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# Impact of Reduced Nitrogen Inputs on Soil Organic Carbon and Nutrient Dynamics in Arable Soil, Northern Thailand: Short-Term Evaluation

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**Abstract:** Based on a soil analysis of individual crops, lower nitrogen (N) inputs may affect soil fertility and the soil's capacity for carbon sequestration. This study investigates the changes in soil nitrogen levels, the amounts of labile and recalcitrant carbon fractions, and their relationship to soil organic carbon (SOC) over the course of a single crop season. We conducted this study on seven crops in the provinces of Chiang Mai, Lamphun, and Lampang in northern Thailand, from February 2022 to December 2023. The farmer plots, which included litchi, mango, banana, maize, cabbage, garlic, and paddy rice, underwent three nitrogen addition treatments: high-nitrogen fertilizer (FP), reduced-nitrogen fertilizer informed via soil analysis (FS), and fertilizer absence (FZ). Soil samples were collected from a depth of 0 to 30 cm following the harvest of each crop. Subsequently, we utilized these samples to distinguish between labile and recalcitrant carbon fractions and assessed the impact of reduction through a one-way ANOVA. This study indicated a reduced availability of nitrogen, with the recalcitrant carbon fractions being the fine fraction (FF) and less labile carbon (LLB_C). The labile organic carbon fraction, referred to as LB_C, exhibited no change in FP treatment, in contrast to the non-fine fraction (NFF) and permanganate-oxidizable carbon (POXC). Our concern was to reduce the quantity of synthetic nitrogen fertilizer to achieve a lower level of soil organic carbon (SOC) and decreased nitrogen availability. These findings underscore the importance of considering N management when assessing soil carbon dynamics in agricultural soils, and, in future work, we should therefore model the optimal N input for crop yield, soil fertility, and soil carbon storage.

**Keywords:** availability N; recalcitrant carbon; fine fraction; permanganate-oxidizable carbon

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## 1. Introduction

Climate change may increase food demand by 30% to 62% [1] as a result of population growth and rising per capita consumption [2]. Thailand's Climate Change Master Plan (CCMP) outlines its national climate change response, aiming for carbon neutrality by 2065. The Agriculture Strategic Plan on Climate Change (ASPCC) aligns with the CCMP, providing a sector-specific input to the National Adaptation Plan (NAP). Thailand's updated Nationally Determined Contribution (NDC) to the UNFCCC in October 2020 identifies "safeguarding food security" and "promoting sustainable agriculture" as core strategies to address climate change impacts. The updated NDC emphasizes enhanced adaptation

and aims for better integration into national planning processes. Thailand is also enhancing resilience through early warning systems, improving adaptive capacity, and building farmer capabilities [3]. The FAO predicts a 50% increase in synthetic nitrogen fertilizer consumption by 2050, which could escalate greenhouse gas emissions from agricultural soils, potentially 'threatening' the Paris Agreement's goal to limit global warming to 1.5 °C or less [4]. Soil left unfertilized for a long duration store less soil organic carbon (SOC) and has reduced levels of essential elements like nitrogen, phosphorus, and potassium [5,6]. Indeed, unfertilized soils have reduced nutrient usage efficiency, physical features, and carbon sequestration capability [7,8], and their uneven carbon-to-nitrogen ratios negatively affect soil microbial activity and nutrient mineralization [7]. In order to feed the world's expanding population, synthetic fertilizers dramatically increase agricultural yields [9]. In order to increase crop yields and ensure global food security, nitrogen fertilizer is crucial, which boosts farmers' profits all over the globe [10].

Synthetic fertilizers play a crucial role in crop production and food security, but their excessive or imbalanced use can escalate production costs and contribute to environmental pollution [11]. Synthetic nitrogen fertilizers, which significantly contribute to high fertilizer application rates, especially in vegetables and fruits, have a disproportionately large impact [12]. The chemical composition of fertilizers and soil conditions also contribute to $CO_2$ emissions [13], and farmers' increasing use of fertilizer is resulting in an excess of nitrogen in soil [14]. Studies have shown that fertilizers contribute to nutrient dispersion in the environment [15–17], leading to soil pollution, reduced fertility, and increased susceptibility to plant diseases [18]. To mitigate the impacts of climate change through synthetic fertilizer management, reducing nitrogen usage and increasing carbon storage is crucial to lowering greenhouse gas emissions from site-specific nutrient management (SSNM). In Thailand, India, and China, intensive cash crop farmers typically apply excessive nitrogen doses, underscoring the potential for nitrogen reduction through practices such as nutrient efficiency (NE) via SSNM [11]. Optimal nitrogen fertilizer application at 225 kg N $ha^{-1}$ is essential for achieving high grain yields and enhancing carbon sequestration in rice cultivation [19]. Nitrogen management is crucial for sustainable agriculture, food security, and environmental protection, and improving nitrogen use efficiency (NUE) can enhance crop productivity and reduce environmental impacts [20]. Agronomic management practices and high-throughput technologies can significantly improve NUE, reducing intensive nitrogen applications [21]. When used with fertilizers for nitrogen, phosphorus, and potassium, fertilizer may help plants store more SOC. This impact has been seen in a wide variety of crops [22]. Fertilizer at the proper quantities may increase soil organic carbon and soil total carbon reserves rather than providing too much nitrogen. Accordingly, obtaining the maximum C stored in the soil requires careful nitrogen management [23].

Accordingly, a previous study demonstrated that a N addition increased both labile (e.g., particulate organic carbon, POC) and non-labile (e.g., mineral-associated organic carbon, MAOC) SOC fractions, though the extent varies [24]. The authors examined the chemical process [19] that affects the availability of nutrients connected with labile carbon fractions by measuring labile and stable carbon fractions in a number of different ways. Small amounts of nitrogen additions raise the activity of labile C-degradation genes and enzymes, which cause more $CO_2$ to be released and labile carbon to be broken down more quickly [25,26]. This decrease in microbial activity and respiration rates reduces labile carbon content in soil, causing soil acidification and altering microbial community composition. Reducing nitrogen application can also cause soil acidification, which reduces bacterial biomass and affects the stability of the labile carbon pool [27,28]. For this study, it is crucial to utilize the fine carbon fraction (FF) in soil, as it is a stable carbon fraction associated with fine particles such as silt and clay [29,30], and it stabilizes organic carbon, influences soil fertility, and enhances carbon storage capacity [31–33]. When nitrogen is added, mineral-associated organic matter slowly changes into fine particulate organic matter (POM), and the carbon content of fine POM changes in response to nitrogen additions [34]. Adding nitrogen raises SOC in fine soil fractions, such as free light fractions and mineral-

associated fractions, which facilitates the overall build-up of SOC [35]. Conversely, the carbon content in the FF influences the reduction in N additions.

The availability of labile carbon supports the mineralization or priming of soil organic matter (SOM), with soils differing in the extent to which labile C stimulates priming [36]. The availability of nutrients also plays a significant role in this process; $NO_3$ accelerates organic matter decomposition, while $NH_4^+$ has distinct effects [37]. Adding nitrate lowers soil enzyme activities, but adding ammonium can increase these activities, affecting the soil organic carbon (SOC) stability [38]. Ammonium-based fertilization reduces mineralization and nitrification rates, which are positively correlated with SOC and total nitrogen concentration [39]. Labile carbon and phosphorus significantly impact soil organic carbon (SOC) [40]; indeed, labile C, like glucose, has a priming effect that makes SOC minerals form more quickly [34,35], and this property is especially true in the subsoil. P, on the other hand, accelerates SOC mineralization by making microbes work harder [41,42]. Labile C and P can have a significant effect on SOC mineralization [43,44], and P can encourage mineralization in tropical forest soils, while N can prevent priming [45]. K fertilization can significantly increase carbon sequestration in cropland soils, benefiting both corn and wheat production [46].

This study investigated how N fertilization affects the soil analyses of cash crops by comparing typical farmer practices, as well as the different types of soil carbon, including labile and stable soil carbon. Our study also examined the availability of plant nutrients and presented the ratio of labile carbon to the final impacted soil carbon in arable soil. We hypothesized that a decreased amount of the synthesis fertilizer by an SSNM recommendation would not impact nitrogen availability and would not lead to any impacts in soil organic carbon (SOC). The goal of this study was to evaluate how the proposed SSNM's reduction in nitrogen addition leads to changes in soil carbon fraction content and SOC in arable soil.

Understanding how organic carbon fraction content changes with reduced nitrogen fertilizer is critical for managing soil health, optimizing crop production, and mitigating climate change through, for example, carbon storage in soil. These revisions provide a more precise focus and structure for this study.

## 2. Materials and Methods

This experiment was conducted between February 2022 and December 2023. The experimental plots were located in Chiang Mai, Lamphun, and Lampang provinces in upper Northern Thailand. First, by interviewing the plot experiment's owner, we gathered information about individual plots' soil practices. The experimental plots included seven crops: litchi (*Litchi chinensis* L.) (20 years old), mango (*Mangifera indica*) (20 years old), banana (*Musa sapientum*) (2, 3, and 5 years old), maize (*Zea mays*), cabbage (*Brassica oleracea*) (20 years old), garlic (*Allium sativum*) (41 and 17 years old), and paddy rice (*Oryza sativa*) (36 years old). Their land-use age ranged from 3 to 41 years old, and before the new crop season, most farmers left straw and stubble on the field and plowed it. Small machinery was regularly used to perform tillage, roughly twice before planting. After crop harvesting, the crop residues remained in the fields until the new crop season. This study investigated how the synthetic fertilizer suggestions from a soil analysis replaced the way farmers used to fertilize crops during a single season, which caused the N levels in the experiment plots to drop.

