

The Impact of Perennial Covers on Soil Microbiome and Nutrient Dynamic

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ABSTRACT

Our study discusses the impact of different cropping practices on soil health indicator and crop yield. The combination of no fertilization and perennialization enhanced soil health indicators and crop yield compared to conventional tillage annual crop fallow. The results showed that excessive nitrogen fertilization increases soil bulk density, while perennialization with no fertilizer added improves it. Higher carbon content was recorded under corn cultivation, but higher carbon-to-nitrogen ratio was shown in perennial covers. The study highlights the benefits of adopting perennial crops without fertilization for soil health and suggests improved management practices for sustainable agriculture. Our finding is particularly significant as it suggests an alternative approach to farming that improve soil health while reducing the reliance on synthetic fertilizer. It provides valuable insights for farmers, policymakers, and researches enabling them to make informed decisions and implement practices that support both productivity and environmental stewardship. Overall, it underscores the importance of responsible fertilizer use and improved management practices for maintaining soil fertility and promoting sustainable agriculture system.

KEYWORD: Perennial, Sustainable agriculture, synthetic fertilizer, agriculture management

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INTRODUCTION

Competing global demands for food, feed and energy have stimulated research on second-generation biofuel systems that promote use of perennial grasses. Perennial grasses play a significant role in enhancing soil carbon levels and promoting carbon sequestration. (Uchida., 2000). As perennial grasses continue to grow year after year without the need for reseeding, they provide continuous root biomass that adds soil organic carbon (SOC) to the soil. (Islam et al., 2015). SOC content increased by 9.7% with the conversion of cropland to perennials but did not change when fallow and grasslands were converted to annual biofuels. (Uchida., 2000). *Panicum virgatum* (switchgrass) and *Miscanthus x giganteus*, have received considerable attention as potential second-generation biofuel crops in Europe and North America because they have high biomass yield potential, and may sequester soil organic carbon (SOC) compared to annual row crops (Blanco-Canqui, 2010). Perennials have deep and extensive root system that penetrate the soil, enabling the, to access deeper carbon pools and effectively sequester carbon below the soil surface (Erisman et al., 2008). The nature of these grasses allows them to continue input of organic matter

into the soil and as a result impact on plants photosynthesis to capture more carbon dioxide and convert it into organic compounds (Skorupka et al., 2021).

When perennial grasses are deployed on the landscape as biofuel crops, it is generally assumed that SOC sequestration will increase and greenhouse gas emissions will decline. However, the direction and magnitude of these changes will likely be altered by an array of soil and climate factors, and by current and previous management choices. The conversion of cropland to biofuel cropping has been proposed to potentially sequester SOC and mitigate greenhouse gas emissions (Anderson-Teixeira *et al.*, 2009). In this situation, scientist can choose between changing the management practices from annual crops to perennial biofuels or continue using fertilizer on annual crops. However, several studies have reported that the application of fertilizer and nano-fertilizer can have both positive and negative effects on soil health and its quality (Amanullah et al., 2016; Wang et al., 2020; Liu et al., 2021; Stewart 2022; Mirbakhsh 2023).

The use of fertilizer has been linked to changes in the physical, chemical, and biological properties of the soil. Nitrogen fertilizers, for example, can contribute to soil

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acidification and decrease in soil organic matter content, which can lead to reduced soil fertility and productivity. Excessive use of phosphorus fertilizers can lead to soil eutrophication, which results in the overgrowth of algae and aquatic plants that depletes oxygen levels in water bodies. It can also lead to soil pollution through the accumulation of heavy metals (Chandini et al., 2019). On the other hand, appropriate use of fertilizers can help to maintain soil fertility and promote healthy plant growth. For instance, the application of potassium-rich fertilizers can improve soil structure, reduce soil erosion and increase crop stress tolerance. It can also lead to soil pollution through the accumulation of heavy metals (L et al., 2016). Inappropriate application of fertilizer can affect soil health indicators, microorganisms, diversity that play an essential part in soil fertility and nutrient cycling (Dinca et al., 2022). We should consider that environmental stressors and different cropping practices have a great impact on soil health, carbon accumulation, and greenhouse gases (GHGs) (Mirbakhsh et al., 2023).

