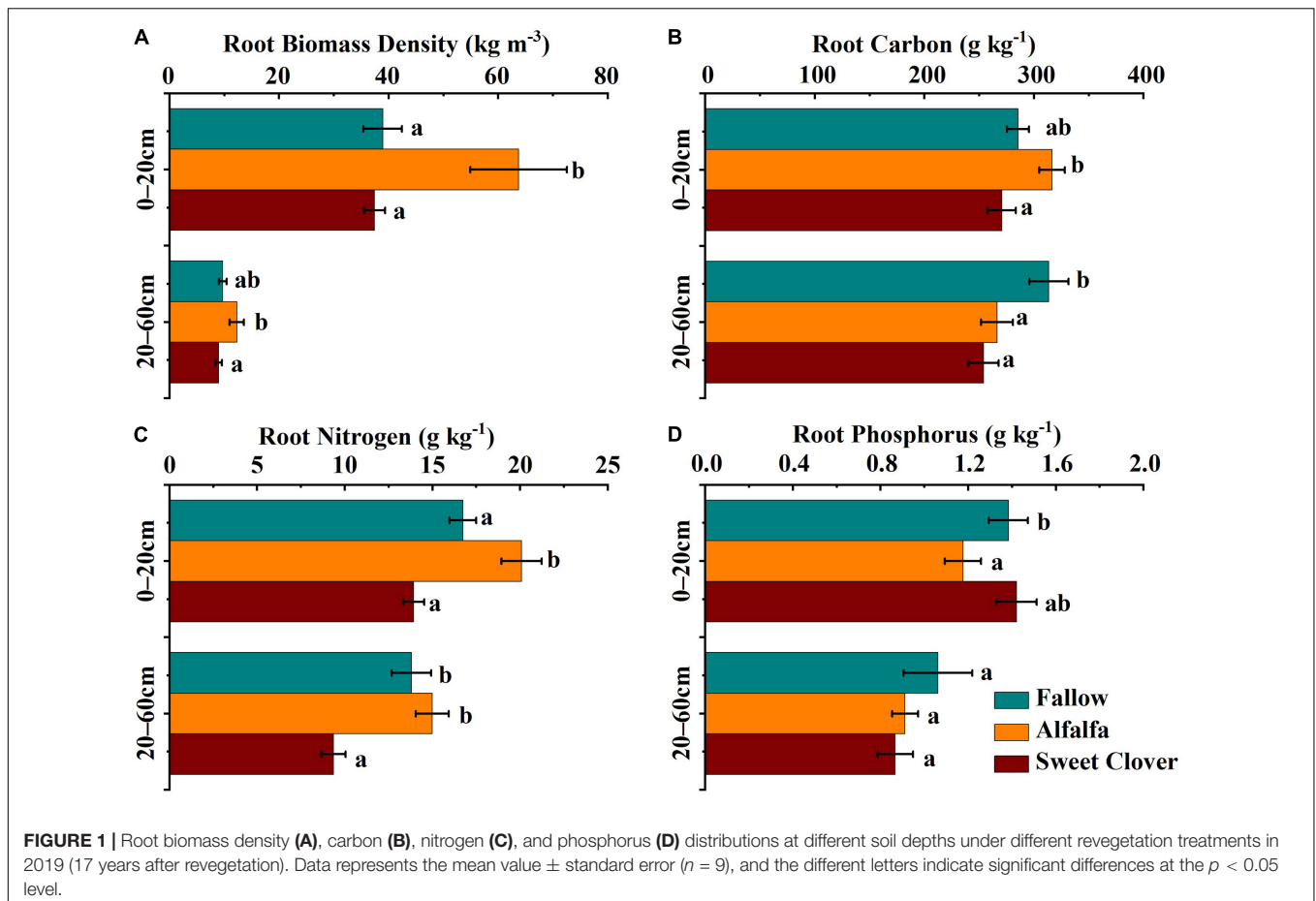
The Loess Plateau in China is an ecologically fragile zone, with serious soil erosion issues (Fu, 1989; Chen et al., 2007; Zhao et al., 2013), and is also sensitive to climate change (He et al., 2006; Liu and Sang, 2013; Miao et al., 2016; Fang et al., 2017; Fang et al., 2018; Gong et al., 2020). Revegetation of degraded land is one of the principal strategies used to control soil erosion and recover ecosystem function, which has had significant effects on soil quality (Yuan et al., 2019), and even on socioeconomic development (Fu et al., 2011; Zhao et al., 2013). The "Grain for Green" project (converting steep farmlands to forest or grassland) was initiated by the Chinese government in 1999 to undertake ecological restoration (Feng et al., 2005; Deng et al., 2014). In this process, legumes such as alfalfa, sweet clover, and *Caragana* species are widely used as pioneer species to restore damaged or degenerated ecosystems due to their strong environmental adaptability, N fixation benefits, and rapid formation of continuous vegetation cover (An et al., 2013; Yuan et al., 2016b; Yao et al., 2020). Vegetation succession may affect the input and decomposition processes of soil C or N; thus, affecting its storage (Fujii et al., 2020). Carbon sequestration requires a large amount of N, resulting in the soil N concentration being one of the limiting factors for soil C storage (van Groenigen et al., 2017). Legumes have the potential to increase the N input into ecosystems, while promoting C and N accumulation in vegetation and soil (Vitousek et al., 2013; Hu et al., 2016). Phosphorus is considered to be a stable element in the soil ecosystem (Vitousek et al., 2010); thus, the process of P fixation and mineralization should also be considered when assessing the introduction of legumes. Vegetation change has a profound impact on the soil biogeochemical cycles of ecosystems at regional and global scales (Yu et al., 2018). In addition, there is a lag effect in the response of soil to vegetation restoration (Knops and Tilman, 2000). Therefore, it is essential to assess the long-term effects of land use change on soil C, N, and P in this region.