We initially conducted a random sampling of the soil from the designated farmer plot to assess the soil organic matter, available phosphorus, and exchangeable potassium for fertilizer recommendations from the Department of Agriculture. Subsequently, the designated plot experiment only compared the nitrogen levels resulting from farmers' nitrogen inputs, which were above the nitrogen levels from SSNM. We applied all management techniques, such as soil preparation, planting time, irrigation, and pest and weed control, to the three treatments on a single plot simultaneously and equally often. For every single crop, we used three plants for each individual treatment, started all the practices at the

same time, and performed the same farmer practices, except for fertilizer input. The soil analysis values determined the nitrogen application, but the chosen plots showed lower nitrogen levels. We ignored using a farmer's plot if its N application fell lower than the soil analysis recommendation and established control plots of identical dimensions in the same location.

*Treatment Allocation:* This experiment included three different fertilizer application patterns, namely, the following: (1) applying fertilizer in accordance with recommendations based on soil analysis results (FS), (2) applying fertilizer in accordance with farmers' practices (FP), and (3) applying no fertilizer at all (FZ), all of which were replicated three times. The number of soil samples for each plot treatment was then 42, and the total number of soil samples for all treatments was 126. These experiments were applied to individual crops, as mentioned earlier, and we therefore applied three different amounts of synthetic fertilizer to a single crop in this experiment. The lone crop was planted, managed, and harvested in a typical amount of time, alongside farmer practices. Additional management procedures, such as pesticide treatment and irrigation, were completed in compliance with customary practices in the area. We emphasized and compared N additions via farmer practices (FPs) and N recommendations based on soil analysis (FS) and found that FS practices decreased the amounts of N added. Therefore, in this study, we extracted the amounts of $P_2O_5$ and $K_2O$ from our treatments, but we also determined the amounts of $P_2O_5$ and $K_2O$ for each individual crop (Table 1). We set up the experiment utilizing farmer plots with sampled individual plots before applying fertilization treatments. At the same time, in addition to farmer practices (FPs), we planted our own maize, cabbage, garlic, and paddy rice for FS. In the meantime, we experimented with the farmer's plants for litchi, mango, and banana, but we adjusted the synthetic fertilizer dosage for FS based on soil analysis. We conducted this study using three treatments and three replications, all within the same plot (owned by the same individual), and, then, we applied the same treatments to more than two to four plots (owners) of the same type of plant. A gathering and summary of the owner's plot interview determines the type of fertilizer used for FP. The interviews with individual plot owners provided information on fertilization practices, such as the type of synthetic fertilizer, timing, and quantity of application. We then converted these data into amounts of N, $P_2O_5$, and $K_2O$ per crop (Table 1). The fertilization recommendation via soil analysis in Thailand is as follows: Based on the soil test results, specific fertilizer formulas can be recommended, including the choice between organic and inorganic fertilizers. For specific recommendations, it is advisable to consult local agricultural extension services or conduct soil testing through certified laboratories to obtain tailored advice based on current soil conditions and crop requirements. Farmers can also determine the timing and method of application to maximize nutrient uptake by crops [47]. According to FS treatment recommendations, this fertilizer formula is normally used because it can provide complete N, $P_2O_5$, and $K_2O$ in accordance with the SSNM concept. This research identifies the kinds of synthetic fertilizers often used in SSNM and FS treatment: urea functions as a nitrogen supply, diammonium phosphate (DAP) supplies both nitrogen and phosphorus, and potassium chloride, referred to as muriate of potash, acts as the potassium source (Table 1).

**Table 1.** Comparing the amounts of N additions via farmer practices (FPs) and N recommendations based on soil analysis (FS) and determining the amounts of $P_2O_5$ and $K_2O$ for every individual crop.

| Crop (No of Plot), | Age: Year | FP | FS | FZ | FP | FS | FZ | FP | FS | FZ | Fertilizer Used in FP Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N (kg ha$^{-1}$) | | | $P_2O_5$ (kg ha$^{-1}$) | | | $K_2O$ (kg ha$^{-1}$) | | | |
| Banana (3) | 2 | 14 | 3 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 46-0-0 |
| | 3 | 14 | 2 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 46-0-0 |
| | 5 | 14 | 2 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 46-0-0 |
| Cabbage (2) | 20 | 575.5 | 93.8 | 0 | 0 | 31.3 | 0 | 0 | 62.5 | 0 | 46-0-0 |
| | 20 | 182.8 | 62.5 | 0 | 182.8 | 31.3 | 0 | 295.3 | 62.5 | 0 | 13-13-21 |

**Table 1.** *Cont.*

| Crop (No of Plot), | Age: Year | FP | FS | FZ | FP | FS | FZ | FP | FS | FZ | Fertilizer Used in FP Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N (kg ha$^{-1}$) | | | $P_2O_5$ (kg ha$^{-1}$) | | | $K_2O$ (kg ha$^{-1}$) | | | |
| Garlic | 17 | 77.5 | 62.5 | 0 | 92.5 | 31.3 | 0 | 112.5 | 31.3 | 0 | 18-24-24,13-13-21 |
| (2) | 41 | 135 | 93.8 | 0 | 135 | 31.3 | 0 | 135.0 | 31.3 | 0 | 18-18-18 |
| Litchi (1) | 20 | 211 | 175 | 0 | 211 | 22 | 0 | 211 | 44.0 | 0 | 15-15-15 |
| Maize | 20 | 168.8 | 31.3 | 0 | 75 | 18.8 | 0 | 75 | 39 | 0 | 46-0-0,15-15-15 |
| (2) | 20 | 115 | 31.3 | 0 | 0 | 62.5 | 0 | 0 | 39 | 0 | 46-0-0 |
| Mango | 20 | 575 | 47 | 0 | 163 | 16 | 0 | 0 | 39 | 0 | 46-0-0,16-20-0 |
| (2) | 20 | 163 | 47 | 0 | 0 | 63 | 0 | 263 | 39 | 0 | 46-0-0,0-0-60 |
| Rice | 36 | 80 | 37.5 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 16-20-0 |
| (2) | 36 | 230 | 37.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 46-0-0 |

**Note**: In FS treatment, the ratios of N, $P_2O_5$, and $K_2O$ applied in synthetic fertilizer form were 46-0-0, 18-46-0, and 0-0-60. Cropping system of individual year: Only one type of crop was present in the studied plots, including banana, litchi, and mango. The individual plots of cabbage, garlic, maize, and rice are cultivated biannually. The number displayed in the light green background area represents the amount of N added to our study.

### 2.1. Soil Sampling and Property Analysis

After harvesting each crop, we obtained soil samples that were indicative of replication. At soil depths of 0–30 cm, an auger was used to drill the soil from a few representative samples. We then brought the soil samples back for air drying and screening, preparing them for chemical and physical examination, and we stored the freshly separated soils at 4 °C until analysis. Due to the absence of fresh soil samples from FZ, the results for phosphorus by malachite green and nitrate ($NO_3^-$) could not be obtained.

### 2.2. Methodology for of Soil Carbon Fraction Pools

*Total organic carbon (TOC):* the amount of total organic carbon (TOC) in the soil by sieving 2 mm of dry soil mixed with $H_2SO_4$ and $K_2Cr_2O_7$, heating it to 130 °C for 30 min, and then letting the mixture sit overnight [48]. We then used the $FeSO_4$ solution to titrate it, following the process described in [49].

*Carbon in the fine fraction (FF, < 0.4 mm) in soil:* The soil samples were air dried, crushed, and separated from plant material using a 2 mm sieve. Large pieces of coarse material were removed, and the dry sieving/winnowing procedure was used to remove light fraction organic material. The residual organic matter in the soil post-process, together with carbon, was designated as fine-fraction soil organic matter (FF < 0.4 mm), believed to contribute to the more stable, slowly decomposing pool of soil organic matter [50]. Dry soil (FF < 0.4 mm) of individual samples was then mixed with $H_2SO_4$ and $K_2Cr_2O_7$, heated to 130 °C for 30 min, and allowed to sit overnight [43]. We then used the $FeSO_4$ solution for titration, following the instructions in [44].

*The P malachite green method as P(M)*: We used the malachite method to extract a solution to determine its P content. To determine the phosphorus content in fresh soil, we mixed a solution of sulfuric acid and ammonium molybdate tetrahydrate with a solution of green malachite green and polyvinyl alcohol. We then measured the absorbance at a wavelength of 630 nm after 30 min [51] using a spectrophotometer with double beam light sources (Thermo spectronic genesys 20, Waltham, MA, USA).

*The Bray (II) method as P (B) in soil is as follows*: The Bray method involves using a solution of 0.1 M HCl and 0.03 M $NH_4F$ to extract phosphorus from the soil, and the extraction time in this study was 60 s. Adequate levels of ammonium, molybdate, boric acid and ascorbic acid then developed a blue color for the P concentration [52], examined via an 880 nm spectrophotometer (Thermo spectronic genesys 20, USA).

*$NH_4^+$ and $NO_3^-$ in soil*: We analyzed the dried and fresh soil samples for $NH_4^+$ and $NO_3^-$ content. We then determined the amounts of $NH_4^+$ and $NO_3^-$ using established procedures and spectrophotometers set at 520 nm and 420 nm, respectively [53]. An easy way to measure $NH_4^+$ is by looking at the color that forms when ammonium, a chlorine

source, and a slightly acidic mixture of Na salicylate react; i.e., Na-nitroprusside intensifies the green hue [54]. We extracted $NO_3^-$ from soil samples using potassium sulfate at a concentration of 0.5 M. The soil's $NO_3$ underwent a reaction with salicylic acid and concentrated sulfonic acid. We determined the $NH_4^+$ and $NO_3^-$ using a spectrophotometer (Thermo spectronic genesys 20, USA).

