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Substitution of Chemical Fertilizer with Organic Fertilizer Affects Soil Total Nitrogen and Its Fractions in Northern China

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Abstract: The impact of chemical to organic fertilizer substitution on soil labile organic and stabilized N pools under intensive farming systems is unclear. Therefore, we analyzed the distribution of soil total N (STN), particulate organic N (PON), microbial biomass N (MBN), dissolved organic N (DON), and mineral N (NO3− and NH4+) levels down to 100 cm profile under wheat–maize rotation system in northern China. The experiment was established with four 270 kg ha−1 N equivalent fertilizer treatments: Organic manure (OM); Organic manure with nitrogen fertilizer (OM + NF); Nitrogen fertilizer (NF); and Control (CK). Results found that the OM and OM + NF treatments had significantly higher STN, PON, MBN, DON, and NO3− contents in 0–20 cm topsoil depths. Conversely, the NF treatment resulted in the highest (p < 0.01) DON and NO3− depositions in 40–100 cm subsoil depths. The NH4+ contents in selected profile depths were significantly highest (p < 0.01) under OM treatment. The correlations between STN and its fractions were positively significant at 0–10 and 10–20 cm topsoil depths. Our results suggest that partial substitution of chemical fertilizer with organic manure could be a sustainable option for soil N management of intensive farming systems.

Keywords: organic manure; nitrogen fertilizer; soil total N; labile organic N; mineral N; soil fertility

1. Introduction

Soil nitrogen (N) availability influences the yield, grain N recovery and protein content of cereal crops [1–3]. Therefore, N fertilization is inevitable for maintaining crop productivity and grain nutritional quality of cereal-based dryland farming systems where N is often yield-limiting [4,5]. However, in wheat–maize growing dryland farming areas of North China Plain (NCP), the application of chemical N fertilizer (often overdosed) has become a regular practice for decades to achieve higher yields [6]. Such long-term fertilization with chemical fertilizer has aggravated acidification, nutrient imbalance, enzyme activities, and compaction of soils, thereby suppressing crop growth [7–10]. Moreover, due to low nitrogen use efficiency (NUE; ~33% on average) of major cereal crops most of the unutilized N often leaks out from the farming systems in various N forms, contributing to environmental contamination, global warming, and human health issues [11–14]. On the other hand, repeated application of organic fertilizer (i.e., manures) was reported to be beneficial.
for soil organic matter (SOM) content, pH buffering, aggregate formation, water holding and nutrient retention capacity. Moreover, crop productivity is identical when equal total nutrients are supplied from organic or chemical sources [15,16]. Therefore, organic manure substitutions for chemical fertilizer have been suggested as a viable approach to ensure sustainable future food security, to restore soil fertility and structural properties, and to reduce environmental impacts of chemical fertilizer [17–20]. Still, their impacts on soil labile organic and stabilized N pools under intensively managed dryland farming systems is less understood.

Although nitrogen is a highly mobile element in soil, soil total N (STN) status changes relatively slowly due to its large pool size. Therefore, STN changes are most frequently reported under long-term soil fertility studies [21,22]. Although continuous N input increases STN content in the profile, in topsoil depths, significantly higher total N is often reported with organic or organic–inorganic combined fertilization [17,22,23]. The reason for that can be attributed to organic fertilizer induced increases in soil organic matter (SOM) and available soil N content, because STN status responds to SOM content positively and correlates strongly with available soil N content [24,25]. The labile organic fractions of STN are actively involved in soil N mineralization and considered as sustainable soil fertility indices, which are described as particulate organic N (PON), microbial biomass N (MBN), and dissolved organic N (DON). The dynamics of these pools vary temporarily, respond sensitively to soil fertility management approaches, and affect the short and long-term N supply [25–27].

Despite variations, soil mineral N pool (NO$_3^-$ and NH$_4^+$) represents only a small fraction of STN which are readily available for plant acquisition. Application of chemical N fertilizer increases mineral N contents in the profile, but it often exhibits a potential risk of NO$_3^-$ leaching and groundwater contamination [28]. In fact, significant NO$_3^-$ leaching has been reported recently under chemically managed wheat–maize rotations from this area [29]. In contrast, manure-containing treatments have been found to increase the mineral N content in topsoil layers while reducing the NO$_3^-$ leaching by influencing N immobilization processes [28–31]. Therefore, chemical fertilizer substitution with organic fertilizer could be a sustainable option to reduce N losses.

Soil labile organic N pools are more sensitive than STN to agricultural management practices [32]. These pools are actively involved in short-term N transformations and play a significant role in soil mineral N supply [32,33]. Despite their importance in soil nutrient pools, soil fertility studies often focused on inorganic N pools (NO$_3^-$ and NH$_4^+$) [29,34]. In recent years, however, DON has been getting special attention among scientists due to its substantial contribution to N leaching pathways of forest, grassland, as well as agroecosystems [35–40]. For example, in Europe, average DON leaching from agricultural ecosystems accounted for about 26% of total dissolved N leached [41]. In another study in Australia, DON makes up to 40% of the deep drainage nitrogen from irrigated Vertisol cotton–wheat–maize production systems [42]. More recently, significant DON leaching has been reported at high N rates from maize–legume cover rotations under Mediterranean course soil conditions [40]. However, the MBN and PON contents of cultivated soils have been identified greater under continuous manuring or organic–inorganic balanced fertilization than exclusively applied fertilizer N [43]. Nevertheless, only a few fertility studies investigated the distribution of labile organic N pools in cultivated soil in diverse environments, some of which only examined topsoil depths. Therefore, information is limited to conclude the effects of chemical fertilizer substitution with organic manure on the distribution of labile organic N pools in the whole soil profile under intensively managed farming systems.

The crop productivity and sustainability of the agroecosystems are highly dependent on the short-term (seasonal) and long-term (years to decades) dynamics of SOM, including the turnover of soil labile organic fractions and the regeneration of stabilized nutrient pools [44]. Understanding the dynamics of these pools is essential for long-term soil fertility management decisions. Although, recent soil management studies reported
that continuous substitution (partial or total) of chemical fertilizer with organic fertilizer significantly increases SOM status, SOC and STN content \[22,43,45,46\]. Still, their impact on the size and distribution of soil N pools especially those labile organic N pools in the whole soil profile under intensively managed wheat–maize farming systems of northern China is unclear. Despite being an indicator of overall soil quality, SOM can be insensitive to new management practices, and STN changes in the soil profile could be prolonged \[47\]. Therefore, in this study, we aimed: (1) to investigate the effects of organic manure substitutions for chemical fertilizer on size and distribution of STN and its labile organic (PON, MBN, and DON) and mineral (NO\(_3^-\) and NH\(_4^+\)) fractions in the whole soil profile; and (2) to evaluate how such fertilizer substitution treatments affect the correlations among STN and its fractions; under intensively managed farming system.

2. Experimental Methods

2.1. Experimental Site

The field experiment was conducted at the dryland water-saving experimental station of Institute of Dryland Farming, Hebei Academy of Agriculture and Forestry Sciences, Hengshui, Hebei province of China (115°10'~116°34' E and 37°03'~38°23' N). The site is located at North China flat plain between 17.5 and 28 m above sea level and belongs to the warm temperate semi-humid climate with a continental monsoon. The recorded annual mean precipitation was 497.0 mm, unevenly distributed, mostly fallen in summer months (July to September), while winter months received 120–160 mm. The annual mean temperature, sunshine duration, evapotranspiration, and frost-free period were 12.8 °C, 2509.4 h, 1785.4 mm, and 201 days, respectively. The soil is deep, slightly alkaline (pH 7.8), loamy, and classified as ‘Fluvo-aquic’ according to the FAO-UNESCO system of soil classification \[48\].

