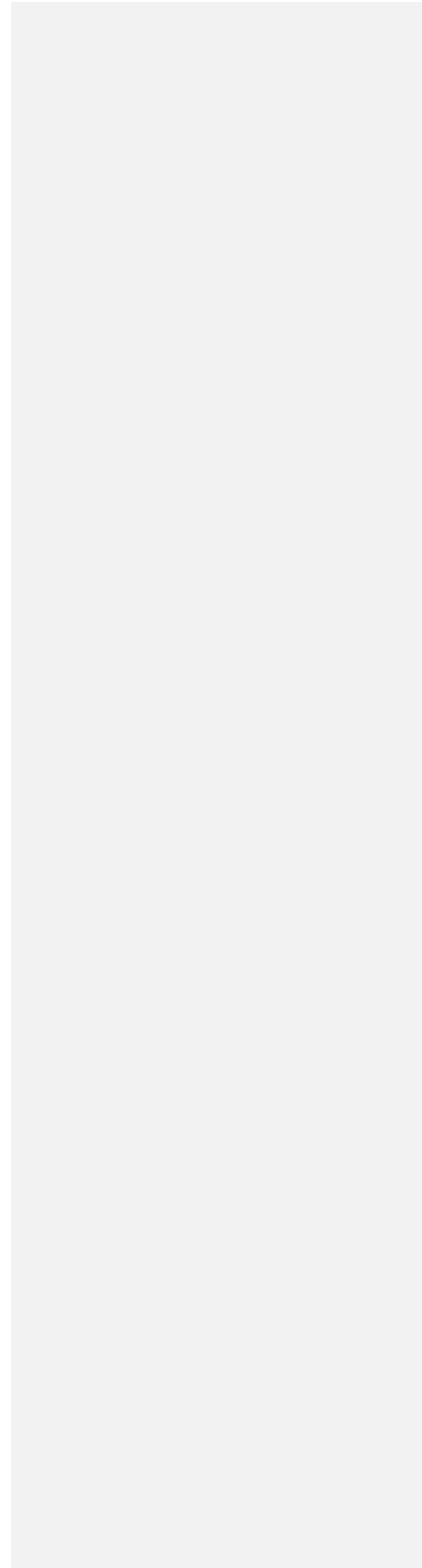


**Positive and negative impact of nitrogen fertilizer on soil properties and nutrient dynamic**



## **Abstract**

Our study was conducted over the period of two years and explored the impact of nitrogen fertilization and non-fertilization systems on soil health indicators and crop yield. Our study hypothesized that no fertilization coupled with cover cropping would enhance soil health, carbon content, and bulk density. This study found that excessive nitrogen fertilization had negative impact on soil health factors such as bulk density increment and carbon content decline through deeper profiles. In contrast, the no-fertilization system exhibited improved bulk density and better carbon distribution near the soil surface, suggesting that reducing or eliminating nitrogen can promote soil health and prevent long-term fertility decline. The study also highlighted the imbalanced carbon to nitrogen ratio resulting from high nitrogen fertilizer rate can negatively impact soil microbial activity and nutrient mineralization.

Our study raised the importance of sustainable soil management practices in agriculture. While nitrogen fertilization can enhance short-term crop yield, it can have detrimental effects on soil health and long-term sustainability. Our study suggests alternative approaches like cover cropping and integrated management improve soil health while maintaining adequate crop yield. Farmers can promote sustainable and productive agriculture systems while minimizing environmental impact.

**Key word:** Cover crops, Sustainable agriculture, nitrogen fertilizer, agriculture management

## Introduction

### Material and methods

Nitrogen is the primary component of plant proteins, chlorophyll, and nucleic acids, making it an essential nutrient for plant growth and development (Uchida, 2000). Although nitrogen is abundant in the atmosphere, it needs to be converted into a usable form by soil microorganisms. This conversion process, known as nitrogen fixation or nitrification, is carried out by nitrogen-fixing bacteria in the soil, such as *Nitrosomonas* and *Nitrobacter* (Islam et al., 2015). However, nitrogen can be lost through leaching and denitrification, causing environmental contamination. Maintaining the soil's microbiome is crucial to ensure the availability of nitrogen to plants, but it can be expensive and time-consuming (Uchida, 2000). Nitrogen fertilizers are widely used in modern agriculture to boost crop yield and maintain soil fertility. However, their use can lead to soil and environmental degradation (Erismann et al., 2008; Zhang et al., 2015). The impact of fertilizers on soil health depends on various factors, including nutrient content, soil type, climate, and management practices (Skorupka et al., 2021). To address these concerns, nitrogen use efficiency (NUE) has gained attention as a way to enhance soil health and reduce nitrogen losses. Nanotechnology is also being explored to improve NUE and contribute to food availability and security (Mirbakhsh, 2023).