Management practices can affect soil properties (Ashworth et al., 2018) and used as an alternative to decrease human needs to fertilizer. The sustainability of soil resources can be maintained by promoting soil health through improved management practices (Norris et al., 2020). The choice of suitable cropping practices for increasing soil organic matter content should focus on several factors such as GHG emissions mitigation, nitrogen capture improvement, and temporal yield stability, and reducing environmental contamination (Knapp and van der Heijden, 2018). For example; well-managed arable land conversion to cover crops has been resulted in accumulation of SOC, due to their low nutrient requirements and subsequent turnover of aboveground biomass (Ledo et al., 2020; Chen et al., 2022) or no-tillage system reduces soil acidification, but increased organic matter and Ca, Mg, and K concentrations compared to conventional tillage (Tarkalson et al., 2006).

This study aimed to compare two main cropping practices that are annual system includes corn cultivation with tillage and fertilization via perennial crops that contains *Panicum virgatum* (switchgrass) and *Miscanthus x giganteus* with no tillage and zero fertilization. We hypothesized that no fertilization coupled with perennialization will enhance soil health indicators and crop yield compared to conventional tillage annual crop-fallow.

MATERIAL AND METHODS

Field experiment

The experiment was conducted in Turkmen Sahara (37° 13' 0"N 55° 0' 0" E) in northeast, Abarkouh (31° 7' 44.04" N 53° 16' 56.64" E) in center and Shush district (32° 11' 39.12" N 48° 14' 36.96" E) Southwest of Iran during 2020-2023 crop year to test our alternative hypothesis. Plots are 10 m wide and 48.5 m long and Each plot is individually drained with plastic agricultural tile lines (0.1 m diameter) installed in the

longitudinal center of the plots at a depth of 0.9 m. Soil samples with 0.9 m depth were collected in May-April 2023 before tillage, planting, and fertilization. Measurements were made for intact and repacked soil cores. The experimental design includes 6 treatments in completely randomized block design that have been applied since 2005. The subset of three treatment chose for corn cultivation that contains corn (CC) with tilling and fertilization. Three subsets of corn (*Zea mays* L.) were chosen (Figure 1a). Within our two experimental years, plots were burned on 8 Apr. 2022 and 13 Apr. 2023. The N sources for corn treatments were urea-ammonium nitrate 28% (w/w) N (UAN) side-dressed at corn growth stage V5 at rates of 157 and 135 kg N ha⁻¹ yr⁻¹ for CC and CS, respectively, and liquid swine manure (C/N ratio: 2:1, 80% [w/w] of N as NH₄⁺) injected into CC at a rate of 255 ± 24 kg N ha⁻¹ yr⁻¹ in either the spring (SM) or the fall (FM). For perennialization *Panicum virgatum* (switchgrass) (Figure 1 b), and *Miscanthus x giganteus* were added in different plots (Figure 1 c). Tillage operations were chisel in the fall and chisel plus disk in the spring. Soil samplings occurred within the week of corn planting in early May (11 May 2019 and 3 May 2020) and again at corn growth stage R1 in late July (25 July 2019 or 27 July 2020). Soils were tested each fall for general fertility using recommended protocols, and results indicated soil P, K, and pH were non-limiting. Mean temperature, relative humidity, and precipitation of study site are presented in Table 1.

Soil sampling and analysis

Four soil collections were done to monitor fractions of soil organic carbon (SOC) and total N (TN). Soil samplings occurred during the week of corn planting in May (11 May 2022 and 3 May 2023) and again at corn growth stage R1 in late July (25 July 2022 or 27 July 2023). The top 0.15 m of soil was sampled by auger hand probe (2.5-cm diam.) with at least 12 cores collected at random positions throughout each experimental plot. Each core was separated into 0- to 5- and 5- to 15-cm depth increments, composited within each depth and sieved in field-moist condition to pass an 8-mm mesh within 24 h after collection. Sieved soils were thoroughly mixed, air dried, and stored at room temperature for physical fractionation. Air-dried soil subsamples were finely ground using a Dyno-Crush 2 Grinder (Customs Laboratory Equipments, Inc., Orange City, FL), passed through a 2-mm sieve and stored at room temperature in 20-mL polyethylene vials for chemical fractionation as well as determination of SOC and TN. Any identifiable plant material in this subsample was removed before grinding. Bulk density was determined in separate, undisturbed soil cores (5 cm i.d. and 2.5 cm length) collected with a double-Cylinder, hammer-driven core sampler from three sampling positions at 0- to 5- and 5- to 15-cm depth increments from each experimental plot. For the deeper soil depth increments (15–30, 30–50, 50–75, and 75–100 cm) bulk density was determined from subsamples of the cores collected by the tractor probe. Total soil profile (0–100 cm) SOC and TN storage were estimated

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as the sum of storages in each layer following correction with bulk density values for equivalent layer mass.