At the beginning of this experiment, the initial soil samples were collected from 0–20 cm topsoil profile, and soil organic matter, alkali-hydrolysable N, available P, available K, and bulk density were recorded as 1.65%, 71.90 mg N kg\(^{-1}\), 22.6 mg P kg\(^{-1}\), 171.6 mg K kg\(^{-1}\), and 1.48 g cm\(^{-3}\), respectively.

2.2. Experimental Design

Four N equivalent treatments were established in 2014 based on farmers’ recommended rate of N (270 kg ha\(^{-1}\)) for dryland farming systems of northern China \[49\]. The treatments were as follows: (1) Organic manure (OM), 100% N from composted cattle manure; (2) Organic manure with nitrogen fertilizer (OM + NF), 50% N from composted cattle manure plus 50% N from urea; (3) Nitrogen fertilizer (NF), 100% N from urea; and (4) Control (CK), with zero N fertilization. Three replicates for each treatment were arranged in a 17.4 × 10 m\(^2\) plot maintaining 0.8 m spacing between plots and borders.

The irrigated winter wheat (*Triticum aestivum* L.) and rain-fed summer maize (*Zea mays* L.) rotation system was followed with alternation of wheat varieties (HengH1401, water-saving; and Cangmai6005, drought-resistant) in each calendar year. Crops were harvested each year, and following each harvest, the maize crop residues were removed while the wheat straws were returned to the field for recycling. After each harvesting of summer maize between early to mid-September, a 15–20 cm deep moldboard ploughing was practiced. Manure treatments which contained 341.6 g organic C kg\(^{-1}\), 19.1 g N kg\(^{-1}\), 10.1 g P kg\(^{-1}\), and 8.0 g K kg\(^{-1}\), were applied before ploughing and during the soil preparation for winter wheat. The N equivalence of organic manure to urea fertilizer (N 46%) was assessed before each application based on the total Kjeldahl nitrogen content of composted cattle manure. For OM + NF treatments, 40% of total urea fertilizer was applied before sowing as a basal dose and the remaining 60% was side dressed during returning green stage of wheat development. Besides, 57.6 kg P ha\(^{-1}\) from triple superphosphate (P\(_2\)O\(_5\) 46%), and 68.5 kg K ha\(^{-1}\) from potassium sulfate (K\(_2\)O 60%) were also applied to wheat across all treatments. Each year, winter wheat was sown between 1 and 10 October at 330 seeds/m\(^2\) with 15 cm row spacing using a mechanical seeder and
harvested at maturity in early June. Small-scale sprinklers were used for wheat irrigation at, before sowing (120 mm), returning green stage (80 mm), and heading stage (80 mm). After winter wheat harvest, summer maize (Zhengdan958) was planted by a mechanical planter with minimum tillage following a 90 mm supplemental irrigation. Chemical pest control measures were applied to control weeds and insect pests.

2.3. Soil Sampling

Soil samples were collected on 27 September 2019 following the harvest of summer maize. Soil cores down to 100 cm soil profile from three random points in each treatment plot were sampled using a standard auger (8 cm diameter). Each soil core of the selected profiles was separated into successional sub-samples at 10 cm depth interval. The three sub-samples of each depth category from each treatment plot were thoroughly mixed to make a composite sample. After removing organic stubbles, each composite sample was divided into two parts. One part was air-dried and kept for analysis of soil chemical properties, and the second part of the fresh sample was passed through a 2 mm sieve and stored at 4 °C for biochemical analysis.

2.4. Soil Analysis

Air-dried soil was used for the analysis of STN, SOC, and PON. For analysis of STN and SOC, soil samples were grounded and passed through a 0.15 mm sieve. STN was determined following the Kjeldahl digestion-distillation procedure as recommended by Bremner and Mulvaney (1982) [50]. SOC was estimated using the K₂Cr₂O₇ oxidation-titration method as described by Blake (1965) [51]. Briefly, 0.1 g sieved soil was digested by boiling with 5 mL 0.8 M K₂Cr₂O₇ and 5 mL concentrated H₂SO₄ in an oil bath at 180 °C for 5 min. The organic C content in the digested soil-solution was measured by titration method using 0.2 M FeSO₄·7H₂O in presence of C₁₂H₈N₂·H₂O indicator. PON was measured using the procedure as described by Bronson et al. (2004) [52]. Briefly, 20 g of air-dried soil (<2 mm) was dispersed in 60 mL sodium hexametaphosphate solution (5 g L⁻¹) using a reciprocal shaker for 16 h. The soil suspension was rinsed with deionized water and passed through a 0.15 mm (53 µm mess) sieve. The remnant materials on the sieve were oven-dried and weighed after removing visible stones. The total nitrogen content in PON was estimated following the same procedure for STN determination.

The MBN, DON, and mineral N (NO₃⁻ and NH₄⁺) content in soil was measured from fresh soils. MBN was determined using the fumigation-extraction method of Vance et al. (1987) [53]. In brief, two sets of 15 g soil samples were re-wetted and incubated for 24 h in the dark at 25 °C and 40–45% water holding capacity. One set of soil samples was subjected to CHCl₃ fumigation, and the next one was CHCl₃-free. After incubation, organic nitrogen in soil samples was extracted with 45 mL 0.5 M K₂SO₄ solution, shaken at 200 rev/min for 30 min, and filtered. The organic nitrogen content in the extracts was estimated by dry combustion using a CN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The microbial biomass nitrogen (MBN) was calculated as: MBN = [(organic N in fumigated soil–organic N in non-fumigated soil)/Kₑ], where Kₑ is 0.57 (Jenkinson, 1988) [54].

The DON content was determined using the procedures described by Gigliotti et al. (2002) [55]. Briefly, 10 g fresh soil was mixed with 50 mL water, shaken for 1 h on a reciprocal shaker, and then the colloidal suspension was centrifuged at 15,000 × g rev/min at 25 °C for 10 min. Next, the translucent solution was passed through a 0.45 µm membrane filter. The DON in the filtrate was determined by a continuous flow analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The mineral N (NO₃⁻ and NH₄⁺) content in soil was estimated using the KCl extraction method [56]. Briefly, 10 g fresh soil was suspended with 2 M KCl solution at 1:10 soil-to-solution ratio using a reciprocal shaker at 180 rev/min for 30 min. The extract was filtered, and the concentration of NO₃⁻ and NH₄⁺ in the filtrates were determined using a continuous flow automated colorimeter (AA3, Automatic chemical analyzer, Easychem Plus, Europe).
2.5. Statistical Analysis

Data of all soil parameters were presented on an oven-dried weight basis. Analysis of variance (ANOVA) was performed using the SPSS Statistics 25.0 software package (SPSS Inc. IBM, Chicago, IL, USA). The main treatment effects on variable means were detected and compared using the least difference (LSD) at the 0.05 probability level.

3. Results

3.1. Soil Total N and Organic C

Five-year continuous substitution of chemical fertilizer with organic manure treatments (OM and OM + NF) significantly improved the soil total N (STN) contents in topsoil depths (Figure 1A). The average STN contents of OM, OM + NF, and NF treatments were increased by 32.9% (0.27 g kg\(^{-1}\)), 8.4% (0.07 g kg\(^{-1}\)), and 8.7% (0.07 g kg\(^{-1}\)), respectively, when compared with the CK. The OM treatment had the significantly highest STN contents in 0–30 cm topsoil depths, which was an average 32% (0.41 g kg\(^{-1}\)), 46.8% (0.54 g kg\(^{-1}\)), and 50.3% (0.57 g kg\(^{-1}\)) greater than OM + NF, NF, and CK treatments, respectively. The STN contents of OM + NF treatment at 0–10 and 10–20 cm topsoil depths were 19.1% (0.24 g kg\(^{-1}\)) and 17.53% (0.22 g kg\(^{-1}\)) greater over CK, and significantly higher than NF treatment (Figure 1A). The STN variations between NF and CK treatments were identical (\(p > 0.05\)) except at 40–50 cm depth of the profile.