*The permanganate-oxidizable carbon (POXC):* We modified the Weil et al. [55] method to extract the permanganate-oxidizable carbon (POXC), also known as active carbon, and then tested it. In a 50 mL centrifuge tube, we placed 3 g of air-dried soil and passed it through a 0.5 mm sieve with 20 mL of 0.02 M $KMnO_4$. We vigorously shook the tubes for 30 min at a rate of 120 revolutions per minute using a reciprocation shaker (HERMEL, Gosheim, Germany) and then left them to facilitate the progression of the oxidation process and the setting of particulate matter via centrifuge (HERMEL Z 206 A, Germany) for 5 min. Afterwards, we transferred 0.1 mL of the sample solution from the tube to another tube containing 9.9 mL of demineralized water to stop the reaction. We used the spectrophotometer (Cecil Aurius Series CE 2021, Peterborough, UK) to measure each sample's absorbance at 550 nm and calculated the POXC (mg $kg^{-1}$ soil) accordingly.

$$\text{POXC} = [0.02\ (\text{mol L}^{-1}) - (\text{a+ b Abs})] \times (9000\ \text{mg C mol}^{-1}) \times (0.02\ \text{L solution Wt}^{-1}(\text{kg})) \quad (1)$$

The $KMnO_4$ solution, starting at 0.02 mol $L^{-1}$, oxidizes nine thousand milligrams of carbon, reducing $Mn_7^+$ to $Mn_4^+$, with soil used in the reaction measured in kilograms (kg).

$$\text{Passive_C} = \text{TOC-active carbon (POXC) (g kg}^{-1}) \quad (2)$$

*Different concentrations of $H_2SO_4$ were used to chemically oxidize carbon fractions for carbon fractionation methods.*

To obtain the oxidizable carbon fractions, we adjusted the method used by [56]. Using potassium dichromate to oxidize organic materials in an acidic solution, we determined C [57,58]. We did not need to heat the solution from the outside, and we employed various concentrations of $H_2SO_4$ [58]. The dichromate concentration did not change, although the $H_2SO_4$ concentration increased to its maximum of 6 and 9 mol $L^{-1}$, which, according to Chan et al. [56], represents 6 N and 9 N $H_2SO_4$, respectively. According to Equations (3) and (4), these ratios represent extremely labile carbon fractions (LB_C) and less labile carbon fractions (LLB_C). The amount of organic carbon that was oxidized at each concentration was split into three parts, each with a different susceptibility level: Lastly, we subtracted the total of Equations (3) (LB_C) and (4) (LLB_C) from the TOC pool in Equation (5) to obtain the non-labile C pool.

$$\text{LB_C (very labile C fraction) = organic C oxidizable under 6 N } H_2SO_4 \quad (3)$$

$$\text{LLB_C (less labile C) = 9 N} - \text{6 N } H_2SO_4 \text{ oxidizable C} \quad (4)$$

$$\text{RC_C (recalcitrant C) is equal to TOC-(LB_C+LLB_C) oxidizable carbon} \quad (5)$$

*Exchangeable $K^+$*: We added dried soil samples using 1 N $NH_4$AOC pH 7. After one hour of shaking and filtering, we measured the soil aliquot using an atomic adsorption spectrophotometer (SavantAA GBC, Keysborough, VIC, Australia).

### 2.3. Calculations and Data Analysis

We performed statistical analyses using Excel 2019 and JASP Version 4, using a one-way analysis of variance (ANOVA) to determine how different fertilization methods affected nutrients, carbon fractions, and the ratio of labile C fraction to nutrient. We used the LLB_C (mg $kg^{-1}$)/nutrient (mg $kg^{-1}$) ratio (without unit). To determine the effect size for carbon fractions, we used eta-squared ($\eta^2$): The one-way ANOVA statistic shows how well nutrients, carbon fractions, and the ratio of labile fraction to nutrient content account for all fertilizer practices. We compared the results to both the Tukey HSD method and the

reported format of homogenous groups and then presented the average and standard error for the evaluation of the results. To create the graphs, the differences in the heatmaps were considered significant at $p < 0.05$, <0.01, and <0.001. JASP Version 4 also calculated and displayed the heatmap of Spearman's rho correlation coefficient in the matrix.

## 3. Results

### *3.1. The Decrease in N Addition Impacts the Carbon Fractions*

Our study on the effects of a nitrogen (N) dose reduction on carbon fractions revealed the following significant findings. TOC: There were FP (18.042 ± 1.007 g $kg^{-1}$), FS (17.717 ± 0.98 g $kg^{-1}$), and FZ (19.064 ± 0.68 g $kg^{-1}$) values that did not differ significantly (F = 0.608, $p = 0.546$, $\eta^2 = 0.100$) (Figure 1a). However, in the FS variant, the TOC showed a decreasing trend. *NFF*: A statistically significant difference was observed, showing that FP (2.609 ± 1.124 g $kg^{-1}$), FS (2.686 ± 1.046 g $kg^{-1}$), and FZ (7.29 ± 0.742 g $kg^{-1}$) significantly affect changes in this fraction (Figure 1b). LLB_C under FP, FS, and FZ consistently showed values of 12.889 ± 1.639, 8.153 ± 0.582, and 2.930 ± 0.606 g $kg^{-1}$, respectively (Figure 1c). In this research, the carbon fractions, especially the reduced LLB_C, decreased very quickly in long-term arable soils that were switched to reduced nitrogen (FS and FZ).

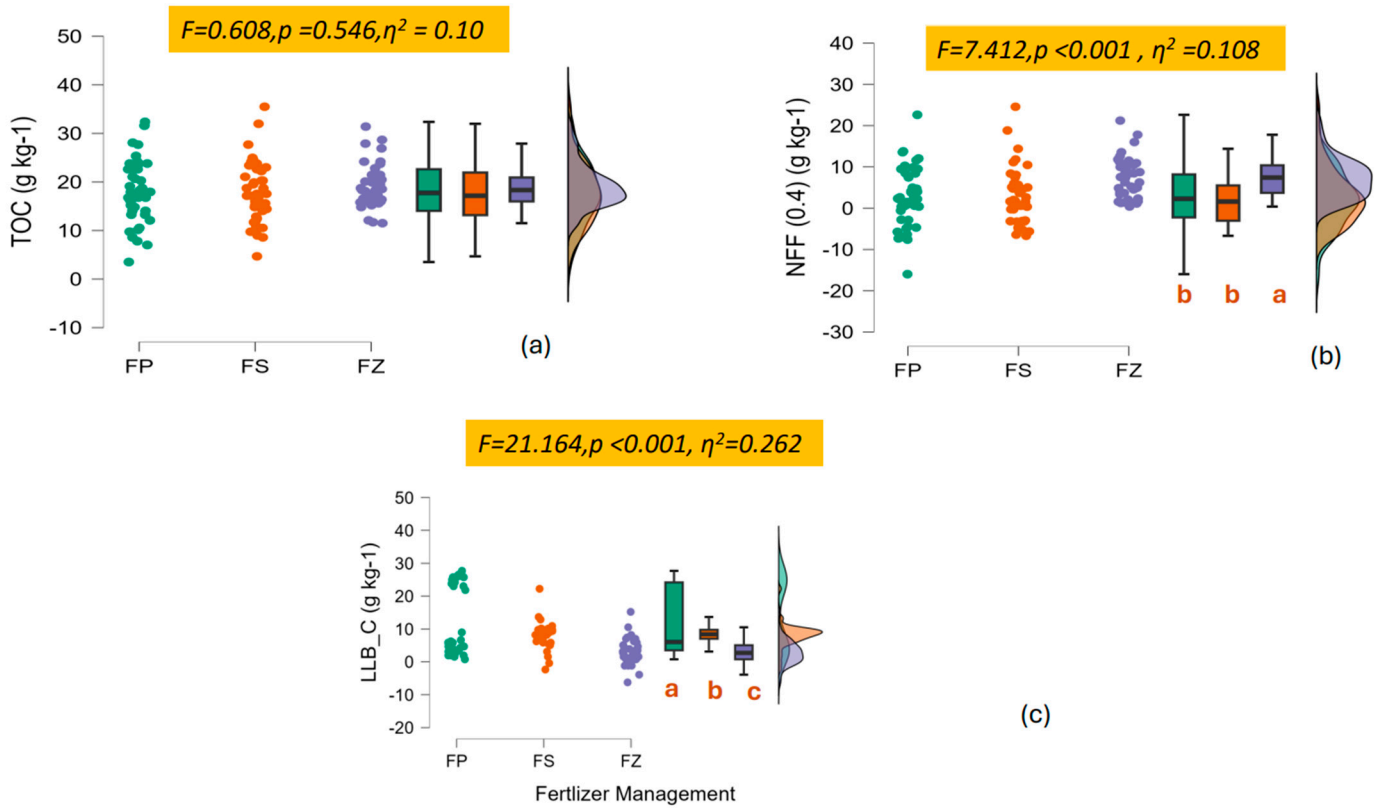



**Figure 1.** The impact of a reduction in N addition on the soil organic carbon and labile carbon fractions: TOC (**a**) and NFF (**b**), along with the LLB_C (less labile carbon) (**c**). *Note*: FP = high N addition via farmer practices; FS = reduced N addition via SSNM; FZ = No additional N fertilizer. The lines above and below the bars represent the maximum and minimum values, respectively. The line inside the bar graph indicates the average value for each individual N addition. $\eta^2$ = estimated effect size. The statistically significant group, which includes different small brown hyphenates, is denoted as a, b, and c.