Studies have shown that both the application of fertilizers and nano-fertilizers can have positive and negative effects on soil health and quality (Amanullah et al., 2016; Wang et al., 2020; Liu et al., 2021; Stewart, 2022). Fertilizers can cause changes in the physical, chemical, and biological properties of the soil. For example, nitrogen fertilizers can contribute to soil acidification and a decrease in soil organic matter content, reducing fertility and productivity. Phosphorus fertilizers, when used excessively, can lead to soil eutrophication and the accumulation of heavy metals (Chandini et al., 2019). However, appropriate fertilizer application can help maintain soil fertility and promote healthy plant growth. Potassium-rich fertilizers, for instance, can improve soil structure, reduce erosion, and increase crop stress tolerance. Nonetheless, improper fertilizer application can negatively affect soil health indicators, microorganisms, and diversity, which play a crucial role in soil fertility and nutrient cycling (Dinca et al., 2022).

Soil health is impacted by environmental changes and stressors like salinization and drought, which limit crop growth, cause land degradation, and affect gene expression (Mirbakhsh and

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Hosseinzadeh, 2013; Cui et al., 2016; Zhou et al., 2016; Luo et al., 2018; Zhu et al., 2018; Mirbakhsh and Sedeh, 2022). Implementing appropriate management practices can influence soil properties and serve as an alternative to excessive fertilizer use. Promoting soil health through improved management practices is essential for the sustainability of soil resources (Norris et al., 2020). Factors such as GHG emissions mitigation, nitrogen capture improvement, and temporal yield stability should be considered when choosing suitable cropping practices to increase soil organic matter content and reduce environmental contamination (Knapp and van der Heijden, 2018; Sun et al., 2018).

The study aims to compare the impact of fertilization and non-fertilization systems under two cropping practices: corn with fertilization in rotation and corn-cover cropping without fertilization, on soil health indicators. The hypothesis suggests that no fertilization coupled with cover cropping will enhance soil health indicators and crop yield compared to conventional tillage annual crop-fallow.

## Materials and methods

### **Field experiment**

The experiment was conducted at a Research Farm (Mashhad University, Mashhad Branch), located at 38° 88' N and 63° 72' E with an altitude of 1 m above sea level, during 2020-2023 crop year to test our alternative hypothesis. Plots are 10 m wide and 48.5 m long and ~~Each 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.) in rotation with legume Persian clover (*Trifolium resupinatum*) was chosen as

corn-cover cropping with no fertilization (Figure 1). 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<sup>-1</sup> yr<sup>-1</sup> for CC and CS, respectively, and liquid swine manure (C/N ratio: 2:1, 80% [w/w] of N as NH<sub>4</sub><sup>+</sup>) injected into CC at a rate of 255 ± 24 kg N ha<sup>-1</sup> yr<sup>-1</sup> in either the spring (SM) or the fall (FM). For cover cropping white clover (*Trifolium repens*) were added to corn in different plots (Figure 1 b). 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) (Hernandez et al., 2009). Soils were tested each fall for general fertility using recommended protocols, and results indicated soil P, K, and pH were non limiting.

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### 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 samples 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.

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Bulk density was determined in separate, undisturbed soil cores (5 cm i.d. and 2.5 cm length)

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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 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 ( $\text{g kg}^{-1}$  soil) were recorded in each individual depth increment. Following Ellert et al. (2002), the equivalent soil mass correction were performed before calculation of cumulative TOC and TN mass storage ( $\text{Mg ha}^{-1}$ ) 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:

$$M = \rho_b \times C_o \times (T_o + T_{adj}) \times 10\,000 \text{ m}^2 \text{ ha}^{-1} \times 0.001 \text{ kg g}^{-1} \text{ [Batjes., 1996]}$$

Where  $\rho_b$  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 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 recorded the negative impact of fertilization on soil bulk density that increased through soil profile and it is clearly shown in shallowest profiles (Figure 2a-b). The interaction of soil bulk density and soil depth is shown in figure 2 c. No fertilization decreased and improved soil bulk density in the most top soil layers (0-30 cm) (Figure 2c). Our results are aligned with the hypothesis of the negative impact of excessive application of nitrogen fertilizer 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 nitrogen fertilizer is like a two-edge sword and can vary depending on various factors such as management practices, soil condition, and environmental factors.