Soil organic C (SOC) differences among treatments were significant across the profile depths, and SOC contents were higher with OM and OM + NF treatments (Figure 1B). The average SOC contents of OM, OM + NF, and NF treatments increased by 65.2% (4.42 g kg\(^{-1}\)), 38% (2.57 g kg\(^{-1}\)), and 21.6% (1.46 g kg\(^{-1}\)), respectively, when compared to the CK. Although all treatments demonstrated higher SOC concentrations in 0–20 cm topsoil depths, the SOC contents of OM and OM + NF treatments were significantly greater (\(p < 0.01\)) than NF and CK treatments. The OM, OM + NF, and NF treatments had an average of 90% (9.85 g kg\(^{-1}\)), 52.9% (5.78 g kg\(^{-1}\)), and 15.8% (1.72 g kg\(^{-1}\)) higher SOC in 0–20 cm topsoil depths. A gradual declining trend in SOC contents with increased profile depths was observed under all treatments.

3.2. Soil Organic C/TN Ratio

Significant variations were observed among fertilizer substitution treatments on soil organic C/TN ratio across the selected profile, and treatment effects were higher in topsoil
layers (Figure 1C). The average soil organic C/TN ratio of OM, OM + NF, and NF treatments were increased by 22.4% (1.99), 24.1% (2.14), and 10.5% (0.93), respectively, when compared to the CK. The soil organic C/TN ratio of each depth under OM and OM + NF treatments were statistically similar, and organic C/TN ratio differences among OM, OM + NF, and NF treatments at 0–10, 10–20, and 20–30 cm soil depths were non-significant. Under all treatments, we found a gradual declining trend in soil organic C/TN ratio with an increase in profile depths.

3.3. Labile Organic N Pools

The variations among fertilizer substitution treatments on the depth distribution of soil labile organic N fractions in the profile were statistically significant (Figure 2A–C). The particulate organic N (PON) differences among treatments were pronounced in 0–20 cm topsoil layers, while a slight variation was observed at 40–50 and 50–60 cm subsoil depths (Figure 2A). The OM and OM + NF treatments had 106.5% (0.49 g kg\(^{-1}\)) and 56.5% (0.26 g kg\(^{-1}\)), and 93.5% (0.43 g kg\(^{-1}\)) and 47.8% (0.22 g kg\(^{-1}\)) higher accumulation of PON at 0–10 and 10–20 cm soil layers, respectively, when compared to the CK. The NF treatment did not significantly affect PON contents at 0–10 and 10–20 cm topsoil layers. The observed PON differences among treatments were statistically identical at 20–30, 30–40, 60–70, 70–80, 80–90, and 90–100 cm profile depths.

![Figure 2. Effect of five years of continuous N equivalent fertilizer substitution treatments on the depth distribution of particulate organic N (A), microbial biomass N (B), and dissolved organic N (C) in the profile. ** Significant differences among treatments at p < 0.01, * significant differences among treatments at p < 0.05. OM, organic manure; OM + NF, organic manure with nitrogen fertilizer; NF, nitrogen fertilizer; CK, control.](image)

Irrespective of soil depths, the MBN differences among treatments were significant, and MBN contents were higher with OM and OM + NF treatments (Figure 2B). The MBN contents under OM and OM + NF treatments at 0–10 and 10–20 cm topsoil depths were identical but significantly highest (p < 0.01) over NF and CK treatments. The average MBN contents of OM, OM + NF, and NF treatments were increased by 32.8% (8.14 mg kg\(^{-1}\)), 39.4% (9.77 mg kg\(^{-1}\)), and 20.1% (10.61 mg kg\(^{-1}\)), respectively, when compared to the CK. We found a gradual declining trend in soil MBN contents with increased in profile depths under all treatments.

The variations among fertilizer substitution treatments on soil DON contents were significant across the profile depths (Figure 2C). The DON contents in 0–30 cm topsoil depths of OM and OM + NF treatments were significantly higher (p < 0.01) than other treatments, while NF treatment deposited significantly highest (p < 0.01) DON in 40–100 cm subsoil depths (Figure 2C). The average DON contents in 0–30 cm profile depths under OM, OM + NF, and NF treatments were increased by 77.3% (40.77 mg kg\(^{-1}\)), 46.9%
(24.72 mg kg\(^{-1}\)), and 20.1% (10.61 mg kg\(^{-1}\)), respectively, when compared to the CK. In contrast, the average DON content in 40–100 cm subsoil depths of NF treatment was 53.7% (31.75 mg kg\(^{-1}\)), 49.1% (29.91 mg kg\(^{-1}\)), and 83.2% (41.26 mg kg\(^{-1}\)) greater than OM, OM + NF, and CK treatments, respectively. The DON contents at 30–40 cm depth of OM, OM + NF, and NF treatments were identical but significantly higher \((p < 0.01)\) than CK treatment.

### 3.4. Mineral N Pools

The variations among treatments on NO\(_3^-\) and NH\(_4^+\) content were significant across the selected profile (Figure 3A, B). The NF treatment had the highest (23.9 mg kg\(^{-1}\)) average NO\(_3^-\) content in the profile, which was 21.4% (4.36 mg kg\(^{-1}\)), 13.1% (3.33 mg kg\(^{-1}\)), and 696.7% (20.89 mg kg\(^{-1}\)) higher than OM, OM + NF, and CK treatments, respectively. However, the average treatment effect on NO\(_3^-\) content in 0–30 cm topsoil layers was OM > OM + NF > NF > CK while below 30 cm soil layers NO\(_3^-\) accumulation followed NF > OM + NF > OM > CK (Figure 3A). Compared to CK, the average NO\(_3^-\) content in 0–30 cm topsoil layers of OM, OM + NF, and NF treatments were increased by 912.9% (33.71 mg kg\(^{-1}\)), 532.1% (19.65 mg kg\(^{-1}\)), and 180% (6.65 mg kg\(^{-1}\)). On the other hand, the NF soil accumulated 85.8% (187.53 mg) of profile total NO\(_3^-\) in 30–100 cm profile depths, which was an average 145.6% (17.63 mg kg\(^{-1}\)), 47.63% (9.51 mg kg\(^{-1}\)), and 998.7% (27.3 mg kg\(^{-1}\)) higher than OM, OM + NF, and CK treatments, respectively. The NO\(_3^-\) content differences among OM, OM + NF, and NF treatments at 30–40 cm depth were non-significant.

![Figure 3](image-url) Effect of five years of continuous N equivalent fertilizer substitution treatments on the depth distribution of NO\(_3^-\) (A) and NH\(_4^+\) (B) in the soil profile. ** Significant differences among treatments at \(p < 0.01\). * Significant differences among treatments at \(p < 0.05\). OM, organic manure; OM + NF, organic manure with nitrogen fertilizer; NF, nitrogen fertilizer; CK, control.

Irrespectively of soil depths, NH\(_4^+\) contents of OM treatment were significantly highest (Figure 3B). The average NH\(_4^+\) content of OM treatment was 2.52 mg kg\(^{-1}\), which was 55.9% (0.86 mg kg\(^{-1}\)), 51.4% (0.84 mg kg\(^{-1}\)), and 36.7% (0.67 mg kg\(^{-1}\)) greater than OM + NF, NF, and CK treatments, respectively. The NH\(_4^+\) content of OM + NF treatment was significant \((p < 0.05)\) only at 0–10 and 10–20 cm topsoil depths (Figure 3B), an average 26.4% (0.5 mg kg\(^{-1}\)) and 24.1% (0.47 mg kg\(^{-1}\)) higher than NF and CK treatments, respectively. However, the average NH\(_4^+\) contents OM + NF and NF treatments in 20–100 cm soil depths were 64.4% (0.96 mg kg\(^{-1}\)) and 50.4% (0.82 mg kg\(^{-1}\)) lower than OM treatment,
respectively. Soil NH$_4^+$ variations between NF and CK treatments were identical ($p > 0.05$) in all depths of the selected profile.