How the aboveground biomass responds to the extent of vegetation restoration or stand age has been extensively studied (Liu et al., 2015; Zhang et al., 2017). Land use change can affect the functional composition of plant communities (Hoelzle et al., 2012; Turner et al., 2019), and would finally influence the chemical characteristics of roots and root biomass in the long-term (Mokany et al., 2006; Qiu et al., 2012). However, there have been few studies of the changes in belowground biomass. Land-use changes could alter the magnitude of C inputs into soil by changing the biomass of plant residues, including both aboveground and belowground biomass (Frasier et al., 2019). The input of aboveground litter, fine root turnover, and root exudates increases the C, N, and P concentration in the surface soil, while roots and root exudates are the main sources of organic matter in the deep soil (Rumpel and Kogel-Knabner, 2011; Schmidt et al., 2011). However, roots store SOC and N more efficiently than shoots (Kong and Six, 2010; Jackson et al., 2017; Sokol and Bradford, 2019) and a specific relationship has been observed between root biomass and SOC accumulation (Fornara and Tilman, 2008). In addition, roots not only directly affect the SOC concentration, but also form aggregates with the soil particles (Gale et al., 2000), driving the physical-chemical stabilization of SOC (Six et al., 2000; Six et al., 2004). Although P is derived from the weathering of parent material and ecosystems exist with a constant P content (Walker and Syers, 1976), root exudates can directly or indirectly affect P availability in the rhizosphere (Schilling et al., 1998; Gransee, 2001). Legume introduction extends the rhizosphere in both the horizontal and vertical directions, leading to a change in the distribution of soil C, N, and P (Pransiska et al., 2016). However, exactly how roots regulate soil C, N, and P after legume introduction still remains unclear.

Land-use change is the main factor that affects plant litter input, root turnover, and exudation, all of which make a large contribution to microbial activity and community composition (Campbell et al., 1997; Pandey et al., 2010; Singh and Ghoshal, 2014). Soil microorganisms are the main source of soil enzymes, which have a key role in C, N, and P transformation, playing an important part in the biogeochemical cycles of terrestrial ecosystems (Kiss et al., 1975). Microbial associations with roots in the soil are complex and can enhance the ability of plants to acquire nutrients from soil through the turnover of microbial biomass within the rhizosphere (Vanveen et al., 1989; Boyrahmadi and Raiesi, 2018). It is therefore necessary to consider the root-microorganism relationship in the process of revegetation.

The impact of land use change on soil properties, root systems, and soil microorganisms is of great importance in ecosystem management and policy making. Therefore, an experiment was conducted in the semi-arid region of the Loess Plateau to determine the long-term effects of legume introduction on soil properties [SOC, total nitrogen (TN), total phosphorus (TP), microbial biomass carbon (MBC) and nitrogen (MBN), mineral N, available phosphorus (AP)] at depths of 0–20 and 20–60 cm and their inter-relationship. We hypothesized that: (1) legume introduction increases the root biomass of communities and microorganisms (i.e., the MBC and MBN), leading to an increase in the concentrations of mineral nutrients; and (2) legume introduction improves soil C and N storage, but has no significant effect on the TP concentration due to its stability.

FIGURE 1 | Root biomass density (A), carbon (B), nitrogen (C), and phosphorus (D) distributions at different soil depths under different revegetation treatments in 2019 (17 years after revegetation). Data represents the mean value ± standard error ($n = 9$), and the different letters indicate significant differences at the $p < 0.05$ level.