Nitrogen fertilizer can enhance crop 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<sub>2</sub> 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 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 (0-15 cm) (Figure 3c).

#### Carbon nitrogen ratio (C: N ratio)

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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 (figure 4a) and higher carbon to nitrogen ratio was recorded under fertilization (figure 4b). Obviously, under nitrogen fertilization, 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 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.

**Mineralization.**

### **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.

### **References**

**Comment [D7]:** Not found in the references section

Amanullah, A.; Iqbal, A.; Ali, S.; Fahad, S.; Parmar, B. Nitrogen source and rate management improve maize productivity of smallholders under semiarid climates. *Front. Plant Sci.* 2016, 7, 1773.

Ashworth, A. J., Allen, F. L., Debruyne, J. M., Owens, P. R., & Sams, C. (2018). Crop rotation and poultry litter affect dynamic soil chemical properties and soil biota long term. *Journal of Environmental Quality*, 47, 1327–1338. <https://doi.org/10.2134/jeq2017.12.0465>.

**Comment [D8]:** Not found anywhere

Balota, E. L., Calegari, A., Nakatani, A. S., & Coyne, M. S. (2014). Benefits of winter cover crops and no-tillage for microbial parameters in a Brazilian Oxisol: A long-term study. *Agriculture, Ecosystems and Environment*, 197, 31–40. <https://doi.org/10.1016/j.agee.2014.07.010>.

Batjes, N.H. 1996. Total carbon and nitrogen in the soils of the world. *Eur. J. Soil Sci.* 47:151–163.

Bisht, N., Chauhan, P. (2020). “Excessive and disproportionate use of chemical cause soil contamination and nutrient stress”. DOI: 10.5772/intechopen.94593.

Chen J., Larke P., Jorgensen U. 2022. “Land conversion from annual to perennial crops: A win-win strategy for biomass yield and soil organic carbon and total nitrogen sequestration”. *Agriculture, Ecosystems & Environment*, <https://doi.org/10.1016/j.agee.2022.107907>.

**Comment [D9]:** Not found anywhere in the m/s

Cui, P., Fan, F., Yin, C., Song, A., Huang, P., Tang, Y., et al. (2016). Long-term organic and inorganic fertilization alters temperature sensitivity of potential N<sub>2</sub>O emissions and associated microbes. *Soil Biol. Biochem.* 93, 131–141. doi: 10.1016/j.soilbio.2015.11.005.

Dinca, L., Grenni, P., Onet, C., Onet, A. (2022). “Fertilization and soil microbial community: A review”. *Appl. Sci.* 2022, 12(3), 1198; <https://doi.org/10.3390/app12031198>

Ellert, B.H., H.H. Janzen, and T. Entz. 2002. Assessment of a method to measure temporal change in soil carbon storage. *Soil Sci. Soc. Am. J.* 66:1687–1695.

Erisman JW, Sutton MA, Galloway J, Klimont Z, Winiwarter W. How a century of ammonia synthesis changed the world. *Nature Geoscience.* 2008;1:636–639. doi: 10.1038/ngeo325.

Farmah, B., Sekaran, U., Franzluebbers, A. (2021). “Cover cropping and conservation tillage improve soil health in the southeastern United States”. <https://doi.org/10.1002/agj2.20865>

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., et al. (2011). Solutions for a cultivated planet. *Nature* 478, 337–342. doi: 10.1038/nature10452

Hochmuth, G.J. Progress in mineral nutrition and nutrient management for vegetable crops in the last 25 years. *Hortscience* 2003, 38, 999–1003.