There was a statistically significant difference in POXC, and FP, FS, and FZ treatments exhibited values of 0.676 ± 0.052, 0.688 ± 0.050, and 0.832 ± 0.031 mg $kg^{-1}$, respectively (Figure 2a). Instead, LB_C did not change significantly, which means that reducing the nitrogen dose does not have a significant impact on this faction (FP = 5.107 ± 0.315,

FS = 5.683 ± 0.391, and FZ = 4.986 ± 0.354 g kg$^{-1}$) (Figure 2b). Therefore, in this study, the labile carbon fractions, especially the increase in POXC content, might change very quickly in long-term arable soils that were switched to no additional nitrogen fertilizer (i.e., FZ). However, LB_C did not change.

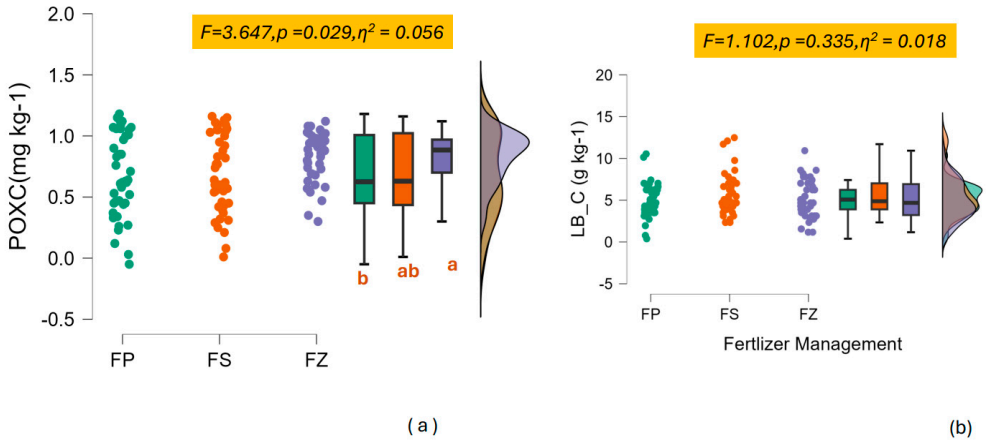



**Figure 2.** The impact of the N addition pattern on the labile carbon fractions: POXC (**a**), and LB_C (**b**). Typical farmer practices (FPs); lower N via SSNM (FS); no fertilizer (FZ). Note: The lines above and below the bars represent the maximum and minimum values, respectively. The line inside the bar graph indicates the average value for each individual N addition. $\eta^2$ = estimated effect size. The statistically significant group, which includes different small brown hyphenates, is denoted as a and b.

For RC_C, there was a statistically significant difference between FP (0.046 ± 1.942 g kg$^{-1}$), FS (3.883 ± 0.744 g kg$^{-1}$), and FZ (11.225 ± 0.673 g kg$^{-1}$) values (Figure 3a). Meanwhile, for the FF, there was a statistically significant difference between the FP (15.434 ± 0.729 g kg$^{-1}$), FS (15.031 ± 0.655 g kg$^{-1}$), and FZ (11.774 ± 0.621 g kg$^{-1}$) values (Figure 3b). We did not find any statistically significant differences in passive carbon between FP (18.041 ± 1.007 g kg$^{-1}$), FS (17.716 ± 0.979 g kg$^{-1}$), and FZ (18.096 ± 0.680 g kg$^{-1}$) values (Figure 3c). This finding suggests that decreasing the N addition causes FF, and particularly FZ, to drop.

For the labile carbon fraction, the NFF increased by 7 and 13% in FS and FZ treatment, respectively. In FS and FZ treatment, respectively, the POXC content grew by 2 and 23%. LB_C and LLB_C in FS treatment decreased by 2 and 37%, respectively, compared to the mean of FP, while the LLB_C dramatically decreased by 77% in FZ. In this study, the reduced N addition resulted in a decrease in FF, LLB_C, and LB_C, and we can rank the effect size in the following order: FF > LLB_C > LB_C. However, POXC (Figure 2) and NFF (Figure 1) increased in this order: POXC > NFF. Figure 3 ranks the effect size as FF > passive_C, but it excludes RC_C. These findings show the amount of N reduction that needs to be considered, along with the different responses of stable carbon fractions, especially the FF, compared to other carbon fractions, such as labile carbon fractions (LLB_C).

### *3.2. The Decrease in N Addition Impacts the Availability of NPK in Soil*

*Phosphorus content was determined* via *the Bray II Method (P(B)):* There was not a statistically significant difference in phosphorus (P) content, but the value is close to suggesting a possible effect. FZ (44.142 ± 8.167 mg kg$^{-1}$) had a higher average than FP (25.238 ± 3.81 mg kg$^{-1}$) and FS (29.619 ± 8.167 mg kg$^{-1}$) (Figure 4a).

*Phosphorus content was determined* via *the Malachite Method (P(M)):* We did not find any statistically important differences between FP (25.392 ± 0.409 mg kg$^{-1}$) and FS (25.101 ± 0.342 mg kg$^{-1}$) (Figure 4b), which means that it is not clear how factor adjustments change P(M) levels in this sample (Figure 4b).

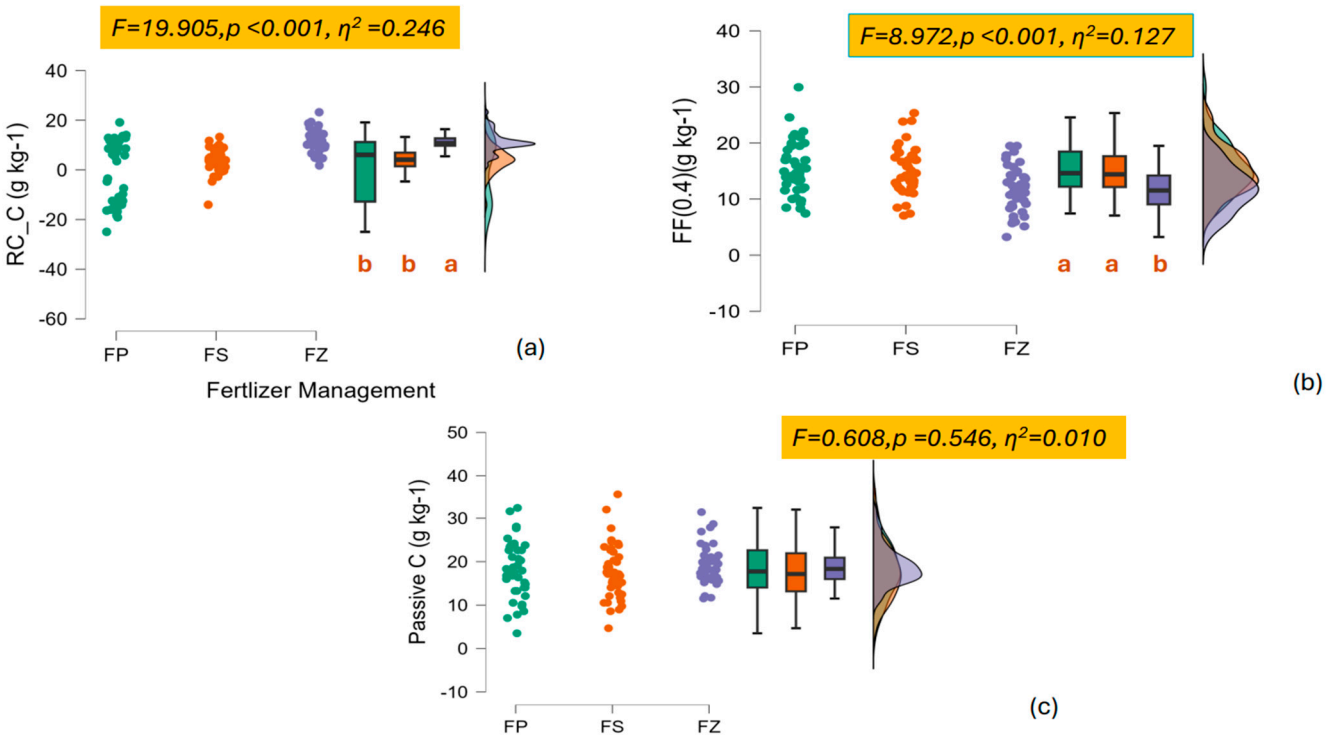



**Figure 3.** The impact of the N addition pattern on the stable carbon fractions: NLB_C (**a**), FF (**b**), and Passive_C (**c**). Typical farmer practices (FPs); N addition via SSNM (FS); no fertilizer added (FZ). Note: The lines above and below the bars represent the maximum and minimum values, respectively. The line inside the bar graph indicates the average value for each individual N addition. $\eta^2$ = estimated effect size. The statistically significant group, which includes different small brown hyphenates, is denoted as a and b.

*The exchangeable potassium (Exch. K):* It was found that fertilizer practices had a statistically important effect on the amounts of Exch. K. The K content in FP and FS treatment variants had the lowest averages (96.133 ± 17.669 and 89.361 ± 16.784 mg $kg^{-1}$), respectively, while FZ had the highest value (142.197 ± 10.387 mg $kg^{-1}$). This difference suggests that N fertilization may have an effect on the changes in Exch. K levels (Figure 4c). Therefore, the pattern of N additions in long-term arable soils that were switched to reduced nitrogen fertilization had a significant effect on Exch. K but not on P content.

*Ammonium in dried soil ($NH_4^+$_DS):* An important difference was found in the amounts of $NH_4^+$-N in dried soil. The results for FP, FS, and FZ were 147.681 ± 7.881, 108.042 ± 4.944, and 77.390 ± 9.381 mg $NH_4$+-N $kg^{-1}$, respectively. In FP treatment, the average amount of $NH_4^+$-N was the highest (Figure 5a).