MATERIALS AND METHODS

The Study Area

The study was conducted at the Gansu Dryland Agroecology Observation and Research Station on the Loess Plateau (36°02′N, 104°25′E, 2,400 m above sea level), Lanzhou University, where a long-term revegetation experiment was established in 2003. The station is located in the northern mountain region of Yuzhong County, Gansu Province, China. This region is characterized by a semi-arid climate, with a mean annual temperature and precipitation of 7.5°C and 378.4 mm, respectively, during the experiment (2003–2019). Most of the precipitation occurred in June to September. The soil had a mean soil bulk density of 1.28 g cm^{-3}, pH of 8.2, and calcium carbonate ($CaCO_3$) concentration of 146 g kg^{-1}. The soil was classified as a Heima [a calcic Kastanozems, according to the World Reference Base for Soil Resources (WRB)], with a field water holding capacity of 23% and a permanent wilting point of 4.5% (Shi et al., 2003). This area is subjected to both wind and soil erosion.
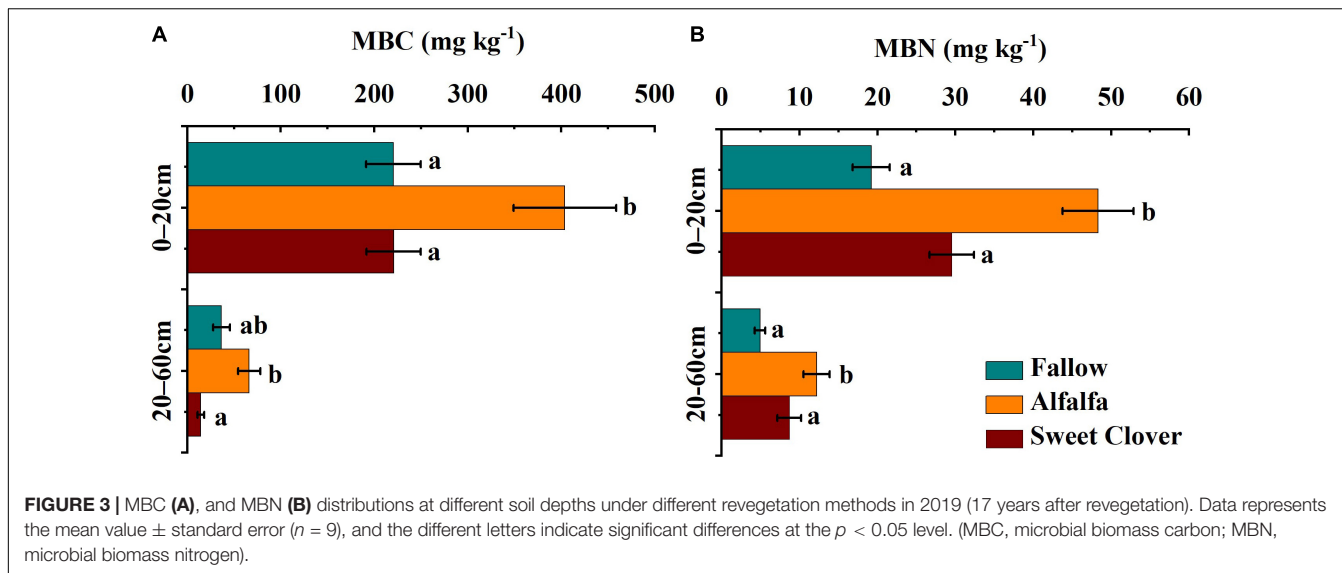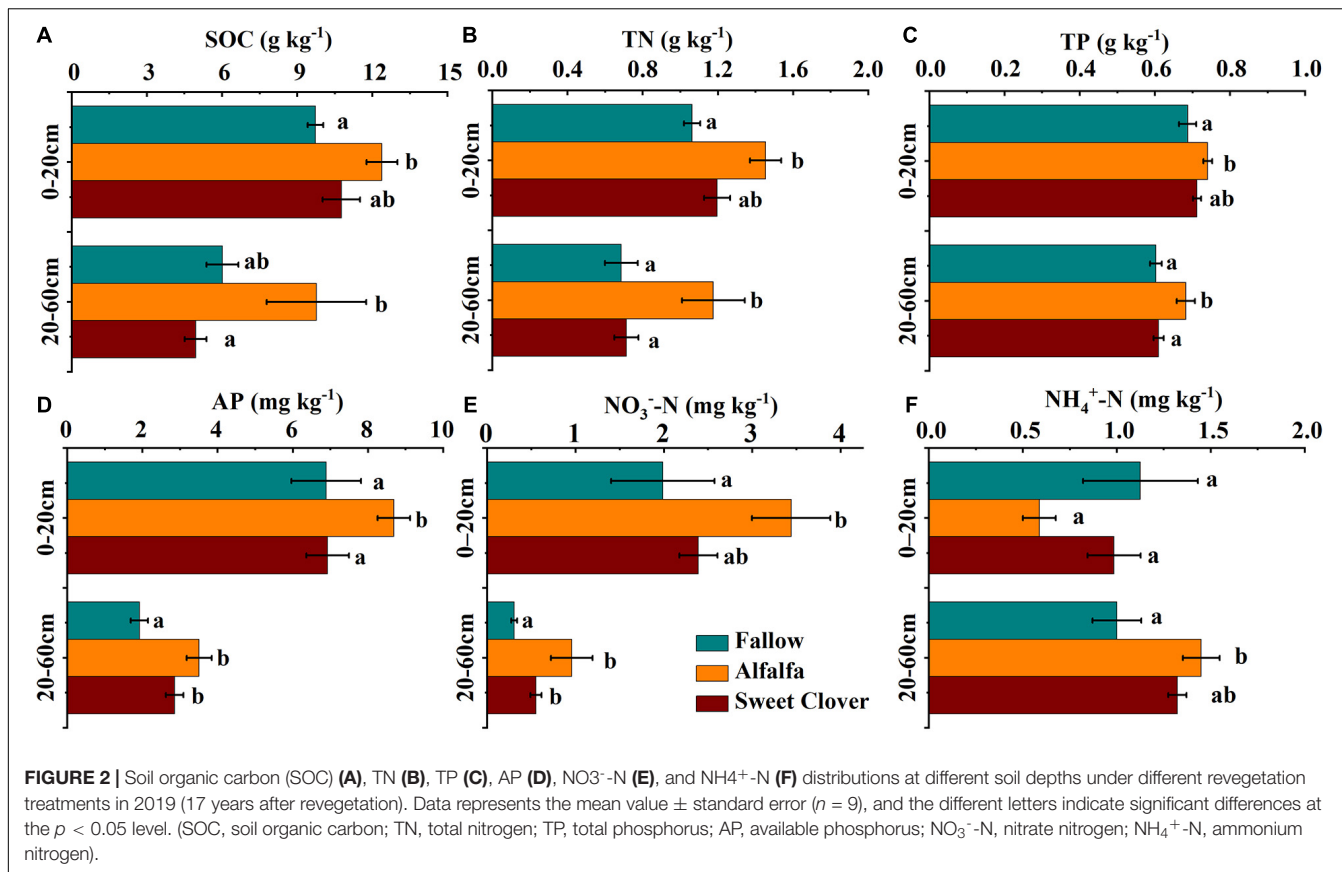
To address the pressure caused by the expanding human population since the 1950s, cropland became the main land use in this region, even in sloping areas. After livelihood conditions improved, croplands with a slope greater than 15° were converted into grasslands as part of the "Grain for Green" project, which was beneficial to both the environment and animal husbandry.

Experimental Settings and Design

In April 2003, three hillside fields were selected for revegetation, following the cultivation of spring wheat. The three fields had different landscape aspects, one was north–east facing, with a slope of 10–14°, and the other two were south-east facing with slopes of 12–16° and 4–8°, respectively. The distance between each site was less than 1,000 m and they all had the same elevation. We divided each field into three plots. The size of each plot was 35 m × 45 m, and they were located next to each other. The three plots were treated randomly with: (i) fallow conditions, (ii) alfalfa (*Medicago sativa* L.) (perennial legume species) introduced at a seed density of 22.5 kg ha^{-1}, and iii) sweet clover (*Mentha suaveolens* L.) (biennial legume species) introduced at a seed density of 11.3 kg ha^{-1}. These densities were optimal for planting based on local farming practices. The plant community succeeded naturally with no further management practices, such as grazing (fencing), tillage, fertilization, or harvesting after legume introduction (Yuan et al., 2016a). A plant community survey was conducted in August every year, taking care to avoid disturbance (Yuan et al., 2015).