**Comment [D10]:** Not found anywhere in the m/s.

Hussain, S., Khan, F., Cao, W., Wu, L., and Geng, M. (2016). Seed priming alters the production and detoxification of reactive oxygen intermediates in rice seedlings grown under sub-optimal temperature and nutrient supply. *Front. Plant Sci.* 7:439. doi: 10.3389/fpls.2016.00439

Islam M. R., Hossain M. B., Siddique A. B., Rahman M. T., Malika M. (2015). Contribution of green manure incorporation in combination with nitrogen fertilizer in rice production. *SAARC J. Agric.* 12 134–142. 10.3329/sja.v12i2.21925.

Knapp, S., van der Heijden, M.G.A., 2018. A global meta-analysis of yield stability in organic and conservation agriculture. *Nat. Commun.* 9, 3632. <https://doi.org/10.1038/s41467-018-05956-1>.

L A, P K, G SB (2016) Evaluation of *Spirulina platensis* as microbial inoculants to enhanced protein levels in *Amaranthus gangeticus*. *African J Agric Res* 11:1353–1360. <https://doi.org/10.5897/ajar2013.7953>.

**Comment [D11]:** Not found anywhere in the m/s

Ledo, A., Smith, P., Zerihun, A., Whitaker, J., Vicente-Vicente, J. L., Qin, Z., McNamara, N. P., Zinn, Y. L., Llorente, M., Liebig, M., Kuhnert, M., Dondini, M., Don, A., Diaz-Pines, E., Datta, A., Bakka, H., Aguilera, E., & Hillier, J. (2020). Changes in soil organic carbon under perennial crops. *Global Change Biology*, 26(7), 4158–4168.

**Comment [D12]:** Not found anywhere in the m/s

Liu, Q.; Xu, H.; Yi, H. Impact of Fertilizer on Crop Yield and C:N:P Stoichiometry in Arid and Semi-Arid Soil. *Int. J. Environ. Res. Public Health* 2021, 18, 4341. [[Google Scholar](#)] [[CrossRef](#)] [[PubMed](#)]

Luo, Z., Liu, H., Li, W., Zhao, Q., Dai, J., Tian, L., et al. (2018). Effects of reduced nitrogen rate on cotton yield and nitrogen use efficiency as mediated by application mode or plant density. *Field Crop Res.* 218, 150–157. doi: 10.1016/j.fcr.2018.01.003.

Mirbakhsh M, Hosseinzadeh Namin M. 2013. INVESTIGATION OF IN VITRO APOCAROTINOID GENE EXPRESSION IN PERIANTH OF SAFFRON (*CROCUS SATIVUS*) IRAN. 2nd National Congress on Medicinal Plants 15, 16 May 2013 Tehran- Iran.

Mirbakhsh, M. (2023). Role of Nano-fertilizer in Plants Nutrient Use Efficiency (NUE). *J Gene Engg Bio Res*, 5(1), 75-81.

Mirbakhsh, M. and Sedeh, S.S.S. (2022). Effect of short and long period of salinity stress on physiological responses and biochemical markers of *Aloe vera* L. *Ilmu Pertanian (Agricultural Science)*, 7(3), pp. 178–187.

Nawaz, J., Hussain, M., Jabbar, A., Nadeem G., Sajad, M., Subtain, M. (2013). “Seed priming a technique”. *International Journal of Agriculture and Crop Sciences*. IJACS/2013/6-20/1373-1381.

Niu, Z., An, F., Su, Y., Liu, T., Yang, Rong, Du, Zeyu., Chen, sh. (2022). “Effect of long-term fertilization on aggregate size distribution and nutrient accumulation in Aeolian sandy soil”. *Plant (Base)*, 2022 Apr; 11(7):909. doi: [10.3390/plants11070909](https://doi.org/10.3390/plants11070909).

Norris, C. E., Bean, G. M., Cappellazzi, S. B., Cope, M., Greub, K. L. H., Liptzin, D., Rieke, E. L., Tracy, P. W., Morgan, C. L. S., & Honeycutt, C. W. (2020). Introducing the North American project to evaluate soil health measurements. *Agronomy Journal*, 112, 3195–3215. <https://doi.org/10.1002/agj2.20234>.

Skorupka, M.; Nosalewicz, A. Ammonia Volatilization from Fertilizer Urea—A New Challenge for Agriculture and Industry in View of Growing Global Demand for Food and Energy Crops. *Agriculture* 2021, *11*, 822.

Snyder, C.S.; Bruulsema, T.W.; Jensen, T.L.; Fixen, P.E. Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agric. Ecosyst. Environ.* 2009, *133*, 247–266.