Data Processing

The fractions of SOC and TN (g kg^{-1} soil) were recorded in each individual depth increment. Following Ellert et al. (2002), the equivalent soil mass correction was performed before calculation of cumulative TOC and TN mass storage (Mg ha^{-1}) in the complete soil profile (0–90 cm) to adjust the layer and get correction. Finally, mass (M) of SOC and TN was calculated in each individual soil depth (0–5, 5–15, 15–30, 30–60, 60–90) cm as follows:

$$M = \rho_b \times C_o \times (T_o + T_{adj}) \times 10\,000 \text{ m}^2 \text{ ha}^{-1} \times 0.001 \text{ kg g}^{-1}$$

[Batjes., 1996]

Where ρ_b is bulk density, C_o is SOC or TN concentration, and T_o is the thickness of a layer.

All variables were assessed for homogeneity of variance and normality by Cook's distance, Bartlett, and Shapiro–Wilk tests, and Tukey. Multivariate analysis of variance (MANOVA in PROC GLM) was run to test for no overall treatment, year, sampling time, and depth effects by Wilk's Lambda test, these main, fixed effects and their interactions, the full model also included block and block by treatment (error) as random effects using R version 4.0 (R core Team 2020).

RESULTS AND DISCUSSION

Bulk density

Soil bulk density and nitrogen fertilization are both important factors in agriculture and soil management also nitrogen application indirectly affect soil bulk density. Soil acidification due to ammonium-based fertilizer contribute to PH alternation by producing nitrate form by soil microorganism, which potentially leading to increased soil bulk density (Zeng et al., 2016; Sun et al., 2015). Moreover, excessive reliance on fertilization in annual crops such as corn without organic matter management can lead to a decline in organic matter content, which can result in soil compaction, porosity reduction and bulk density increment over time (Nawaz et al., 2013). Our results of comparing variance of two different cropping practices recorded great and significant difference (P -value = 0.003) between annual and perennials (Table 2).

Our results recorded the negative impact of corn cultivation on soil bulk density that increased through soil profile and it is clearly shown in shallowest profiles with Tukey test (table 3). The interaction of soil bulk density and soil depth is shown in figure 2a. No fertilization decreased and improved soil bulk density in the most top soil layers (0–15 cm) (Figure 2b). Our results are aligned with the hypothesis of the negative impact of excessive application of nitrogen fertilizer coupled with corn cultivation and soil health (Wang et al., 2018; Zhang et al., 2015; Snyder et al., 2009). Excessive fertilization can lead to vigorous plant growth and increase biomass production, which make excessive plant residues and

organic matter accumulation that increase bulk density and negatively impact soil structures (Niu et al., 2022).

Soil Carbon

The impact of perennialization is like a two-edge sword and can vary depending on various factors such as environmental factors, soil condition, temperature, precipitation, and type of treatments. On the other side, nitrogen fertilizer can enhance corn growth and productivity leading to higher residues, which can increase soil carbon content over time (Wang et al., 2014). Organic carbon decomposition can stimulate microbial activity resulting in more efficient decomposition of organic matter and CO_2 releasing into the soil. Moreover, nitrogen fertilizers can promote root development and proliferation leading to increase root biomass that positively influence soil carbon level. On the other side, excessive nitrogen fertilization can accelerate decomposition which can lead to microbial activity and rapid breakdown of organic matter over long time (Hussain et al., 2016). Nitrous oxide emission under improper condition can indirectly impact on soil carbon by reducing ecosystem carbon sequestration potential (Foley et al., 2011). Our results showed greater amount of soil carbon in annual crops under fertilization during two years of experiment (Figure 3a). However, carbon content decreased through soil profiles and the lower amount of carbon was recorded in (30–60 cm) (Figure 3b). The results of interaction plot of carbon content, soil depths, and different treatments recorded the greatest difference between the treatments in the shallowest layer (0–30 cm) (Figure 3b).