Root and Soil Sampling

After 17 years of vegetation restoration, root biomass was measured in the most vigorous vegetation-growing period in August 2019. Root biomass was measured using a root drill

FIGURE 2 | Soil organic carbon (SOC) **(A)**, TN **(B)**, TP **(C)**, AP **(D)**, NO3⁻-N **(E)**, and NH4⁺-N **(F)** distributions at different soil depths under different revegetation treatments in 2019 (17 years after revegetation). Data represents the mean value ± standard error ($n = 9$), and the different letters indicate significant differences at the $p < 0.05$ level. (SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; AP, available phosphorus; NO3⁻-N, nitrate nitrogen; NH4⁺-N, ammonium nitrogen).

FIGURE 3 | MBC **(A)**, and MBN **(B)** distributions at different soil depths under different revegetation methods in 2019 (17 years after revegetation). Data represents the mean value ± standard error ($n = 9$), and the different letters indicate significant differences at the $p < 0.05$ level. (MBC, microbial biomass carbon; MBN, microbial biomass nitrogen).

(70 mm inner diameter; 100 mm height, Kezheng Instrument Co. Ltd., Shangyu, Shaoxing). The sample points were 3 m from the margin so avoiding "*Edge effects.*" Three random samplings were conducted at the upper, middle, and lower sites in each sample plot, with sampling increments at soil depths of 0–20 and 20–60 cm. We got 3 soil samples and mixed them as a sample each site. So, 54 root samples were obtained (3 replication, 3

treatments, 3 landscapes, and 2 soil layers). A root mesh bag, with a 0.15 mm mesh size, was used to wash the soil attached to the roots. Then, the roots were dried to a constant mass at 75°C and root biomass was measured.

In April 2019, i.e., the initiation of the growing season, soil samples were collected in the 0–20 and 20–60 cm soil layers using a soil auger, with an inner diameter of 4 cm. The sampling
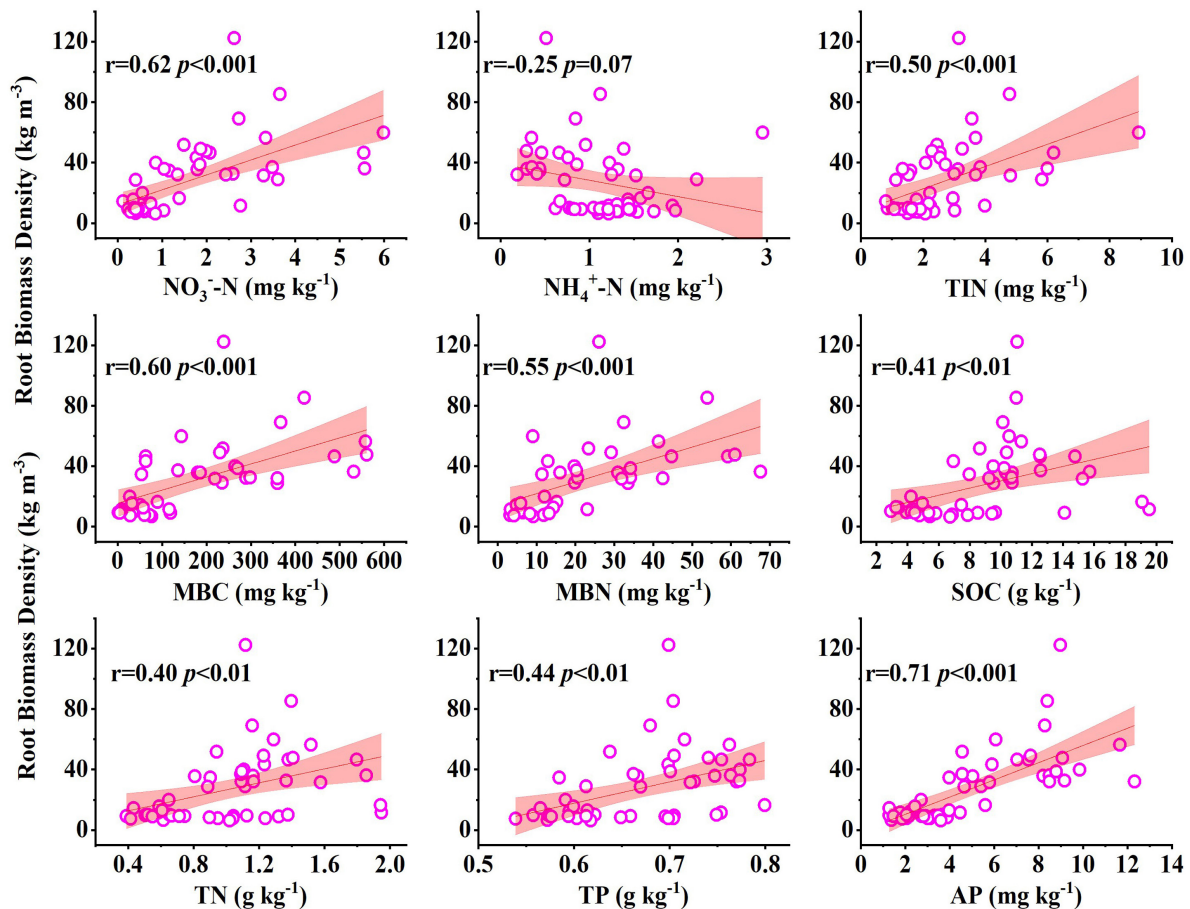
FIGURE 4 | Relationships of root biomass density with soil properties (data were collected in 2019, 17 years after revegetation). NO_3^--N, nitrate nitrogen; NH_4^+-N, ammonium nitrogen; TIN, total inorganic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; AP, available phosphorus.