*$NH_4^+$-N in soil ($NH_4^+$_FS):* The amounts of $NH_4^+$_FS found were very different according to the N fertilizer management of the experiments, with the variant FP (145.525 ± 6.397 mg $kg^{-1}$) having the highest average compared to FS (138.146 ± 9.678 mg $kg^{-1}$) and FZ (77.39 ± 9.381 mg $kg^{-1}$) (Figure 5b). *Nitrate from dried soil* ($NO_3^-$_DS): The amounts of nitrate in dried soil ($NO_3^-$–DS) were not statistically different between the variants FP (36.577 ± 3.563 mg $kg^{-1}$), FS (37.280 ± 4.655 mg $kg^{-1}$), and FZ (42.181 ± 3.164 mg $kg^{-1}$) (Figure 5c). This finding means that the fertilizer methods that were investigated do not impact the amounts of $NO_3^-$_DS. *Nitrate–Nitrogen in fresh soil ($NO_3^-$_FS):* Statistically significant changes were also observed, with the average FZ (42.198 ± 3.164 mg $kg^{-1}$) as the lowest, differing markedly from FS (113.198 ± 13.972 mg $kg^{-1}$) and FP (82.747 ± 4.492 mg $kg^{-1}$) treatments (Figure 5d). In long-term arable soils that were switched to reduced nitrogen fertilization, the pattern of N additions had a significant effect on the availability of $NH_4^+$_DS, $NH_4^+$_FS, and $NO_3^-$_FS, but not on $NO_3^-$_DS. This finding enables us to draw some conclusions about the reduced $NH_4^+$ and $NO_3^-$, the higher K, and the fact that it did not

affect the availability of P in the reduced N additions, such as variants FS and FZ compared to FP.

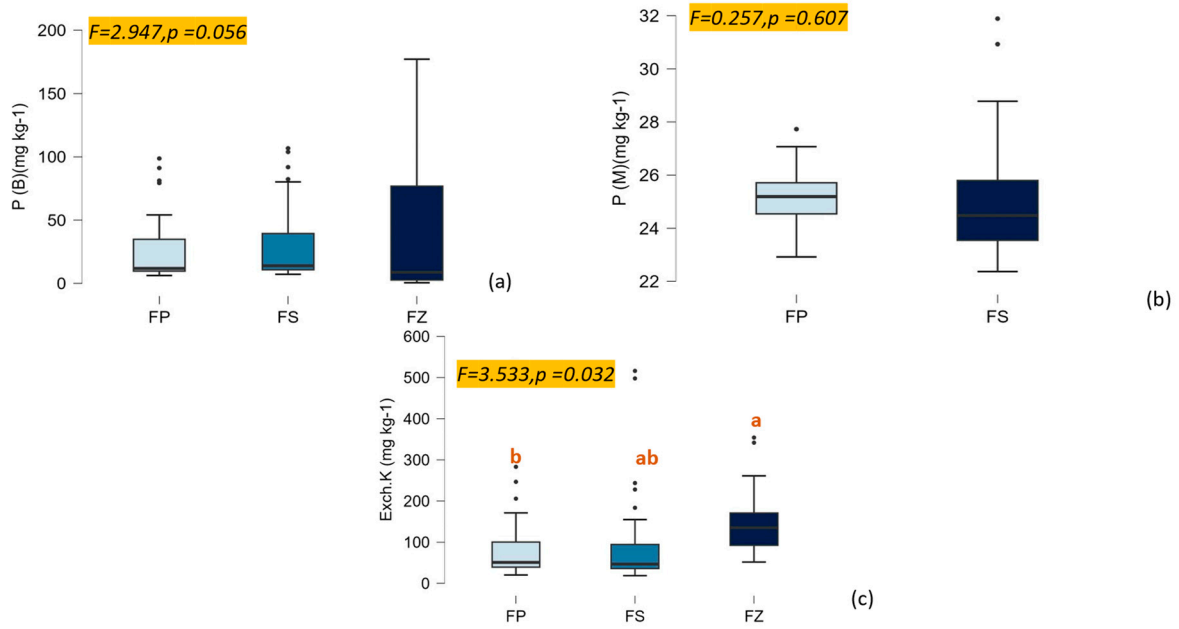



**Figure 4.** The impact of the N addition pattern on the availability of P and exchangeable K; the P via Bray II method (**a**), the P via Malachite method (**b**), and Exch. K (**c**). Typical farmer practices (FPs); N addition via SSNM (FS); no fertilizer added (FZ). The lines above and below the bars represent the maximum and minimum values, respectively. The line inside the bar graph indicates the average value for each individual N addition. The statistically significant group, which includes different small brown hyphenates, is denoted as a and b. (**b**) does not display any available P(M) data for the FZ treatment.

### *3.3. Relationships Exist Between Carbon Fractions and the Effects of Reducing N in Synthetic Fertilizer on the Dynamics of Soil Carbon*

*Spearman's rho correlation coefficient:* We discovered that the independent factors FF (0.038, $p$ = 0.05 *), LB_C (0.662, $p$ = 0.001 ***), Passive C (0.999, $p$ = 0.001 ***), NFF (0.721, $p$ = 0.01 **), RC_C (0.594, $p$ = 0.001 ***), and POXC (0.741, $p$ = 0.001 ***) all had a significantly positive relationship with TOC (dependent variable) for FP. For FS, the dependent factors FF (0.252, ns), LB_C (0.908, $p$ = 0.001 ***), Passive C (0.999, $p$ = 0.001 ***), NFF (0.698, $p$ = 0.001 ***), RC_C (0.838, $p$ = 0.001 ***), POXC (0.892, $p$ = 0.001 ***), and LLB_C (0.336, $p$ = 0.05 *) exhibited a positive relationship with TOC (dependent variable) (Figure 6b). For FZ, LB_C (−0.364, $p$ = 0.05 *), NFF (0.476, $p$ = 0.01 **), FF (0.399, $p$ = 0.01 **), Passive C (0.998, $p$ = 0.001 ***), RC_C (0.771, $p$ = 0.001 ***), and POXC (−0.285, $p$ = ns) were positively correlated with TOC (independent variable) (Figure 6c). An increase in reduced nitrogen inputs, specifically FS and FZ, demonstrated a positive correlation with soil carbon fractions—namely, LLB_C and FF—in relation to TOC, whereas POXC and LB_C exhibited a negative correlation with TOC. LLB_C and the FF in FS and FZ treatment exhibited a downward trend, while an increase in POXC,LB_C correlated with a reduction in TOC content.

Therefore, this study examined the impact of reducing nitrogen in synthetic fertilizer on carbon fractions. The results showed a positive relationship between the non-fine fraction (NFF) and TOC across all fertilization groups (FP, FS, and FZ). This study also found a similar pattern for fine fraction carbon (FF), with increased TOC leading to increased carbon intensity in FP and FS treatment. This study also found a strong link between labile carbon (LB_C) and TOC, with FP and FS showing the most noticeable changes.

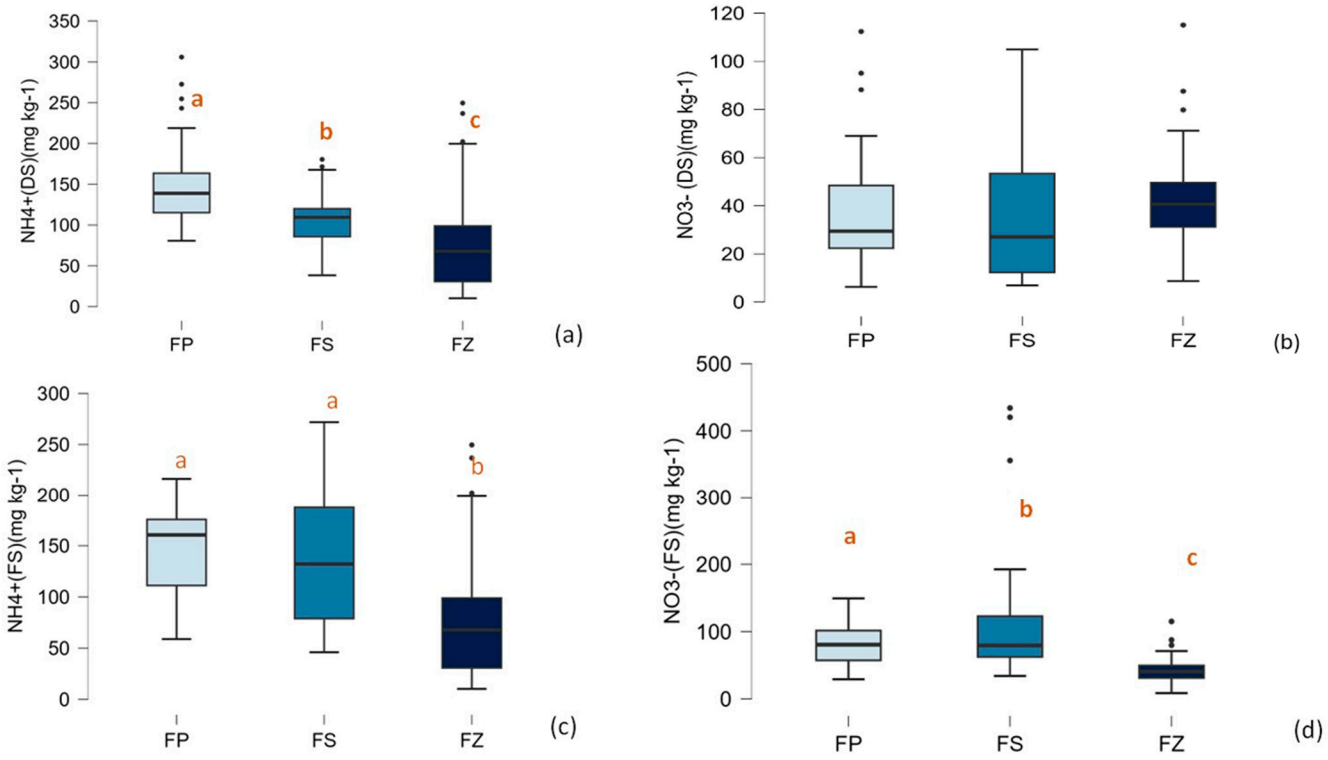
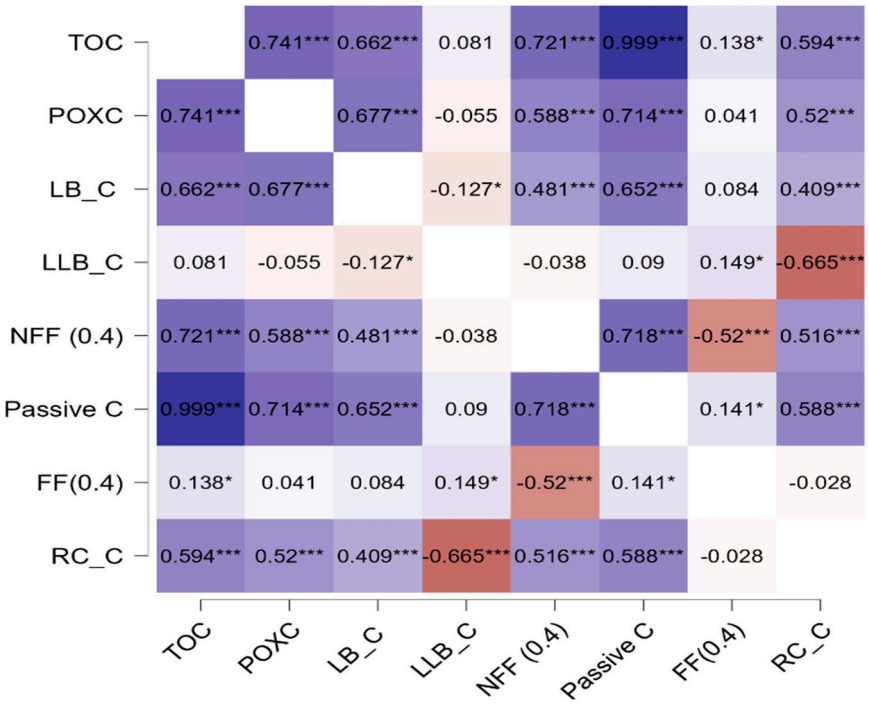