C: N

The impact of nitrogen fertilization on soil carbon to nitrogen (C: N) ratio can vary depending on several factors. It is important to consider that the impact on C: N is influenced by various factors and specific outcomes may depend on soil type, climate, crop management practices, and initial soil condition. Increased nitrogen content from nitrogen fertilizer can decrease the C: N ratio. Moreover, adequate nitrogen supply stimulates microbial activity, accelerating organic matter decomposition and potentially reducing the C: N.

On the other side, to mention to the negative impact; excessive nitrogen fertilization without nutrient management can lead to imbalanced nutrient ratio, limiting microbial activity and potential increase C: N. In some case, nitrogen fertilization can promote the preservation of organic matter resulting in higher C: N.

Our results recorded more variation of the results in no-fertilization, and higher carbon to nitrogen ratio was recorded under perennialization (figure 4a). Obviously, under nitrogen perennialization, the carbon to nitrogen ratio typically increases, which means the higher proportion of carbon relative to nitrogen is available in the soil or plant matter. Nitrogen uptake by the plants accelerate in comparison to carbon assimilation and as a result the carbon content in plant tissues become relatively higher compared to nitrogen content. Nitrogen mineralization is the other reason that can

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stimulate microbial activity in the soil, which leading to increased decomposition of organic material resulting in decrease in carbon content relative to nitrogen due to carbon dioxide emission. The other possible reason could be nitrogen immobilization that is caused by microbes which can rapidly consume and immobilize the added nitrogen because microbes require carbon for their growth and energy needs and they may scavenge carbon from soil organic matter. The lower C: N ration was recorded in the shallowest soil profiles (0-60 cm), which increased through the profile depth and reached the highest ration near to 20:1 in 60-90 cm (figure 4b).

CONCLUSION

The impact of fertilizers on soil health and the environment is dependent on its concentration, type, and mode of application. Excessive use of fertilizers can lead to soil degradation, loss of biodiversity, and environmental pollution (Bisht and Chauhan, 2020). Sustainable management practices like soil testing, integrated nutrient management, conservation tillage, and crop rotation can help to mitigate the adverse effects of fertilizers on soil health (Farmaha et al., 2021). To mitigate the potential negative impacts of fertilization and maximize the positive effects of management practices on soil health, it is important to follow the best and less expensive management strategies. It is essential to adopt sustainable nutrient management methods to promote healthy soil, ensure environmental sustainability, and enhance food security. Cover cropping is one of the management practices, which can improve soil health through erosion, nutrient cycling, weed suppression (Balota et al., 2014). It increases soil carbon content, promote bulk density, and enrich soil with nutrient and could be considered as an alternative for nitrogen fertilizer. In conclusion, our study underscores the importance of sustainable soil management practices in agriculture. Excessive nitrogen fertilization and lack of organic matter management negatively impact soil bulk density, while balanced nutrient management promotes optimal soil health. Nitrogen fertilization can influence soil carbon content, with both positive and negative effects depending on the management practices. The C: N ration is affected by nitrogen fertilization, and maintaining a balanced nutrient ratio is essential for sustainable soil management. Overall, our finding provides valuable insights into the intricate dynamic of soil health and highlight the need for targeted approaches to promote sustainable agriculture practices.

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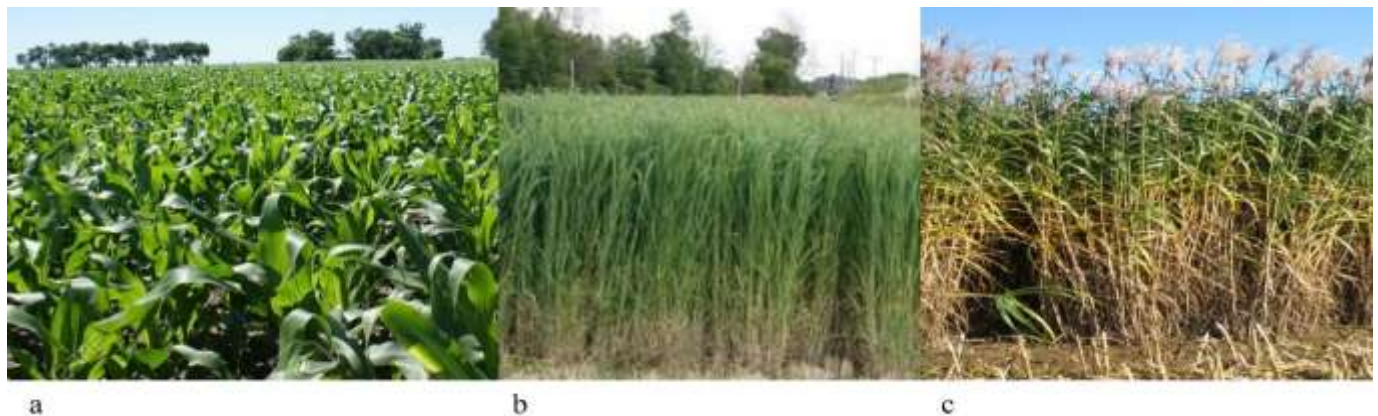