3.5. Correlations among STN and Its Fractions

The STN was positively and significantly correlated with its labile organic N (PON, MBN, and DON) and mineral N (NO$_3^-$ and NH$_4^+$) fractions in topsoil layers (0–10 and 10–20 cm) (Table 1). Among the labile organic N fractions, the PON showed the highest correlation with STN in the topsoil layers, followed by DON and MBN. The correlation between STN and NO$_3^-$ was more significant than between STN and NH$_4^+$. The labile organic N fractions showed a higher correlation with NO$_3^-$ than NH$_4^+$ in topsoil layers. However, correlations among the parameters were weak and non-significant in most depths below 20 cm of the selected soil profile.

Table 1. Correlations (Pearson’s) among STN and its fractions in 0–10 and 10–20 cm soil depths.

| Parameter | STN | PON | MBN | DON | NO$_3^-$ | NH$_4^+$ |
|-----------|-----|-----|-----|-----|----------|----------|
| 0–10 cm   |     |     |     |     |          |          |
| STN       | 1   |     |     |     |          |          |
| PON       | 0.952 ** | 1   |     |     |          |          |
| MBN       | 0.795 ** | 0.861 ** | 1   |     |          |          |
| DON       | 0.800 ** | 0.747 ** | 0.631 * | 1   |          |          |
| NO$_3^-$  | 0.903 ** | 0.933 ** | 0.802 ** | 0.845 ** | 1        |          |
| NH$_4^+$  | 0.728 ** | 0.795 ** | 0.805 ** | 0.789 ** | 0.896 ** | 1        |

| 10–20 cm  |     |     |     |     |          |          |
| STN       | 1   |     |     |     |          |          |
| PON       | 0.901 ** | 1   |     |     |          |          |
| MBN       | 0.752 ** | 0.839 ** | 1   |     |          |          |
| DON       | 0.760 ** | 0.692 * | 0.621 * | 1   |          |          |
| NO$_3^-$  | 0.934 ** | 0.883 ** | 0.848 ** | 0.889 ** | 1        |          |
| NH$_4^+$  | 0.844 ** | 0.842 ** | 0.802 ** | 0.561 | 0.787 ** | 1        |

* STN, soil total N; PON, particulate organic N; MBN, microbial biomass N; DON, dissolved organic N.
** Significant at $p < 0.01$, * Significant at $p < 0.05$.

4. Discussion

4.1. Effect of Fertilizer Substitution Treatments on STN, SOC, and Organic C/TN Ratio

Our study suggests that chemical to organic fertilizer substitutions (OM and OM + NF treatments) increases soil total N (STN) content in topsoil layers which is in agreement with previous studies [22,43,57]. The possible reason can be attributed to the significant influence of continuous manuring on soil N fractions because continuous manure inputs (exclusive or combined with chemical fertilizer) increase soil organic matter (SOM) content [22,43]. SOM could enhance soil microbial abundance and activity by providing C sources for microbial metabolism [58]. As the SOM decomposes, it releases particulate organic matter (POC and PON) and dissolved organic matter (DOC and DON) along with mineral nutrients [59–61]. The leftovers (concentrations) of those nitrogenous compounds (PON, MBN, and DON) that remained in the topsoil profile following soil–microorganism–plant interactions were higher under the OM and OM + NF treatments, likely contributed to STN storage. The positive and significant correlations found among STN and its fractions support this assumption. Previous research has found that the STN content is closely related to soil N availability, which is consistent with our findings [62]. However, we did not find a major improvement in STN content under the NF soil profile. Previous research found that in a chemically fertilized maize-based cropping system, aboveground N uptake was significantly greater than N immobilization [63]. Moreover, the SOC content under the NF soil profile was substantially lower compared to the OM and OM + NF soil profiles. Therefore, high aboveground N uptake by crops along with low SOC content possibly affected the microbial N immobilization of NF soil. Moreover, due to maize residue removal (in the current study), crop litter or root exudation contributions under NF treatment might
be limited to maintaining SOM status and supporting soil aggregation (due to reduced PON content). As a result, most fertilizer N that remains at the topsoil profile after crop uptake and microbial utilization is perhaps not immobilized but leached down to subsoil layers or contributed to other soil N loss pathways.

Composed cattle manure is a rich source of organic C. Therefore, by substituting N fertilizer with manure-containing treatments (OM or OM + NF) in the wheat–maize growing system for five years, we found significant increase in SOC contents in the profile, specifically in 0–20 cm topsoil depths. Many previous studies with organic–inorganic fertilization also reported substantial improvement in SOC with manure-containing treatments compared to the exclusive chemical fertilization [17,23]. Our results found an improvement ($p < 0.05$) in SOC contents in 20–50 cm subsoil depths of NF treatment than CK. The possible reason might be attributed to increased rhizodeposition and belowground biomass production of wheat–maize cropping system under N fertilization. However, average SOC contents in the profile of OM and OM + NF treatments were 35.9% (2.95 g kg$^{-1}$) and 13.5% (1.11 g kg$^{-1}$) greater than the NF treatment. Such SOC changes explained in this paper indicate that organic manure substitutions for chemical N fertilizer can play a key role in improving soil fertility and productivity through soil C sequestration.

Soil organic C/TN ratio represents the interaction of soil C and N cycling and the stability of SOM [64]. Nitrogen fertilization increases SOM stock in maize-based cropping systems by influencing net primary production and rhizodeposition, affecting soil C/N ratio through SOC changes [65]. Thus, irrespective of N sources applied (OM, OM + NF, or NF), we found an improvement in soil organic C/TN ratios in the profile. Still, the average soil organic C/TN ratio of the OM and OM + NF treatments was higher than the NF treatment. The main reason for that is likely the constant input of high C/N organic sources, because manure-containing treatments (OM and OM + NF) increased the average SOC content in the profile substantially higher than NF treatment alone. However, translocation of high C/N organic compounds from topsoil layers to subsoil layers with percolating water from irrigation or rainfall could contribute to higher organic C/TN distribution in subsoil layers as found under OM and OM + NF treatments. Meanwhile, deposition of clay associated low C/N containing SOM clay fractions in subsoil layers likely came up with the gradual declination of soil organic C/TN ratios with an increase in profile depths as previously suggested [25,66].

4.2. Effect of Fertilizer Substitution Treatments on Labile Organic N Pools

After a 5-year substitution of chemical fertilizer with organic manure, we found significant ($p < 0.01$) improvement in PON content mostly at topsoil depths (0–10 and 10–20 cm) of the profile. Qiu et al. (2016) and Hai et al. (2010) also found higher PON content with manure containing treatments than chemical fertilization alone [43,67]. The PON contents were highest with OM treatment, second by OM + NF treatment, and marginal with NF treatment, indicating that the organic manure was the primary factor affecting N concentration in particulate organic matter (POM). Previous studies agree with our explanation [67,68]. Manure application increases soil organic matter (SOM) content, whose turnover releases particulate organic matter (POM; POC and PON), which can remain in topsoil layers for several years due to their short turnover time (<10 years) [22,59,69,70]. Moreover, POM being associated with clay minerals promotes soil aggregation, thus could reduce PON turnover by strengthening its physical protection against microbial oxidation [44]. Hence N concentration in aggregate associated POM is greater than free POM [67]. These findings indicate that higher PON accumulation in topsoil layers of OM and OM + NF treatments likely facilitated through manure-induced soil aggregation. However, soils under NF and CK treatments probably had reduced SOM and soil aggregates due to limited organic C sources. Consequently, lower PON contents were observed in those profiles.