**Figure 5.** The impact of the N addition patterns on the availability of N: $NH_4^+$_DS (**a**), $NO_3^-$_DS (**b**), $NH_4^+$_FS (**c**), and $NO_3$_ FS (**d**). *Note*: FP = high N addition via farmer practices; FS = reduced N addition via SSNM; FZ = no additional N fertilizer. The lines above and below the bars represent the maximum and minimum values, respectively. The line inside the bar graph indicates the average value for each individual N addition. The statistically significant group, which includes different small brown hyphenates, is denoted as a, b, and c.

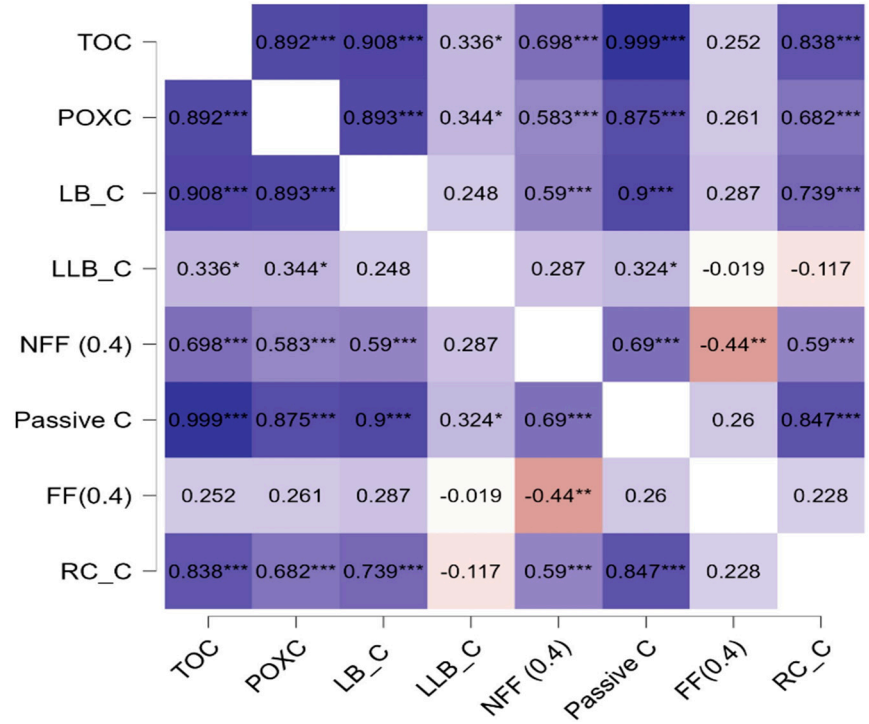
### *3.4. Ratio of Less Labile Carbon (LLB_C) to the NPK Content in Soil*

*Ratio of LLB_C/K:* According to the LLB/K ratio (labile carbon/potassium ratio), the values for the treatments FP (305 ± 44), FS (281 ± 27), and FZ (68 ± 7) were significantly different, as previously noted. We found that FP exhibited the highest ratio, with the greatest average value (Figure 7a). *Ratio of LLB_C/$NO_3^-$ (fresh soil)*: The acronym "LLB_C/$NO_3^-$ fresh soil" stands for the less labile carbon nitrate ratio. When comparing the treatment FS (171 ± 18) with FP (321 ± 31) and FZ (559 ± 50), the latter values were certainly lower, as shown by the substantial statistical differences (Figure 7b). *Ratio of LLB_C/P(B)*: There was a statistically significant difference between the ratio values for the FP (1410 ± 213), FS (876 ± 103), and FZ (2001 ± 456) fertilizer management treatments, with FZ having the highest (Figure 7c). *Ratio of LLB_C/P (M)*: This study found that fertilizer management has a lower effect on the LLB_C/P (M) ratio in the FS (553 ± 27) than in the FP treatment (1063 ± 75) (Figure 7d). By switching long-term arable soils to reduced-nitrogen fertilization (i.e., FS and FZ), the LLB_C/K, LB/P (B), and LLB_C/P (M) ratios were examined in the research; FP had the greatest ratio, and the FZ treatment had the highest average value. It was discovered that FS had a lower LLB/$NO_3^-$ than FP and FZ. Additionally, we discovered that the LLB_C/P (M) ratio in the FS treatment was less affected by fertilizer management than it was in FP treatment. When we looked at the percentage decrease, we found that the ratios of LLB_C/$NO_3^-$_FS and LLB_C/P (B) in FS treatment all fell by 47% and 38%, respectively, compared to the mean of the FP. In the FZ variant, these ratios experienced an increase of 74% and 42%, respectively. While LLB_C/P (M) decreased by 48% compared to the mean of FP, the LLB_C/K ratio decreased in FS and FZ treatment by 8% and 78% compared to FP, respectively. In this study, as we reduced the N addition, the effect size decreased in the sequence LLB_C/P (M) > LLB_C/$NO_3^-$ > LLB_C/K >

LLB_C/P (B), in comparison to the FP treatment. FS revealed a decrease in LLB_C/$NO_3^-$, LLB_C/P (M), LLB_C/P (B), and LLB_C/K, while FZ was higher in LLB_C/$NO_3^-$ and LLB_C/P (B) compared to FP. These findings underscore the importance of considering them for assessing soil carbon dynamics in agricultural soils.




FP (**a**)




FS (**b**)

**Figure 6.** *Cont.*

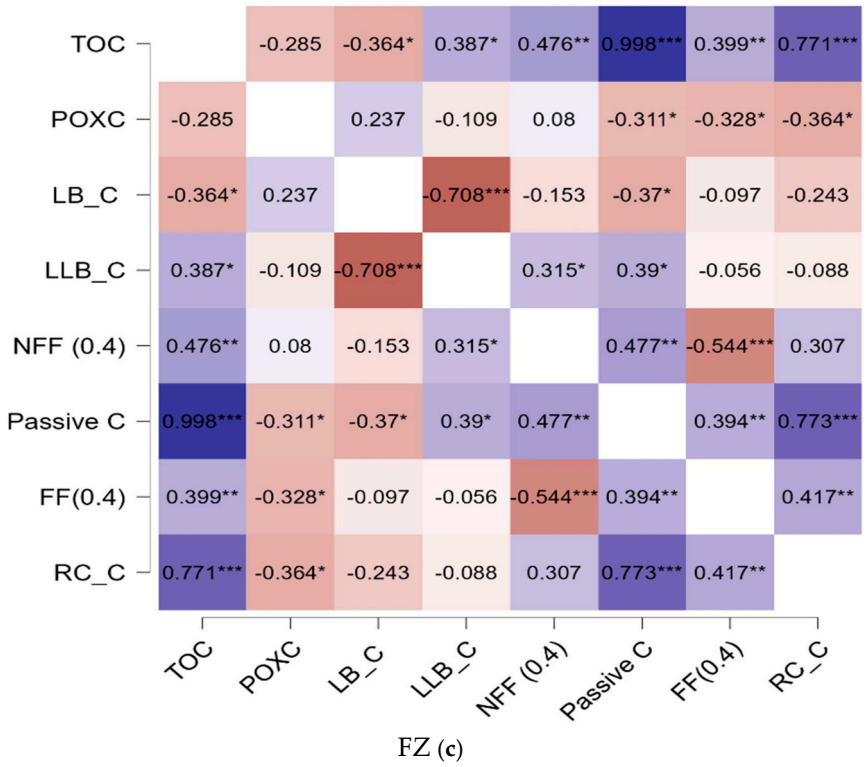



**Figure 6.** The heatmap illustrates the correlation coefficient relationship between carbon fractions and TOC in FZ (**a**), FS (**b**), and FZ (**c**). *Note*: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; blue = positive correlation; brown = negative correlation; dark blue = stronger positive correlation; dark brown = stronger negative correlation.