Figure 1. The subset of treatment chose for corn cultivation that contains corn (*Zea mays* L.) (CC) with tilling and fertilization (a). The subset of *Panicum virgatum* (switchgrass) (b), and *Miscanthus x giganteus* (c) for perennialization were cultivated with zero fertilization and no tilling operation.

Table 1. Meteorological parameters for the field sites during experiment (Abarkouh province Meteorological Office)

Months	Mean temperature (°C)		Relative humidity (%)		Precipitation (mm)	
	2019	2020	2019	2020	2019	2020
	November	12.98	14.06	79.87	84.32	148.36
December	10.52	12.13	85.10	79.80	185.30	122.90
January	8.90	10.60	80.30	82.40	95.20	94.30
February	10.30	12.20	84.70	88.20	87.90	94.30
March	8.10	11.20	83.40	86.50	110.10	118.40
April	11.40	14.15	83.10	79.90	70.30	125.10
May	16.40	18.90	80.70	82.10	18.10	41.20
June	24.20	21.50	73.20	79.30	10.30	26.30

Table 2. The variance component of comparing two cropping practices (annual vs. perennial).

Source	Var	% of Total	SE Var	Z-Value	P-Value
Cropping systems	1.228929	2.16%	5.285855	0.232494	0.003
Error	55.541548	97.84%	9.888496	5.616784	0.000
Total	56.770477				

Table 3. Grouping information using the Tukey method and 95% confidence.

depths	N	Mean	Grouping
60-90 cm	17	1.49433	A
30-60 cm	25	1.48067	A
15-30 cm	25	1.47847	A
0-15 cm	25	1.26614	B

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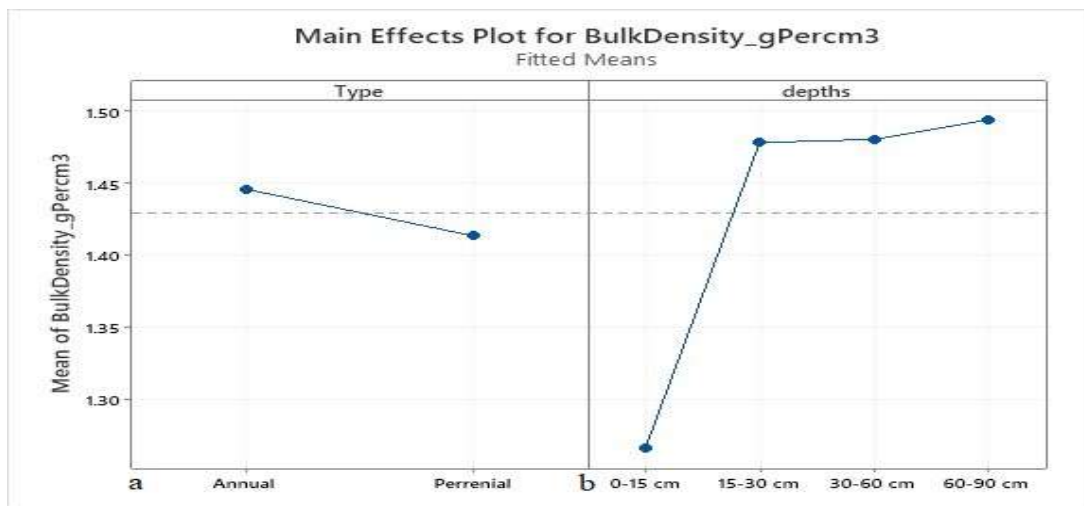


Figure 2. The impact of fertilization on bulk density according to the type of cropping systems (a). Change of bulk density through soil profile from top soil to deep soil (b).