The differences in soil MBN content among fertilizer substitution treatments were significant across the selected profile. MBN contents were higher with manure-containing treatments in all sampling depths, and variations between OM and OM + NF treatments
were insignificant except at 20–30 cm soil depth. Conversely, the NF treatment had no significant influence on soil MBN content over the CK treatment. A substantial increase in soil MBN contents with organic manure and organic–inorganic combined fertilizer treatments were reported by Guo et al. (2019) [57]. Liang et al. (2011) and Qiu et al. (2016) also found significantly higher MBN content in topsoil depths under combined fertilization [43,71]. Manure application increases soil microbial abundance and diversity, primarily by improving organic C availability for microbial metabolism [22,46,69]. Moreover, the highest SOC content (found in this study) and constant SOC turnover rate were resultant with organic (manure) or organic–inorganic combined fertilizer treatments [57]. These findings indicate that OM and OM + NF treatments likely supplied readily mineralizable organic carbon for microbial metabolism in a relatively consistent manner, possibly the main reason for higher MBN contents in those soils. Moreover, conventional tillage (practiced in this study) perhaps accelerated manure effects on microbial growth and activity by increasing aeration. Therefore, applying OM and OM + NF treatments, we found the highest MBN concentrations at topsoil depths (0–10 and 10–20 cm). The higher SOM decomposition, soil respiration, enzyme activities, and biomass content as recorded in topsoil layers of previous studies [72], further supports our assumption. However, some dissolved organic C (DOC) in manure soils can be deposited to deeper layers by earthworm borrow, decaying root holes, and leaching [73]. Such deposition of DOC likely leads to microbial biomass distribution in the subsoil layers. Apparently, soil microbial growth and activity were reduced by limited microbial resources (organic C, in particular) under NF or CK treatments. As a result, no significant improvement in MBN content was noticed in those profiles.

The DON contents in topsoil depths were substantially increased with OM and OM + NF treatments, while in subsoil depths (below 40 cm), DON contents were dominated ($p < 0.01$) by NF treatment. These variations among treatments clearly indicate that substituting organic manure for chemical fertilizer improves soil DON content and increases the DON retention capacity of the soil. SOM is the major source of DOM (DOC and DON) [61]. The DOM being released from decomposing SOM could incorporate into the soil aggregates due to their reactive nature to soil particles [44,60]. Therefore, improved soil aggregation and aggregate stability under manure treatment likely promoted higher accumulation of DON in topsoil layers of OM and OM + NF treatments. However, DON is highly mobile and significant DON leaching was often reported under N fertilization [34,40]. We also found significant DON deposition in all selected depths of OM, OM + NF, and NF treatments over the CK (control). However, average DON deposition in 40–100 cm subsoil depths of chemical fertilizer (NF) treatment was 49.1% (29.91 mg kg$^{-1}$) to 53.7% (31.75 mg kg$^{-1}$) greater than its organic substitutes. In other words, chemical fertilizer substitution with organic fertilizer substantially reduced potentially leachable DON content in the profile. Although root exudates and turnover are likely the primary sources of DON in chemically fertilized agricultural soils, the exact mechanism of how chemical fertilizer influences DON distribution is unclear [74,75]. Still, less availability of leached DON for plant and microbes than mineral N forms, as previously found in agroecosystems [40], could cause DON accumulation in subsoil layers.

4.3. Effect of Fertilizer Substitution Treatments on Mineral N Pools

Our research indicated that NO$_3^-$ contents in 0–30 cm topsoil depths were increased ($p < 0.01$) with chemical to organic fertilizer substitution (OM or OM + NF), while NO$_3^-$ depositions in 40–100 cm subsoil depths were enhanced ($p < 0.01$) with exclusive nitrogen fertilizer (NF) treatment. Organic substitutions retained significantly higher NO$_3^-$ in surface layers, possibly for manure-induced enhanced soil aggregation and SOM content, which could provide some physical protection against leaching. Moreover, soil NO$_3^-$ held in the aggregates is covered from microbial reduction, which may further assist in retaining NO$_3^-$ in the topsoil profile. Conversely, the relatively poor soil conditions under continuous chemical fertilization likely had inadequate physical protection against NO$_3^-$ leaching from the persistent downward flow of water from precipitation or irriga-
tion, because percolating water can readily translocate NO$_3^-$ to deep soil layers [76]. As a result, the significantly highest NO$_3^-$ accumulation occurred in subsoil layers of chemical fertilizer treatment.

Although soil NH$_4^+$ has multiple fates, nitrification is believed to be a single major fate of available NH$_4^+$ due to cultivated soils’ high nitrification potential [77]. Moreover, soil microbes prefer NH$_4^+$ for N assimilation than other N forms [78]. Therefore, lower available NH$_4^+$ contents compared to NO$_3^-$ were often reported in soil fertility studies [22,43]. The report presented in this paper is also in line with previous studies. In our selected profiles, the depth distribution of available NH$_4^+$ ranged between 1.34 and 2.96 mg kg$^{-1}$ soil. Still, OM treatment contained significantly higher NH$_4^+$ in all selected depths. Gradually released NH$_4^+$ from organic manure likely supported more active microbial biomass with greater N demand that was probably met by NO$_3^-$ immobilization. Thus, the turnover of high microbial biomass could contribute to the soil NH$_4^+$ pool. Furthermore, because NH$_4^+$ and K$^+$ compete for the same ion-exchange sites of 2:1 clay minerals due to their identical size and valence properties [79,80], the potassium (K) content (8.0 g kg$^{-1}$) of applied manure could also contribute to the release of clay fixed NH$_4^+$ in subsoil depths of OM treatment.

4.4. The Correlations among STN and Its Fractions

The correlations among STN and its fractions were positive and significant only at 0–10 and 10–20 cm surface layers (Table 1), most possibly for topsoil properties (the higher microbial abundance and activity with enhanced resource availability) that support SOM decomposition and nutrient mineralization [81,82]. These correlations indicate that STN was the primary determinant of profile’s labile organic N (PON, MBN, and DON) and mineral N (NO$_3^-$ and NH$_4^+$) content. In other words, changes (positive or negative) in labile organic N and mineral N pools by soil management practices could impact soil total N stocks. As microbial turnover of particulate organic matter releases dissolved organic matter, that influences soil microbial biomass [59,83,84]; the positive and significant correlations found among PON, MBN, and DON confirm that they are closely linked.

5. Conclusions

Our observations revealed that chemical N fertilizer, when 100% substituted with organic manure, exhibited the most significant improvements in STN, SOC, labile organic N (PON, MBN, and DON), and mineral N (NO$_3^-$ and NH$_4^+$) content of the profile, especially in 0–30 cm topsoil layers. Organic manure combined with chemical fertilizer (50% substitution) moderately improved topsoil labile organic and mineral N pools, still significant over chemical N fertilizer treatment. Application of chemical fertilizer alone showed little or no improvement in STN, PON, and MBN content of the profile but significantly increased DON and NO$_3^-$ concentration in subsoil layers leading to a potential risk of N leaching and groundwater contaminations. As labile organic pools are the early indicators of long-term changes in stabilized nutrient pools, our findings suggest that chemical fertilizer substitution with organic manure (100% or 50%) could improve the sustainability of intensively managed farming systems by improving labile organic N and mineral N pools while reducing the potential risk of N leaching. However, we recommend 50% organic substitution for chemical fertilizer because it improves topsoil N pools significantly as well as substantially reduces leachate (DON, NO$_3^-$, and NH$_4^+$) deposition in deep soil.