principle is consistent with the root sample. Three soil cores were collected and mixed together in each plot. All the visible litter and roots were removed by hand, soil samples were sieved through a 2-mm aperture screen (plant residue on the sieve was discarded). Each soil sample was then divided into two parts. One was stored at 4°C to determine soil moisture, MBC and MBN, and inorganic N at the field moisture level. The other was air dried for the determination of SOC, TN, and P.
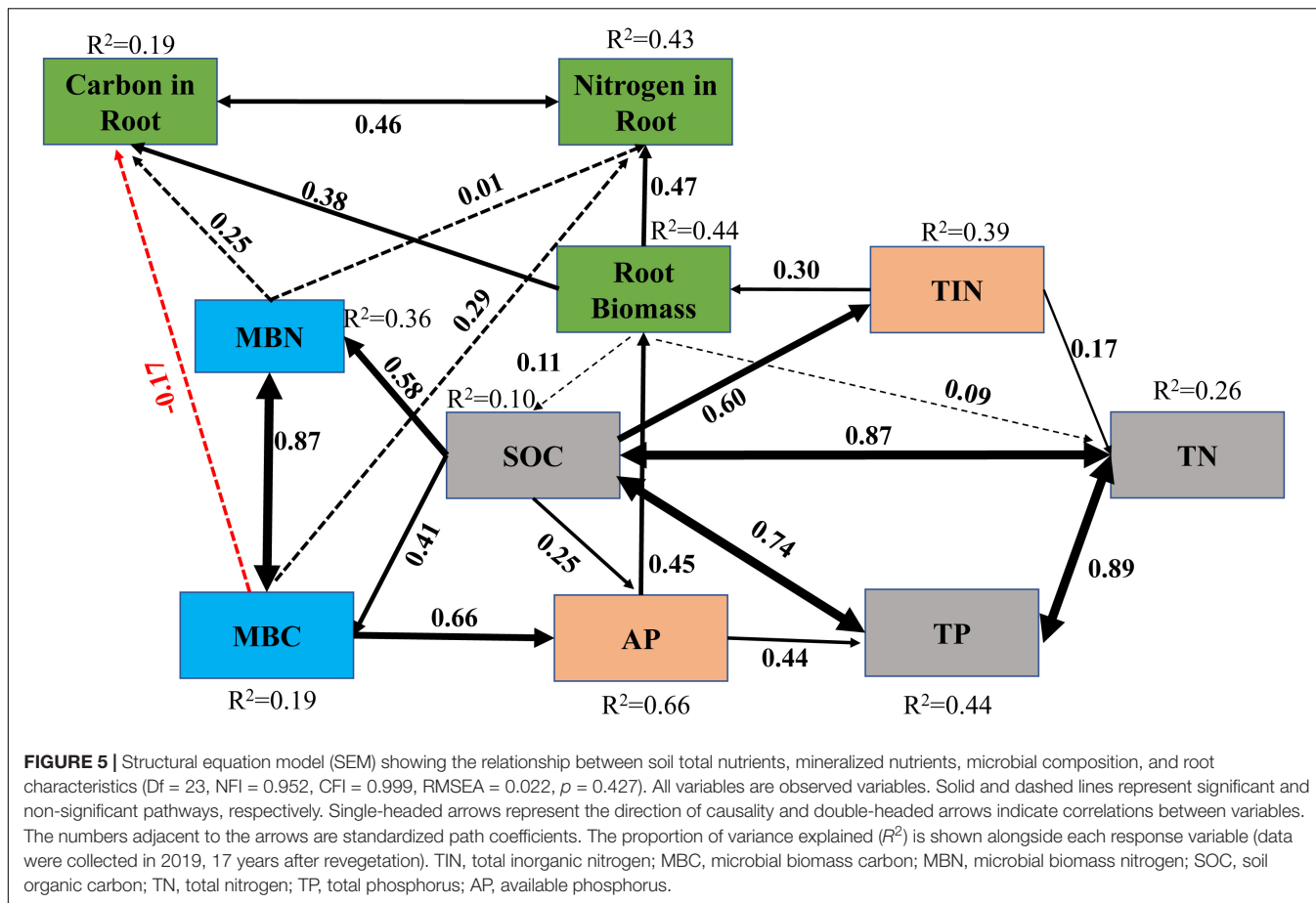
The Determination of Root and Soil Properties

The dried plant samples were crushed by an ultra-centrifugal mill to enable the determination of C, N, and P in the root system. The root C was determined by the potassium dichromate oxidation method, and root N and P were measured after digestion in a $H_2SO_4-H_2O_2$ mixture using the Auto-Kjeldahl and the molybdenum-antimony spectrophotometry methods (Thomas et al., 1967).

Soil samples were soaked in a 2 M KCl solution, shaken in a concentrator at 200 rpm for 1 h and then filtered into centrifugal tubes. An auto-flow injection system (Skalar, Breda, Netherlands) was then used to measure nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N). The fumigation—extraction method was used to determine MBC and MBN. The soil samples were extracted by 0.5 M K_2SO_4, shaking 1 h at 200 rpm. And then, MBC and MBN were directly measured using a CHN Analyzer (LECO CHN–1000, Michigan, United States). The difference between total organic C and TN extracted with 0.5 M K_2SO_4 was determined through chloroform-fumigated and non-fumigated soil samples. Factors of 0.45 and 0.54 were applied to adjust the recovery of MBC and MBN (Brookes et al., 1985; Beck et al., 1997). The values were calculated based on air-dry soil.

Air-dry samples (<0.15 mm) were used for the measurement of SOC by the Walkley and Black dichromate oxidation method (Nelson and Sommers, 1982), TN by the $K_2SO_4-CuSO_4-Se$ (100:10:1) distillation method (Bremner and Mulvaney, 1982), and TP by a colorimetric method at 700 nm (UV-1800, Mapada, Shanghai, China) after soil digestion in an $HClO_4-H_2SO_4$ mixture (O'Halloran and Cade-Menun, 2006). Available phosphorus (<2.00 mm) was extracted with $NaHCO_3$ according to the Olsen method (Olsen, 1954).