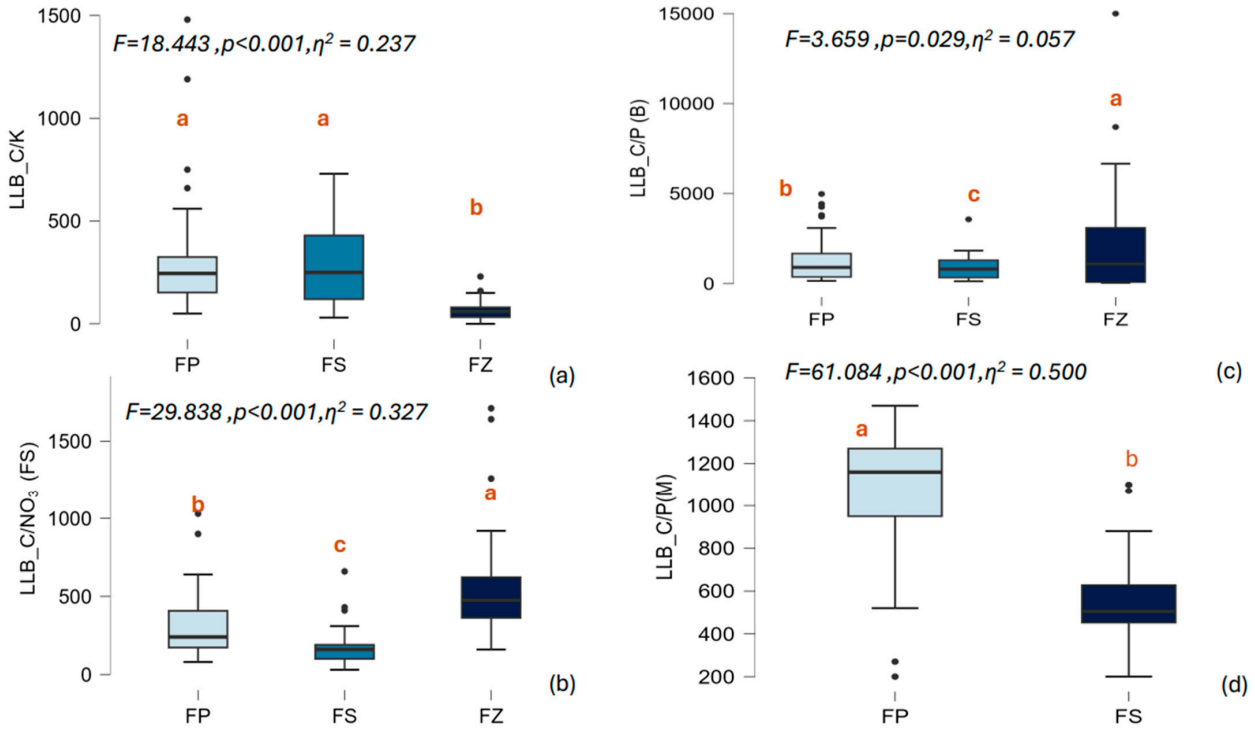



**Figure 7.** The impact of the N addition patterns on LLB_C/K (**a**), LLB_C/$NO_3^-$_FS (**b**), LLB_C/P(B) (**c**), and LLB_C/P(M) (**d**). Typical farmer practices (FPs); N added via SSNM (FS); no fertilizer added

(FZ). *Note*: The lines above and below the bars represent the maximum and minimum values, respectively. The line inside the bar graph indicates the average value for each individual N addition. $\eta^2$ = estimated effect size. The statistically significant group, which includes different small brown hyphenates, is denoted as a, b, and c. (**d**) shows no available data for the FZ treatment.

*Spearman's rho correlation coefficient* for the ratio of LLB_C to N and P in soil is as follows: Fertilizer management has different effects on plant nutrient availability and what kinds of organic matter are in the soil. For example, the ratio of LLB_C to P(M) is positively related to TOC, reducing N in FS treatment. The LLB_C/K ratio exhibits a positive correlation with TOC when no N is added (FZ), but it shows a negative correlation with TOC in the FP and FS treatments (Figure 8a–c). For LLB_C/$NO_3^-$_FS, a trending positive correlation was observed with TOC across all fertilizer practices.

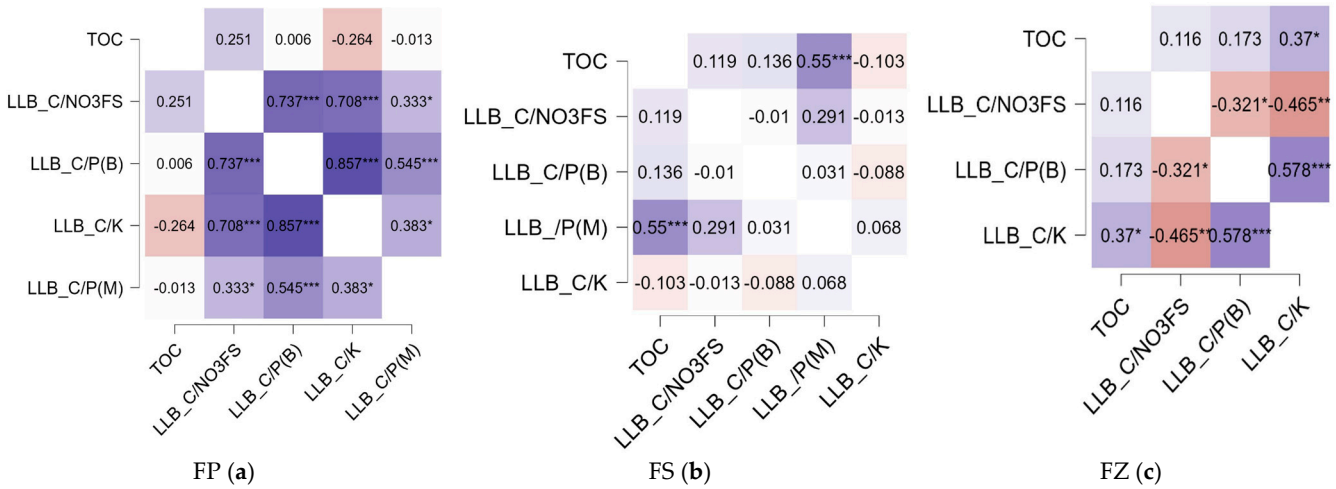



**Figure 8.** The heatmap displays the correlation coefficient link in FP (**a**), FS (**b**), and FZ (**c**) treatments between the ratio of LLB_C to NO3-_FS, P (via Bray II and Malachite methods), exchangeable K, and TOC. *Note*: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; blue = positive correlation; brown = negative correlation.

Therefore, this study demonstrated that LLB_C/P(M) was a crucial factor for keeping SOC under a lower N addition (FS) but not FP. When there was no N addition, the LLB_C/K ratio had a positive relationship with TOC, whereas FP and FS both showed negative relationships. While the LLB_C/$NO_3^-$_FS ratio and LLB_C/P (B) were trending positive with TOC in all the different N addition patterns, FP exhibited the highest correlation coefficient compared with FS and FZ (Figure 8a–c).

## 4. Discussion

### *4.1. The Labile and Recalcitrant Carbon Content Decreased When Switched to SSNM*

Labile Carbon Under Lower N Addition

Less nitrogen can lead to more SOM mineralization as microbes "mine" for nitrogen, which makes the priming effect (PE) stronger [59,60]. This trait led to an increase in the SOC mineralization in FS treatment. There was not enough nitrogen in the soil, so the rates of microbial biomass and soil respiration slowed down. This action caused labile carbon reservoirs to temporarily rise [27,28]. The other study found that adding low nitrogen can raise the carbon management index (CMI) and lower labile carbon fractions like MBC and DOC [61]. This study therefore showed that the drop in labile carbon content under a low N addition (FS) could be because labile carbon inputs under low N addition induced a priming effect that significantly contributed to the depletion of labile carbon pools. The availability of labile carbon supported the mineralization or priming of soil organic matter (SOM), with soils differing in the extent to which labile C stimulates priming [36]. N deficiency in root

exudates can cause microbes to accelerate SOM decomposition to meet their N needs, a phenomenon [59] also observed and which we discovered in this study. Zeng et al. [62] showed that the deficiency in N promoted total cumulative carbon release, and the low nitrogen addition raised the activity of labile C-degradation genes and enzymes, which caused more $CO_2$ to be released and labile carbon to break down more quickly [25,26]. Zeng et al. [62] reported that the notable rise in microbial carbon use efficiency and soil microbial biomass nitrogen did not influence or may have even reduced labile carbon decomposition. Our results did not show a significant difference based on the nitrogen application patterns, while the NFF remained the highest in FZ. These labile carbons did not decompose due to the limited availability of N. On the other hand, the availability of N decreased. Begum et al. [63] established that a nitrogen level of 120–140% raised labile carbon as well as POXC and that POXC was more sensitive to N management systems, as shown by the physical and microbial properties of the soil. The research also showed that POXC, as a sign of healthy soil, accurately detected changes in the soil's labile carbon pool caused by N management [63]. Compared to the NFF, the labile organic carbon fraction, such as LB_C, significantly decreased, reflecting the different responses to levels of nitrogen supply patterns.