**Author Contributions:** M.E.H. conceptualized and investigated the study and prepared the original draft of the manuscript with contributions from all co-authors. X.M. and E.L. performed supervision, project administration, funding acquisition, and resources. W.Z. and W.D. designed the study and managed the resources. M.E.H., Z.Y. and X.L. performed methodology and formal analysis. S.R., S.G. and all co-authors participated in result analysis and the writing—review and editing. All authors have read and agreed to the published version of the manuscript.
**References**

1. Zörb, C.; Ludewig, U.; Hawkesford, M.J. Perspective on wheat yield and quality with reduced nitrogen supply. *Trends Plant Sci.* **2018**, 23, 1029–1037. [CrossRef] [PubMed]

2. Barraclough, P.B.; Lopez-Bellido, R.; Hawkesford, M.J. Genotypic variation in the uptake, partitioning and remobilisation of nitrogen during grain-filling in wheat. *Field Crops Res.* **2014**, 156, 242–248. [CrossRef]

3. Xue, C.; Erley, G.S.A.; Rücker, S.; Koehler, P.; Obenauf, U.; Mühling, K.H. Late nitrogen application increased protein concentration but not baking quality of wheat. *J. Plant. Nutr. Soil Sci.* **2016**, 179, 591–601. [CrossRef]

4. Sainju, U.M.; Lenssen, A.W.; Caesar-TonThat, T.; Jabro, J.D.; Larrey, R.T.; Evans, R.G.; Allen, B.L. Dryland soil nitrogen cycling influenced by tillage, crop rotation, and cultural practice. *Nutr. Cycl. Agroecosyst.* **2012**, 93, 309–322. [CrossRef]

5. Ohyama, T. Nitrogen as a major essential element of plants. In *Nitrogen Assimilation in Plants*; Ohyama, T., Sueyoshi, K., Eds.; Research Signpost: Kerala, India, 2010; pp. 1–18.

6. Gao, B.; Ju, X.; Su, F.; Meng, Q.; Onema, O.; Christie, P.; Chen, X.; Zhang, F. Nitrous oxide and methane emissions from optimized and alternative cereal cropping systems on the North China Plain: A two-year field study. *Sci. Total Environ.* **2014**, 472, 112–124. [CrossRef]

7. Gu, X.B.; Cai, H.J.; Du, Y.D.; Li, Y.N. Effects of film mulching and nitrogen fertilization on rhizosphere soil environment, root growth and nutrient uptake of winter oilseed rape in northwest China. *Soil Till. Res.* **2019**, 187, 194–203. [CrossRef]

8. Guo, J.H.; Liu, X.J.; Zhang, Y.; Shen, J.L.; Han, W.X.; Zhang, W.F.; Christie, P.; Goulding, K.W.T.; Vitousek, P.M.; Zhang, F.S. Significant acidification in major Chinese croplands. *Science* **2010**, 327, 1008. [CrossRef] [PubMed]

9. Nivelle, E.; Verzeaux, J.; Chabot, A.; Roger, D.; Chesnais, Q.; Amelina, A.; Lacoux, J.; Nova-Saucedo, J.E.; Tétu, T.; Catterou, M. Effects of glyphosate application and nitrogen fertilization on the soil and the consequences on aboveground and belowground interactions. *Geoderma* **2018**, 311, 45–57. [CrossRef]

10. Shen, W.; Lin, X.; Shi, W.; Min, J.; Gao, N.; Zhang, H.; Yin, R.; He, X. Higher rates of nitrogen fertilization decrease soil enzyme activities, microbial functional diversity and nitrification capacity in a Chinese polytunnel greenhouse vegetable land. *Plant Soil* **2010**, 337, 137–150. [CrossRef]

11. Raun, W.; Solie, J.; Johnson, G.; Stone, M.; Mullen, R.; Freeman, K.; Thomason, W.; Lukina, E. Improving nitrogen use efficiency in cereal grain production with optical sensing and variable rate application. *Agron. J.* **2002**, 94, 815–820. [CrossRef]

12. Raun, W.R.; Johnson, G.V. Improving nitrogen use efficiency for cereal production. *Agron. J.* **1999**, 91, 357–363. [CrossRef]

13. Hu, C.; Anapalli, S.; Green, T.; Ma, L.; Li, X.; Ahuja, L. Evaluating nitrogen and water management in a double-cropping system using RZWQM. * Vadose Zone J.* **2006**, 5, 493–505. [CrossRef]

14. Reay, D.S.; Davidson, E.A.; Smith, K.A.; Smith, P.; Melillo, J.M.; Dentener, F.; Crutzen, P.J. Global agriculture and nitrous oxide emissions. *Nat. Clim. Chang.* **2012**, 4, 410–416. [CrossRef]

15. Cai, A.; Xu, M.; Wang, B.; Zhang, W.; Liang, G.; Hou, E.; Luo, Y. Manure acts as a better fertilizer for increasing crop yields than synthetic fertilizer does by improving soil fertility. *Soil Till. Res.* **2019**, 189, 168–175. [CrossRef]

16. Sistani, K.R.; Jn-Baptiste, M.; Simmons, J.R. Corn response to enhanced-efficiency nitrogen fertilizers and poultry litter. *Agron. J.* **2014**, 106, 761–770. [CrossRef]

17. Du, Y.; Cui, B.; Zhang, Q.; Wang, Z.; Sun, J.; Niu, W. Effects of manure fertilizer on crop yield and soil properties in China: A meta-analysis. *Catena* **2020**, 193, 104617. [CrossRef]

18. Lal, R. Enhancing crop yields in the developing countries through restoration of the soil organic carbon pool in agricultural lands. *Land Degrad. Dev.* **2006**, 17, 197–209. [CrossRef]
19. Diacono, M.; Montemurro, F. Long-term effects of organic amendments on soil fertility: A review. *Agron. Sustain. Dev.* 2010, 30, 401-422. [CrossRef]

20. He, Z.; Pagliarini, P.H.; Waldrip, H.M. Applied and environmental chemistry of animal manure: A review. *Pedosphere* 2016, 26, 779-816. [CrossRef]

21. Gai, X.; Liu, H.; Liu, J.; Zhai, L.; Yang, B.; Wu, S.; Ren, T.; Lei, Q.; Wang, H. Long-term benefits of combining chemical fertilizer and manure applications on crop yields and soil carbon and nitrogen stocks in North China Plain. *Agric. Water Manag.* 2018, 208, 384-392. [CrossRef]

22. Yang, Q.; Zheng, F.; Jia, X.; Liu, P.; Dong, S.; Zhang, J.; Zhao, B. The combined application of organic and inorganic fertilizers increases soil organic matter and improves soil microenvironment in wheat-maize field. *J. Soils Sediments* 2020, 20, 2395-2404. [CrossRef]

23. Li, K.; Wang, C.; Li, X.; Li, H.; Dong, M.; Jin, S.; Liu, L.; Zhu, C.; Xue, R. Long-term effect of integrated fertilization on maize yield and soil fertility in a calcareous fluvisol. *Arch. Agron. Soil Sci.* 2020, 67, 1400–1410. [CrossRef]

24. Wieder, W.R.; Cleveland, C.C.; Taylor, P.G.; Nemergut, D.R.; Hinckley, E.L.; Philippot, L.; Bru, D.; Weintraub, S.R.; Martin, M.; Townsend, A.R. Experimental removal and addition of leaf litter inputs reduces nitrate production and loss in a lowland tropical forest. *Biogeochemistry* 2013, 113, 629–642. [CrossRef]