Stewart, R.E. Fertilizer. Encyclopedia Britannica. Available online: <https://www.britannica.com/topic/fertilizer> (accessed on 18 March 2022).

Sun R. B., Zhang X. X., Guo X. S., Wang D. Z., Chu H. Y. (2015). Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biol. Biochem.* 88, 9–18. 10.1016/j.soilbio.2015.05.007.

Sun, J., Zou, L., Li, W., Yang, J., Wang, Y., Xia, Q., et al. (2018). Rhizosphere soil properties and banana Fusarium wilt suppression influenced by combined chemical and organic fertilizations. *Agric. Ecosyst. Environ.* 254, 60–68. doi: 10.1016/j.agee.2017.10.010.

Tarkalson, D. D., Hergert, G. W., & Cassman, K. G. (2006). Long-term effects of tillage on soil chemical properties and grain yields of a dryland winter wheat-sorghum/corn-fallow rotation in the Great Plains. *Agronomy Journal*, 98, 26–33. <https://doi.org/10.2134/agronj2004.0240>.

Tung, S. A., Huang, Y., Ali, S., Hafeez, A., Shah, A. N., Song, X., et al. (2018). Mepiquat chloride application does not favor leaf photosynthesis and carbohydrate metabolism as well as lint yield in late-planted cotton at high plant density. *Field Crop Res.* 221, 108–118. doi: 10.1016/j.fcr.2018.02.027

Uchida, R. (2000) Essential Nutrients for Plant Growth: Nutrient Functions and Deficiency Symptoms. In: Silva, J.A. and Uchida, R., Eds., *Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture*, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, 31-55.

Wang, X.; Zou, C.; Gao, X.; Guan, X.; Zhang, W.; Zhang, Y.; Shi, X.; Chen, X. Nitrous oxide emissions in Chinese vegetable systems: A meta-analysis. *Environ. Pollut.* 2018, *239*, 375–383

Wang, Y., Liu, B., Ren, T., Li, X., Cong, R., Zhang, M., et al. (2014). Establishment method affects oilseed rape yield and the response to nitrogen fertilizer. *Agron. J.* 106, 131–142. doi: 10.2134/agronj2013.0374

Wang, Z.; Hassan, M.U.; Nadeem, F.; Wu, L.; Zhang, F.; Li, X. Magnesium Fertilization Improves Crop Yield in Most Production Systems: A Meta-Analysis. *Front. Plant Sci.* 2020, *10*, 1727. [[Google Scholar](#)] [[CrossRef](#)] [[PubMed](#)][[Green Version](#)]

Zeng J., Liu X., Song L., Lin X., Zhang H., Shen C., et al. (2016). Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biol. Biochem.* 92, 41–49. 10.1016/j.soilbio.2015.09.018.

Zhang, X.; Davidson, E.A.; Mauzerall, D.L.; Searchinger, T.D.; Dumas, P.; Shen, Y. Managing nitrogen for sustainable development. *Nature* 2015, *15*, 91.

**Comment [D13]:** Not found anywhere in the m/s

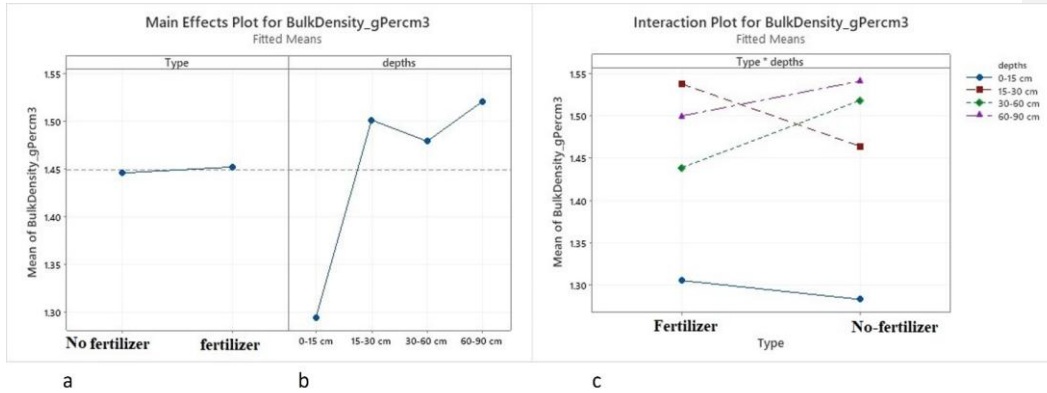
**Comment [D14]:** Not found anywhere in the m/s