FIGURE 5 | Structural equation model (SEM) showing the relationship between soil total nutrients, mineralized nutrients, microbial composition, and root characteristics (Df = 23, NFI = 0.952, CFI = 0.999, RMSEA = 0.022, p = 0.427). All variables are observed variables. Solid and dashed lines represent significant and non-significant pathways, respectively. Single-headed arrows represent the direction of causality and double-headed arrows indicate correlations between variables. The numbers adjacent to the arrows are standardized path coefficients. The proportion of variance explained (R^2) is shown alongside each response variable (data were collected in 2019, 17 years after revegetation). TIN, total inorganic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; AP, available phosphorus.

Statistical Analyses

Before analysis, the data were analyzed for normal distribution by the Shapiro–Wilk test. If not normally distributed, the data were transformed by log10 before analysis. A one-way analysis of variance (ANOVA, in randomized blocks) was applied to test the differences in root biomass density, root C, N, and P, MBC and MBN, SOC, TN, and TP among the fallow, alfalfa, and sweet clover treatments using the Genstat18.0 software (VSN International, Hemel Hempstead, United Kingdom). The landscape was set as a block. Significant differences were compared by Tukey test at $P < 0.05$. A Pearson's correlation analysis was used to evaluate the correlations among root biomass density and soil nutrients. Principal Component Analysis (PCA) was conducted using CANOCO 4.5 (Plant Research International, Wageningen, Netherlands). Structural equation modeling (SEM) was performed using AMOS 21.0 (Amos Development Corporation, Chicago, IL, United States) to quantify the relative importance of the potential direct and indirect pathways, which could affect root biomass based on the Pearson's correlation analysis, conceptual modeling (**Supplementary Figure A**), the goodness of model fit, and logical reasoning. The Origin 9.0 was used to drawn all graphs (OriginLab OriginPro 2015, United States).

RESULTS

The C, N, and P Concentrations in the Root Biomass

Both the root biomass density and its C, N, and P concentration varied throughout the soil profile among the revegetation methods (**Figures 1A–D**). The concentrations of all three elements decreased with soil depth. Seventeen years after legume introduction, the root biomass density in the alfalfa field was significantly higher than in the fallow and sweet clover fields (**Figure 1A**). In the 0–20 cm layer, root C and N in the alfalfa field were significantly higher than in the fallow and sweet clover fields (**Figures 1B,C**). The root P concentration was lowest in the alfalfa field, but the differences in the 0–20 cm layer were not significant (**Figure 1D**). In the 20–60 cm layer, the root N concentration in the alfalfa field showed no differences from the fallow field, but were much higher than in the sweet clover field (**Figure 1B**). The root C and P concentrations in the 20–60 cm layer had intermediate values and displayed the same tendency as N (**Figures 1A,C**), but the root C concentration in the alfalfa and sweet clover fields was much lower than in the fallow field. There were no differences in the root P concentration in the 0–20 cm layer
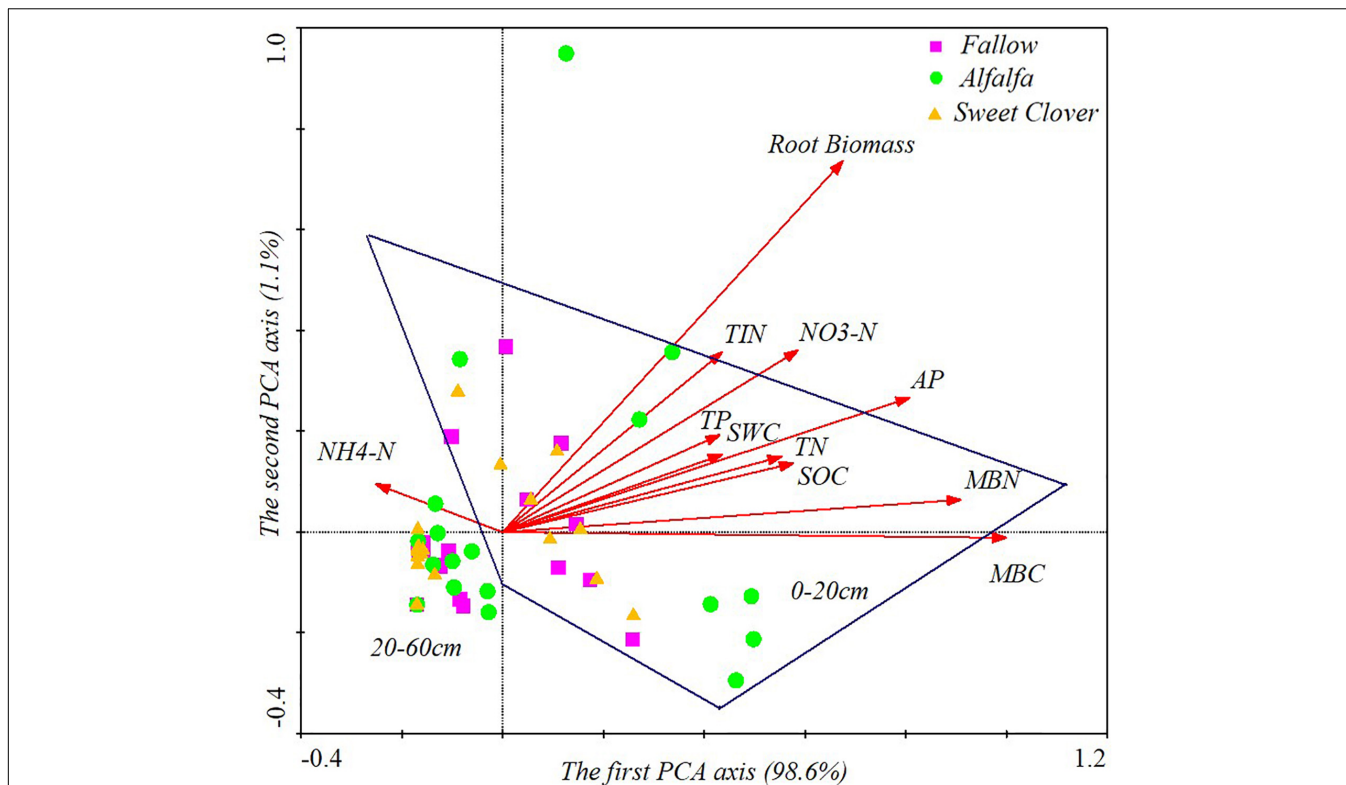
FIGURE 6 | Principal component analysis (PCA) results, showing the relationships for root biomass with soil properties in different soil layers (data were collected in 2019, 17 years after revegetation). NO_3^--N, nitrate nitrogen; NH_4^+-N, ammonium nitrogen; TIN, total inorganic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; AP, available phosphorus.