25. Zhang, H.; Zhang, Y.; Yan, C.; Liu, E.; Chen, B. Soil nitrogen and its fractions between long-term conventional and no-tillage systems with straw retention in dryland farming in northern China. *Geoderma* 2016, 269, 138–144. [CrossRef]

26. Culman, S.; Snapp, S.; Green, J.; Gentry, L. Short- and long-term labile soil carbon and nitrogen dynamics reflect management and predict corn agronomic performance. *Agron. J.* 2013, 105, 493–502. [CrossRef]

27. Yang, X.; Ren, W.; Sun, B.; Zhang, S. Effects of contrasting soil management regimes on total and labile soil organic carbon fractions in a loess soil in China. *Geoderma* 2012, 177-178, 49–56. [CrossRef]

28. Wen, Z.; Shen, J.; Blackwell, M.; Li, H.; Zhao, B.; Yuan, H. Combined applications of nitrogen and phosphorus fertilizers with manure increase maize yield and nutrient uptake via stimulating root growth in a long-term experiment. *Pedosphere* 2016, 26, 62–73. [CrossRef]

29. Huang, T.; Ju, X.; Yang, H. Nitrate leaching in a winter wheat-summer maize rotation on a calcareous soil as affected by nitrogen and straw management. *Sci. Rep.* 2017, 7, 42247. [CrossRef]

30. Hao, M.D.; Jun, F.; Wei, X.R.; Pen, L.F.; Lai, L. Effect of fertilization on soil fertility and wheat yield of dryland in the Loess Plateau. *Pedosphere* 2005, 15, 189–195.

31. Yanan, T.; Emteryd, O.; Dianqing, L.; Grip, H. Effect of organic manure and chemical fertilizer on nitrogen uptake and nitrate leaching in a Eum-orthic anthrosols profile. *Nutr. Cycl. Agroecosyst.* 1997, 48, 225–229. [CrossRef]

32. Sainju, U.M.; Lenssen, A.W. Soil nitrogen dynamics under dryland alfalfa and durum-forage cropping sequences. *Soil Sci. Soc. Am. J.* 2011, 75, 669–677. [CrossRef]

33. Liu, X.; Dong, W.; Jia, S.; Liu, Q.; Li, Y.; Hossain, M.E.; Liu, E.; Kuzyakov, Y. Transformations of N derived from straw under long-term conventional and no-tillage soils: A 15N labelling study. *Sci. Total. Environ.* 2021, 786, 147428. [CrossRef]

34. Hussain, M.Z.; Robertson, G.P.; Basso, B.; Hamilton, S.K. Leaching losses of dissolved organic carbon and nitrogen from agricultural soils in the upper US Midwest. *Sci. Total. Environ.* 2020, 734, 139379. [CrossRef] [PubMed]

35. Campbell, J.L.; Hornbeck, J.W.; McDowell, W.H.; Buso, D.C.; Shanley, J.B.; Likens, G.E. Dissolved organic nitrogen budgets for upland, forested ecosystems in New England. *Biogeochemistry* 2000, 49, 123–142. [CrossRef]

36. Kiikkilä, O.; Smolander, A.; Kitunen, V. Degradability, molecular weight and adsorption properties of dissolved organic carbon and nitrogen leached from different types of decomposing litter. *Plant. Soil* 2013, 373, 787–798. [CrossRef]

37. Qualls, R.G.; Haines, B.L.; Swank, W.T.; Tyler, S.W. Soluble organic and inorganic nutrient fluxes in clearcut and mature deciduous forests. *Soil Sci. Soc. Am. J.* 2000, 64, 1068–1077. [CrossRef]

38. Hoeft, I.; Keuter, A.; Quiñones, C.M.; Schmidt-Walter, P.; Veldkamp, E.; Corre, M.D. Nitrogen retention efficiency and nitrogen losses of a managed and phytodiverse temperate grassland. *Basic Appl. Ecol.* 2014, 15, 207–218. [CrossRef]

39. Riaz, M.; Mian, I.A.; Bhatti, A.; Cresser, M.S. An exploration of how litter controls drainage water DIN, DON and DOC dynamics in freely draining acid grassland soils. *Biogeochemistry* 2012, 107, 165–185. [CrossRef]

40. Salazar, O.; Balboa, L.; Peralta, K.; Rossi, M.; Casanova, M.; Tapia, Y.; Singh, R.; Que mada, M. Effect of cover crops on leaching of dissolved organic nitrogen and carbon in a maize-cover crop rotation in Mediterranean Central Chile. *Agric. Water Manag.* 2019, 212, 399–406. [CrossRef]

41. van Kessel, C.; Clough, T.; van Groenigen, J.W. Dissolved organic nitrogen: An overlooked pathway of nitrogen loss from agricultural systems? *J. Environ. Qual.* 2009, 38, 393–401. [CrossRef]

42. Macdonald, B.C.T.; Ringrose-Voase, A.J.; Nadelko, A.J.; Farrell, M.; Tuomi, S.; Nachimuthu, G. Dissolved organic nitrogen contributes significantly to leaching from furrow-irrigated cotton-wheat-maize rotations. *Soil Res.* 2017, 55, 70–77. [CrossRef]

43. Qiu, S.; Gao, H.; Zhu, P.; Hou, Y.; Zhao, S.; Rong, X.; Zhang, Y.; He, P.; Zhou, W. Changes in soil carbon and nitrogen pools in a Mollisol after long-term fallow or application of chemical fertilizers, straw or manures. *Soil Till. Res.* 2016, 163, 255–265. [CrossRef]

44. Wander, M. Soil organic matter fractions and their relevance to soil function. In *Soil Organic Matter in Sustainable Agriculture*; Magdoff, F.R., Weil, R.R., Eds.; CRC Press: Boca Raton, FL, USA, 2004; pp. 67–102.
45. Wei, W.; Yan, Y.; Cao, J.; Christie, P.; Zhang, F.; Fan, M. Effects of combined application of organic amendments and fertilizers on crop yield and soil organic matter: An integrated analysis of long-term experiments. Agric. Ecosystems. Environment. 2016, 225, 86–92. [CrossRef]

46. Liu, E.; Yan, C.; Mei, X.; Zhang, Y.; Fan, T. Long-term effect of manure and fertilizer on soil organic carbon pools in dryland farming in Northwest China. PloS ONE 2013, 8, e65536. [CrossRef]

47. Wander, M.M.; Drinkwater, L.E. Fostering soil stewardship through soil quality assessment. Appl. Soil Ecol. 2000, 15, 61–73. [CrossRef]

48. FAO-Unesco. Soil Map of the World 1:5,000,000; Legend: Paris, France, 1974; Volume 1.

49. Liu, C.; Wang, K.; Zheng, X. Responses of N₂O and CH₄ fluxes to fertilizer nitrogen addition rates in an irrigated wheat-maize cropping system in northern China. Biogeoosciences 2012, 9, 839–850. [CrossRef]

50. Bremer, E.H.; Mulvaney, C.S. Nitrate and exchangeable ammonium nitrogen. In Methods of Soil Analysis, Page, A.L., Miller, R.H., Keeny, D.R., Eds.; American Society of Agronomy and Soil Science Society of America: Madison, WI, USA, 1982; pp. 1119–1123.

51. Blake, C.A. Methods of Soil Analysis: Part I and II; American Society of Agronomy: Madison, WI, USA, 1965.

52. Bronson, K.F.; Zobeck, T.M.; Chua, T.T.; Acosta-Martinez, V.; van Pelt, R.S.; Booker, J.D. Carbon and nitrogen pools of southern high plains cropland and grassland soils. Soil Sci. Soc. Am. J. 2004, 68, 1695–1704. [CrossRef]

53. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 1987, 19, 703–707. [CrossRef]

54. Jenkinson, D.S. Determination of microbial biomass carbon and nitrogen in soil. In Advances in Nitrogen Cycling in Agricultural Ecosystems; Wilson, J.R., Ed.; CAB International: Wallingford, UK, 1988; pp. 368–386.