Zhou, M., Zhu, B., Bruggemann, N., Dannenmann, M., Wang, Y., and Butterbach-Bahl, K. (2016). Sustaining crop productivity while reducing environmental nitrogen losses in the subtropical wheat-maize cropping systems: a comprehensive case study of nitrogen cycling and balance. *Agric. Ecosyst. Environ.* 231, 1–14. doi: 10.1016/j.agee.2016.06.022.

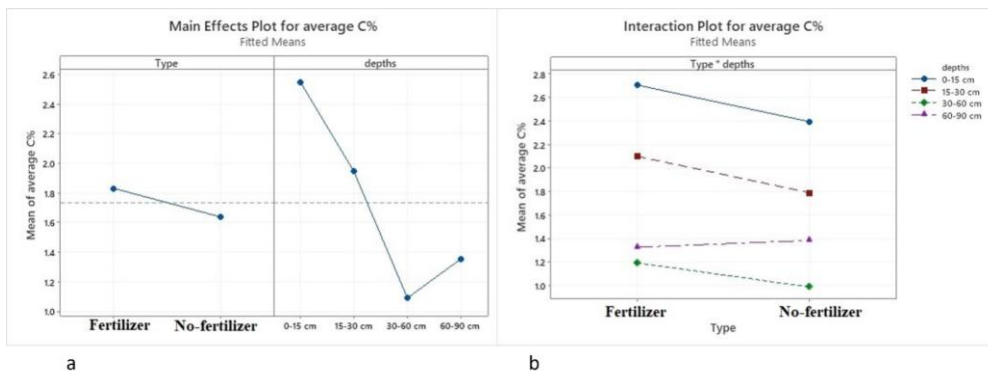
Zhu, Q., Liu, X., Hao, T., Zeng, M., Shen, J., Zhang, F., et al. (2018). Modeling soil acidification in typical Chinese cropping systems. *Sci. Total Environ.* 614, 1339–1348. doi: 10.1016/j.scitotenv.2017.06.257



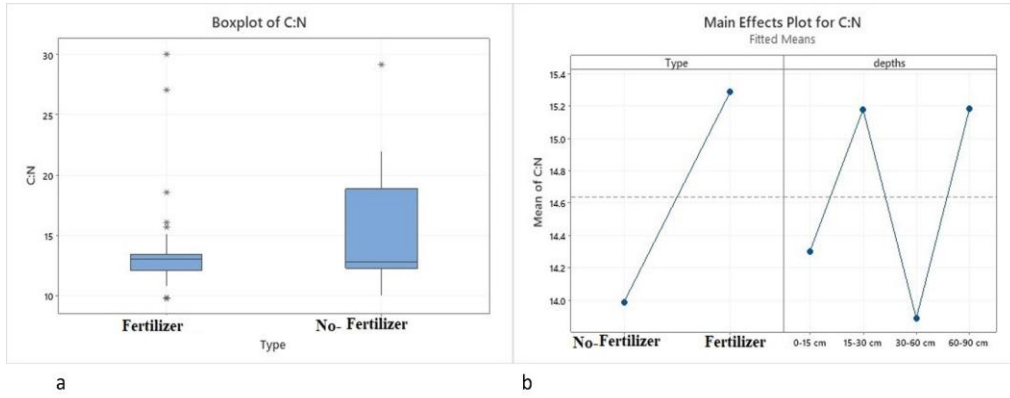
**Figure 1.** The subset of treatment chose for corn cultivation that contains corn (*Zea mays* L.) (CC) with tilling and fertilization (a). Subsets of corn in rotation with legume Persian clover (*Trifolium resupinatum* L.) was chosen as corn-cover cropping with no fertilization (b).



**Figure 2.** The impact of fertilization on bulk density according to the type of fertilization treatments (a). Change of bulk density through soil profile from top soil to deep soil (b). Interaction plot of soil bulk density, soil depth, and type of treatment (c).



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



**Figure 4.** The impact of fertilization on soil C: N ratio according to the type of fertilization treatments in a box plot(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 15-30 cm and 60-90 cm (b).