in the alfalfa field compared with the fallow and sweet clover fields (**Figure 1D**).

The SOC, TN, TP, Nitrate-Nitrogen (NO_3^--N), Ammonia-Nitrogen (NH_4^+-N), and AP Concentrations in Soils

The SOC, TN, and TP concentrations generally declined with soil depth. The highest SOC concentration in the 0–20 and 20–60 cm layers were observed in the alfalfa field, with average values of 12.38 and 9.77 g kg^{-1}, respectively, followed by fallow (9.73 and 6.01 g kg^{-1}, respectively) and sweet clover (10.76 and 4.94 g kg^{-1}, respectively) (**Figure 2A**). The tendency of TN was similar to that of SOC (**Figure 2B**). There was little change in TP compared with SOC and TN, but the concentration in the alfalfa field was significantly higher than in the other two treatments (**Figure 2C**).

The changes in the NO_3^--N and NH_4^+-N concentrations in the 0–20 and 20–60 cm layers are presented in **Figures 2E,F**. The lowest NH_4^+-N concentration was observed in the 0–20 cm layer in the alfalfa field and increased with soil depth. Total inorganic nitrogen (TIN: NO_3^--N + NH_4^+-N) in the alfalfa field was still significantly high. Compared with the fallow field, the AP concentration in the 0–20 and 20–60 cm layers also increased after legume introduction (**Figure 2D**). Alfalfa

introduction resulted in an increase in the AP concentration in both soil layers, while sweet clover introduction had little effect.

Soil Microbial Properties

The highest MBC and MBN values were observed in the 0–20 cm layer in the alfalfa field (402.72 and 48.32 mg kg^{-1}, respectively). There was no significant difference in MBC or MBN between the fallow and sweet clover fields. Both MBC and MBN decreased with soil depth in all three treatments (**Figures 3A,B**).

The Relationship Between Root Biomass and Soil Properties

There were significant positive correlations between soil nutrients and root biomass density ($p < 0.05$), while there was a negative correlation between soil NH_4^+-N and root biomass density ($r = -0.25$, $p = 0.07$) (**Figure 4**). The SEM showed that the organic matter of the soil substrates could affect the available nutrients (AP and TIN) and further affect TN and TP by affecting the MBC and MBN. On the other hand, the AP and TIN directly affected the belowground biomass. Together, these indicators explained 44% of the belowground variance in root biomass. In addition, MBN had a direct effect on the root N concentration. MBC indirectly affected the belowground root biomass by affecting AP, further affecting root C and N concentrations (**Figure 5**).

DISCUSSION

Our first hypothesis that legume introduction would increase the root biomass of communities and the soil MBC and MBN concentrations, leading to an increase in the TIN and AP concentrations was supported by the results under alfalfa fields. After 17 years, alfalfa introduction significantly increased root biomass, which was in line with previous reports of aboveground biomass (Yuan et al., 2016a). The root system plays an essential role in changing SOC and N storage following land use change (Fornara and Tilman, 2008; Cong et al., 2015). Alfalfa is a perennial leguminous plant, with a large root biomass density (**Figure 1A**) (Honde et al., 2020), increasing the C input to soil. Soil organic matter is mineralized by microorganisms to form mineral nutrients (Pearce et al., 2015). In semi-arid areas, organic matter is beneficial to water retention and soil permeability, which could provide more suitable environmental conditions for microbial activities. Available phosphorus and TIN were the main factors affecting root biomass (**Figure 5**). In addition, there was competition between microorganisms and plant roots for the acquisition of soil nutrients (Hodge et al., 2000; Winkler et al., 2010), which could affect the root C and N concentrations. Microorganisms could decompose plant roots (Kusliene et al., 2014; Lupascu et al., 2014), releasing nutrients into the soil (de Kroon et al., 2012). An increase in the supply of organic substrates may alter the competitive relationship between different microbial functional groups (Zak et al., 2003; Waldrop et al., 2006). In this experiment, the highest MBC and MBN was observed in the alfalfa field (**Figures 3A,B**), indicating that the microorganisms in the alfalfa field were more active. There was a significant positive correlation between the microbial index (MBC and MBN) and soil properties (SOC and TN) (**Figure 6**), implying that microbial biomass is one of the sources of soil nutrient elements. Therefore, microbial activity is the driving factor that mediates the plant-soil relationship in this region and regulates the soil biogeochemical cycling process. It plays an important role in the mineralization of soil C and N.