55. Gigliotti, G.; Kaiser, K.; Guggenberger, G.; Haumaier, L. Difference in the chemical composition of dissolved organic matter from waste material of different sources. Biol. Fertil. Soils 2002, 36, 321–328. [CrossRef]

56. Maynard, D.G.; Kalra, Y.P.; Crumbaugh, J.A. Nitrate and exchangeable ammonium nitrogen. In Methods of Soil Analysis, Page, A.L., Miller, R.H., Keeny, D.R., Eds.; American Society of Agronomy and Soil Science Society of America: Madison, WI, USA, 1982; pp. 1119–1123.

57. Zhao, J.; Ni, T.; Li, Y.; Xiong, W.; Ran, W.; Shen, B.; Shen, Q.; Zhang, R. Responses of bacterial communities in arable soils in a rice-wheat cropping system to different fertilizer regimes and sampling times. PloS ONE 2014, 9, e85301. [CrossRef]

58. Haynes, R.J.; Bernacchi, C.J. Laboratory analysis of soil organic matter fractions: A review. Adv. Agron. 2005, 85, 221–268.

59. Gmach, M.R.; Cherubin, M.R.; Kaiser, K.; Cerri, C.E.P. Processes that influence dissolved organic matter in the soil: A review. Sci. Agric. 2020, 77, 1–10. [CrossRef]

60. Filep, T.; Rekási, M. Factors controlling dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and DOC/DON ratio in arable soils based on a dataset from Hungary. Geoderma 2011, 162, 312–318. [CrossRef]

61. Kicklighter, D.W.; Melillo, J.M.; Monier, E.; Sokolov, A.P.; Zhuang, Q. Future nitrogen availability and its effect on carbon sequestration in Northern Eurasia. Nat. Commun. 2019, 10, 3024. [CrossRef]

62. Duan, Y.; Xu, M.; Wang, B.; Yang, X.; Huang, S.; Gao, S. Long-term evaluation of manure application on maize yield and nitrogen use efficiency in China. Soil Sci. Soc. Am. J. 2011, 75, 1562–1573. [CrossRef]

63. Russell, A.E.; Laird, D.A.; Parkin, T.B.; Mallarino, A.P. Impact of Nitrogen Fertilization and Cycling System on Carbon Sequestration in Midwestern Mollisols. Soil Sci. Soc. Am. J. 2005, 69, 413–422. [CrossRef]

64. Poffenbarger, H.J.; Parker, D.W.; Helmers, M.J.; Miguez, F.E.; Olk, D.C.; Sawyer, J.E.; Six, J.; Castellano, M.J. Maximum soil organic carbon storage in Midwest, U.S. cropping systems when crops are optimally nitrogen-fertilized. PLoS ONE 2017, 12, e0172293. [CrossRef]

65. Lou, Y.; Xu, M.; Chen, X.; He, X.; Zhao, K. Stratification of soil organic C, N and C:N ratio as affected by conservation tillage in two maize fields of China. Cateura 2012, 14, 124–130. [CrossRef]

66. Hai, L.; Li, X.G.; Li, F.M.; Suo, D.R.; Guggenberger, G. Long-term fertilization and manuring effects on physically-separated soil organic matter pools under a wheat-wheat-maize cropping system in an arid region of China. Soil Biol. Biochem. 2010, 42, 253–259. [CrossRef]

67. Gong, W.; Yan, X.Y.; Cai, Z.C.; Wang, J.Y.; Hu, T.X.; Gong, Y.B.; Ran, H. Effects of long-term fertilization on soil particle organic carbon and nitrogen in a wheat-maize cropping system. J. Appl. Ecol. 2008, 19, 2375–2381.

68. Luo, H.; Gao, W.; Huang, S.; Tang, J.; Li, M.; Zhang, H.; Chen, X.; Masiliuñas, D. Substitution of manure for chemical fertilizer affects soil microbial community diversity, structure and function in greenhouse vegetable production systems. PLoS ONE 2020, 15, e0214041. [CrossRef] [PubMed]

69. Janzen, H.H.; Campbell, C.A.; Ellert, B.H.; Bremer, E. Soil organic matter dynamics and their relationship to soil quality. In Soil Quality for Crop Production and Ecosystem Health; Gregorich, E.G., Carter, M.R., Eds.; Elsevier: Amsterdam, The Netherlands, 1997; pp. 277–291.

70. Jiang, B.; Yang, X.; He, X.; Zhou, J. Effects of 17-year fertilization on soil microbial biomass C and N and soluble organic C and N in loessial soil during maize growth. Biol. Fertil. Soils 2011, 47, 121–128. [CrossRef]

71. Zhen, Z.; Liu, H.; Wang, N.; Guo, L.; Meng, J.; Ding, N.; Wu, G.; Jiang, G. Effects of manure compost application on soil microbial community diversity and soil microenvironments in a temperate cropland in China. PLoS ONE 2014, 9, e108555.
73. Lorenz, K.; Lal, R. The depth distribution of soil organic carbon in relation to land use and management and the potential of carbon sequestration in subsoil horizons. *Adv. Agron.* 2005, 88, 35–66.
74. Christou, M.; Avramidis, E.J.; Jones, D.L. Dissolved organic nitrogen dynamics in a Mediterranean vineyard soil. *Soil Biol. Biochem.* 2006, 38, 2265–2277. [CrossRef]
75. Kalbitz, K.; Solinger, S.; Park, J.H.; Michalzik, B.; Matzner, E. Controls on the dynamics of dissolved organic matter in soils: A review. *Soil Sci.* 2000, 165, 277–304. [CrossRef]
76. Yang, X.; Lu, Y.; Tong, Y.A.; Yin, X. A 5-year lysimeter monitoring of nitrate leaching from wheat-maize rotation system: Comparison between optimum N fertilization and conventional farmer N fertilization. *Agric. Ecosyst. Environ.* 2015, 199, 34–42. [CrossRef]
77. Robertson, P.G. Nitrogen use efficiency in row-crop agriculture: Crop nitrogen use and soil nitrogen loss. In *Ecology in Agriculture*; Jackson, L.E., Ed.; Elsevier: Amsterdam, The Netherlands; Academic Press: San Diego, CA, USA, 1997; pp. 347–365.
78. Azam, F.; Simmons, F.W.; Mulvaney, R.L. Immobilization of ammonium and nitrate and their interaction with native N in three Illinois Mollisols. *Biol. Fertil. Soils* 1993, 15, 50–54. [CrossRef]
79. Nieder, R.; Benbi, D.K.; Scherer, H.W. Fixation and defixation of ammonium in soils: A review. *Biol. Fertil. Soils* 2011, 47, 1–14. [CrossRef]
80. Scherer, H.W.; Feils, E.; Beuters, P. Ammonium fixation and release by clay minerals as influenced by potassium. *Plant. Soil Environ.* 2014, 60, 325–331.
81. Cheng, W. Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. *Soil Biol. Biochem.* 2009, 41, 1795–1801. [CrossRef]
82. Kuzyakov, Y. Priming effects: Interactions between living and dead organic matter. *Soil Biol. Biochem.* 2010, 42, 1363–1371. [CrossRef]
83. Alvarez, C.R.; Alvarez, R.; Grigera, M.S.; Lavado, R.S. Associations between organic matter fractions and the active soil microbial biomass. *Soil Biol. Biochem.* 1998, 30, 767–773. [CrossRef]
84. Zsolnay, Á. Dissolved organic matter: Artefacts, definitions, and functions. *Geoderma* 2003, 113, 187–209. [CrossRef]