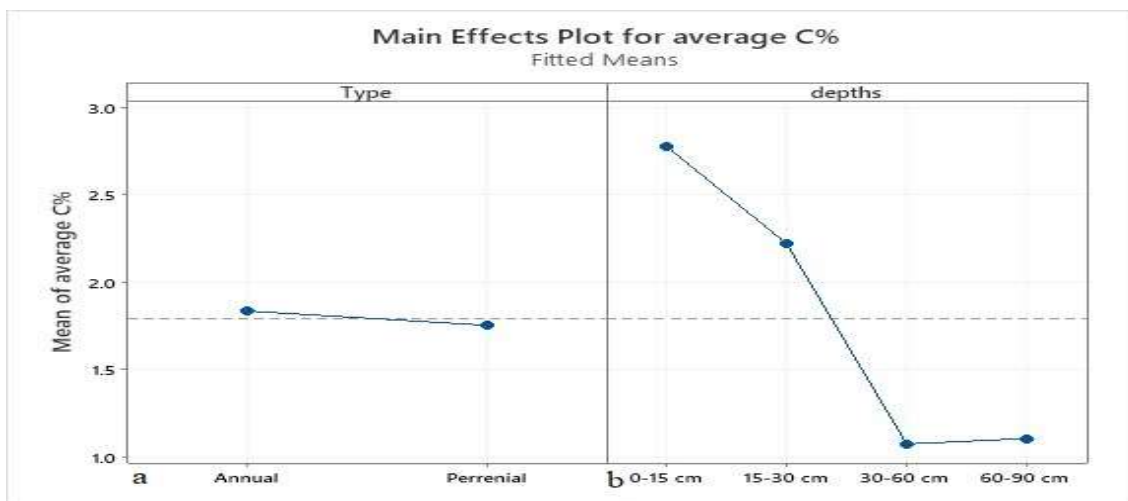


Figure 3. The impact of fertilization on soil carbon according to the type of cropping systems (a). Change of soil carbon through soil profile from top soil to deep soil and interaction plot of soil carbon content, soil depth, and type of treatment (b).

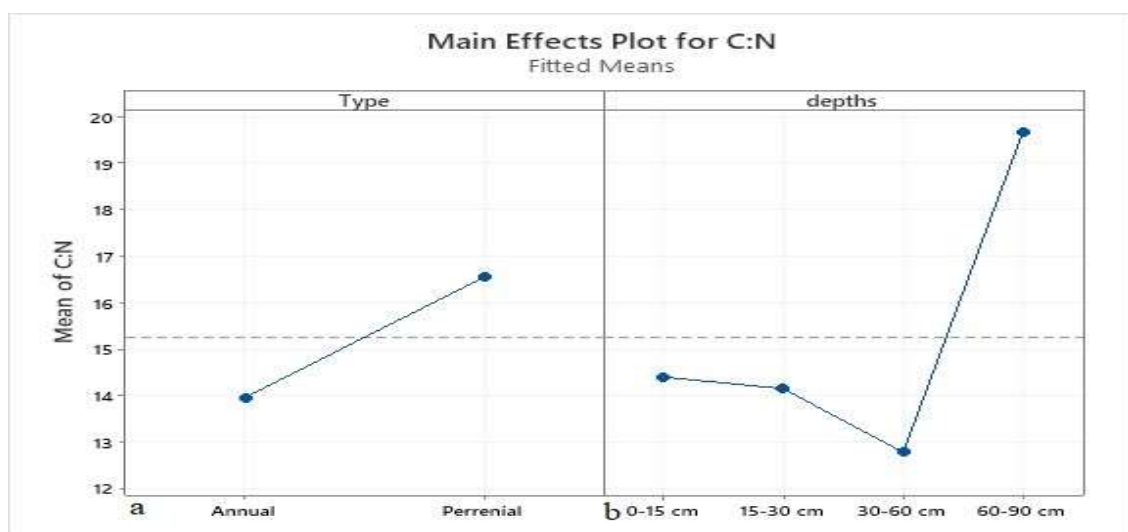


Figure 4. The impact of fertilization on soil C: N ratio according to the type of cropping systems between annual and perennial (a). Change of soil C: N through soil profile from top soil to deep soil, the lowest C: N is shown in 30-60 cm and the highest are shown in 60-90 cm (b).