The second hypothesis that legume introduction would promote soil C and N accumulation was supported in alfalfa fields, and legumes introduction have no effect on soil TP was supported by the results only in sweet clover fields. Consistent with our hypothesis, the introduction of legumes (alfalfa) increased the SOC and TN concentrations, which was in agreement with previous research results (Post and Kwon, 2000; Guo and Gifford, 2002; Wang et al., 2015; Han et al., 2018). In our study, the highest SOC concentration was observed in both the 0–20 and 20–60 cm layers after alfalfa introduction, followed by sweet clover and fallow (**Figure 2A**). Revegetation after farmland abandonment can rapidly improve vegetation coverage (Zheng et al., 2019), which is beneficial to soil erosion control on sloping cropland. Vegetation restoration can also promote the input of litter and root exudates (Zhang et al., 2011), which are conducive to an increase in soil C storage. A "*priming effect*" on the respiration of microorganisms has been observed with an increase in root biomass; thus, promoting the decomposition of the old SOC (Kuzyakov et al., 2000; Fontaine et al., 2003; Kuzyakov, 2010). In this experiment, the "*priming*

effect" was not taken into consideration, and it should therefore be further studied. The increase in root biomass could also lead to a higher root respiration rate (Luo et al., 1996; Pregitzer et al., 2008), inducing a decline in soil C storage. The combined effect of these changes in the belowground C cycle eventually led to more C storage in soils following alfalfa introduction (**Figure 2A**), which implies that the loss of SOC caused by the "*priming effect*" and respiration may be compensated for by the C input. Changes in SOC may therefore reflect the balance of accumulation and degradation rates (Bevivino et al., 2014).

The highest TN was also observed in both the 0–20 and 20–60 cm layers in the alfalfa field, followed by the sweet clover and fallow fields. Both alfalfa and sweet clover are legume species, capable of N-fixation (Sinclair and Serraj, 1995; Carlsson and Huss-Danell, 2003); therefore, improving N storage. However, sweet clover is a biennial plant and would therefore disappear from the plant communities after its 2-year lifespan (Li et al., 1996). Compared with the perennial alfalfa, the N-fixation effect of sweet clover may be lower in the long-term. This explains the lower TN concentration that was observed in the sweet clover fields (**Figure 2B**). The increase in the amount of N-fixing microorganisms could lead to a high TN concentration in alfalfa fields, alleviating the N limitation of the ecosystem (Wei et al., 2019). Alfalfa roots had a high N concentration (**Figure 1C**), and when they decompose it would be rapidly released (McLaren and Turkington, 2010; Makkonen et al., 2012). The belowground environment in alfalfa fields could provide opportunities for decomposers to obtain water, organic matter, and mineralized N, which would increase the release of NO_3^--N and NH_4^+-N by decomposition of the root system (Silver and Miya, 2001; Mulder et al., 2002; Mueller et al., 2013). The alfalfa field had a higher soil NO_3^--N concentration in the 0–20 and 20–60 cm layers than the other two treatments (**Figure 2E**), but there was a lower NH_4^+-N concentration in the 0–20 cm layer (**Figure 2F**). On the one hand, the low soil moisture content and high porosity in the semi-arid area of the study site provided a strong aerobic environment for the conversion of NH_4^+-N to NO_3^--N through nitrification (Wei et al., 2019). On the other hand, the roots in the 0–20 cm layer had a high mineralized N utilization rate, which was mainly NH_4^+-N (Geisseler et al., 2010). Therefore, legume introduction would significantly increase soil NO_3^--N, but not soil NH_4^+-N. The large root biomass in the alfalfa field would be beneficial to soil C input and N fixation, reducing the net N mineralization rate, but the available N released by fine roots could reduce the inhibitory effect on the N cycle induced by the increased root biomass (Fornara et al., 2009). Furthermore, the fine root turnover of leguminous plants is higher than that of C4 grasses (Fornara and Tilman, 2008). Therefore, alfalfa may have the potential to improve the N cycle.

The TP concentration increased in the 0–20 and 20–60 cm layers in the alfalfa field (**Figure 2C**). This result does not support our second hypothesis. This may be associated with P utilization in deep soil layers (He and Li, 2016; Zhou et al., 2018). Alfalfa has a strong and deep root system, which can utilize P even more than 5 m belowground. In addition, the root could be considered a "*nutrient pump*" (Farley and Kelly, 2004; Jobbagy and Jackson, 2004), promoting the transfer of P in the deep

soil layer to the surface soil layer through residue inputs in the alfalfa field. Soil microorganisms and plant roots can therefore effect P transformation and then influence the P availability (Richardson, 2001). Microorganisms not only store immobilized P but can also provide inorganic or organic forms of P for plant roots by dissolving and mineralizing soil TP (Richardson, 2001). Phosphorus in humic-metal complexes could be mobilized by organic anions, inducing an increase in soil P availability (Gerke, 1993). Microorganisms play an important role in rapidly metabolizing various organic anions or releasing them through their own traits (Jones, 1998). Roots can release acid substances and decrease the soil pH (Phillips et al., 2004; Gu et al., 2018). Microorganisms, such as fungi, could enhance the area of plant roots, which is beneficial to the release of acid substances and uptake of mobilized P (Solaiman and Abbott, 2003). In alfalfa grassland, the soil calcium carbonate concentration is low (Gu et al., 2018), therefore the release of acidic substances by plants and microorganisms would lead to low pH which is conducive to the activation of TP. Thus, the increased AP concentration after alfalfa introduction might be due to rhizosphere acidification.

CONCLUSION

The long-term (17-years) effects of legume introduction on the root biomass and soil C, N, and P cycles were investigated. After 17 years, the soil C, N, and P concentrations were enriched in the 0–20 and 20–60 cm layers of the alfalfa field compared with sweet clover and fallow fields. This was induced by the increase in root biomass and soil microbial biomass. Alfalfa introduction promoted the accumulation of soil organic matter and soil organic matter mineralization. Therefore, Alfalfa introduction is a low-cost and rapid revegetation method to enhance the soil C, N, and P pool, enabling sustainable land use in the semi-arid areas of the Loess Plateau.

DATA AVAILABILITY STATEMENT

The original contributions generated for this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

XS, CF, and F-ML designed the experiments. XS performed the experiments. XS, CF, Z-QY, and F-ML jointly analyzed data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2021.649346/full#supplementary-material

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