*Recalcitrant Carbon under lower N addition*: Our study showed in a short period (1-crop season) a contrast in the amounts of stable carbon, with a low N input, an increase in NLB, and a decrease in Passive_C and the FF. Consistently with these findings, a study using ion-exchange membranes found that the total cumulative carbon release was promoted via the N deficiency treatment but inhibited via the high N treatment, and N deficiency increased soil recalcitrant carbon decomposition [62]. The fine fraction is positively correlated with the accumulation of soil organic carbon in mineral horizons, indicating its importance in carbon stabilization [29,30]. The N addition generally decreases the recalcitrant carbon pool in soils. This finding is attributed to enhanced enzyme activities that promote the decomposition of recalcitrant carbon and increased dissolved organic carbon that stimulates this carbon fraction pool decomposition [26,27,64]. For the FF, nutrient addition increased FF levels in all soils, with a significant interaction between soil type and nutrient treatment. Nutrient addition (as N) significantly increased FF sequestration in soils over seven consecutive incubation cycles [32]. On the other hand, inorganic nitrogen sources tend to inhibit soil carbon cycle processes, leading to the accumulation of recalcitrant organic carbon [65]. Microbial regulation strategies under a N imbalance drove soil labile C release [62]. Adding nitrogen raises SOC in fine soil fractions, such as mineral-associated fractions, which facilitates the overall SOC build-up [35]. The shortage of available soil N (i.e., FZ) showed the lowest level of recalcitrant C decomposition (i.e., the FF). A meta-analysis of 48 sites in China found that the synthetic nitrogen fertilizer reduction decreased soil organic carbon [66] while also finding that short-term chemical nitrogen fertilizer reduction negatively impacted SOC levels. This finding explains why soil organic C decomposition was more sensitive to N scarcity than to N abundance [62].

Increased soil organic carbon from organic amendments can enhance nitrification by supplying additional substrates for microbial activity [67,68]. However, the total content of soil carbon and the ratio of soil carbon to nitrogen were insufficient to account for the occurrence of heterotrophic nitrification [69].

When comparing this finding to the application of high levels of nitrogen, as practiced by farmers, Begum et al. [63] found that nitrogen fertilization (120 and 140%) significantly increased the labile C pools compared to the control and lower rates. High N levels also increased the MBC, C pool, lability, and management indices, indicating improved soil biological activities. Higher N fertilization rates led to higher MBC and POC stocks and labile C pool stocks and would consequently increase SOC in rice-based systems in subtropical climates, which suggests that an optimal N fertilization treatment can improve soil quality. The reason for this finding is partially because continuous fertilization combined with crop residue incorporation can enhance labile carbon fractions and related enzyme activities, suggesting that proper management practices can mitigate labile carbon loss [70].

Exogenous nitrogen in litter did not affect litter and soil organic matter decomposition, except for stalk litter treatment, which reduced litter carbon loss and increased soil organic matter carbon loss [71]. There were also significant differences in SOC stability among N addition treatments, and SOC could be most stable via a mid-level N addition [61]. N fertilization at 120 to 140% over a period of seven years markedly decreased C loss (as $CO_2$) via improving anabolism with an associated increase in the size of the labile pool of TOC, especially the POC, MBC, and POXC contents, when compared to the control (100% N) under a wheat–mungbean–rice agroecosystem in subtropical soil and climatic conditions. Increased labile carbon fraction contents translated into stratification [63]. Mid- and high-level N additions decreased soil microbial richness and evenness; however, the low N addition increased the carbon management index [61]. These reasons would explain the high organic carbon fraction and TOC in high N doses applied in FP treatment. This study showed that, due to the lower amount of N in FS and FZ treatments, the scarcity of soil N would hasten the breakdown of recalcitrant C (i.e., the FF) and LLB_C. However, labile carbon fractions (i.e., POXC and LB_C) would not break down as quickly via "nitrogen mining", as hypothesized by [62].

### *4.2. The Impact of Availability of NPK Balanced with Carbon on SOC*

Soil organic C decomposition responded to changes in N availability asymmetrically [62]. Meanwhile, high nitrogen availability reduced PE by decreasing the need for microbial nutrient mining [60], and the high N availability enhanced microbial C use efficiency and reduced SOC mineralization [60]. This study found significant differences in $NH_4^+$ concentrations upon addition, and that turning, lowering, and adding FS resulted in a lower of availability of $NH_4^+$ and $NO_3^-$.

Higher levels of ammonium alter the process by which microbes break down organic matter, altering the structure of the microbiome and often increasing the ratio of fungi to bacteria [72]. Additionally, it may hinder the decomposition of plant residues and soil organic matter, thereby reducing the rates of carbon mineralization [38,73]. Adding ammonium can render enzymes that break down carbon less active, such as β-glucosidase and phenol oxidase, and this finding is especially true in soils that are already acidic [74]. Ammonium additions can lead to a decrease in soil pH value, which in turn affects microbial enzyme activities and carbon mineralization rates [38]. On the other hand, it was reported that adding nitrates tends to raise the SOC content and has less of an impact on microbial activity and carbon mineralization. Unlike ammonium, it reduces the SOC in the soil organic layer by a significant amount, causing more minerals to form and the loss of organic carbon [75]. Because there is less nitrogen in FS and FZ treatments, the priming effect becomes stronger; less nitrogen can lead to increased SOM mineralization as microbes "mine" for nitrogen, which strengthens makes the priming effect (PE) [59,60]. Zeng et al. [62] demonstrated that nitrogen deficiency enhanced the total cumulative carbon release, whereas nitrogen addition suppressed it. The lack of N significantly enhanced the decomposition of recalcitrant carbon through an increase in soil pH, whereas a sufficient level of N had no effect or reduced the decomposition of labile carbon. Due to the lower C availability and the dominance of *K*-strategists in microbial communities, the driving mechanism of SOC decomposition will follow the "microbial N mining" hypothesis. The increase in N availability will decrease the mineralization of recalcitrant C, simultaneously depleting more labile C (i.e., LB_C), which Zeng et al. [62] found in FS treatment. However, the increase in N availability would stimulate SOC decomposition, which is supposed to be regulated via "stoichiometric decomposition" [62], while the decrease in N availability would increase the storage of SOC in FZ [62]. N deficiency in root exudates can cause microbes to accelerate SOM decomposition to meet their N needs. Chertov et al. [59] also observed this phenomenon, which we expect to find in our study, particularly in FZ.

Heterotrophic nitrification rates correlated with the composition of soil organic carbon [69]. It was found that the recalcitrant carbon fractions, specifically carbonyl C and aromatic C, exhibited a significant positive correlation with heterotrophic nitrification rates,

which resulted in a decrease in nitrification rates in FS and FZ treatment. Subsequently, FS and FZ might exhibit an increase in $NO_3^-$ limitation. In contrast, labile carbon is easily degradable to carbon sources, such as glucose, and it promotes heterotrophic nitrification while suppressing autotrophic nitrification. Additionally, the labile carbon content would increase the microbial immobilization of ammonium, leading to a reduction in its availability for autotrophic nitrifiers [61,76]. This study showed the remaining labile carbon content, including POXC and LB_C, was elevated in FS treatment, while FZ treatment may partially inhibit autotrophic nitrification [61]. The incorporation of readily degradable carbon into soil enhances heterotrophic nitrification and $N_2O$ emissions while suppressing autotrophic nitrification, which results in elevated nitrification rates and increased $NO_3^-$ accumulation [76].

It is important to consider the significance of accounting for various factors when evaluating the soil carbon content in arable crops. The LLB_C/P (M) ratio affects the P/C acquisition ratio [77]. Extracellular hydrolases control the amount of available carbon (C) and phosphorus (P) in the soil, making it easier for microbes and plants to obtain C and P [77].

According to this study, changes in soil organic carbon mineralization affect the amounts of nutrients, such as the availability of N, P, and K. These changes can be seen in the LLB_C/$NO_3^-$ and LLB_C/K ratios. This study revealed that the levels of LB_C, NFF, POXC, and DOC remained high, indicating a reduction in nitrogen management. Decreased nitrogen availability can cause imbalances, preventing substrates from meeting N requirements for microbial growth. Microbes immobilize nitrogen in new biomass, inhibiting growth and activity and slowing soil organic carbon decomposition [62]. The increase in DOC is due to the reduction in N fertilization, yet despite the lack of significant phosphorus availability in this study, the released DOC in FS treatment (524.506 mg $kg^{-1}$) was higher than that in FP treatment (468.072 mg $kg^{-1}$) and did not significantly differ from the data not included in this article. The decrease in LLB_C and FF contents could potentially explain the decline in LLB_C/P (B) and LLB_C/P (M), particularly in FS treatment.

This study found no significant phosphorus availability in P (B) but not in P (M). It is important to acknowledge the role of phosphorus fertilization. The increase in DOC is due to the reduction in N fertilization. It is also important to acknowledge the role of phosphorus fertilization. Adding phosphate can raise the amount of labile carbon and dissolved organic carbon (DOC) in the soil solution [44]. In temperate grassland soils, the dissolved organic carbon pool is quickly turned over, with soil microorganisms being the main consumers [78]. P fertilization enhances the recalcitrant carbon fraction associated with MAOC formation [79,80]. However, the phosphorus amendment increases the decomposition of the soil carbon fraction, thus constituting a longer-term sink, which suggests that the availability of P could significantly influence the strength of SOC sinks. The long-term P addition resulted in enhanced DOC biodegradation in an N-limited temperate wetland [81], which are physiochemical aspects. The co-limitation of N and phosphorus results in the highest priming effect (PE) when both nutrients are present in subsoils, suggesting that a match between nutrient availability and microbial demand boosts SOC turnover [82]. However, no evidence was found for phosphorus mining, as P fertilization increases both short- and long-term mineralization [83]. There are dynamic relationships between carbon that is recalcitrant and carbon that is labile when nitrogen addition is reduced. Additions of N and P resulted in microbial changes that accelerated the decomposition of recalcitrant soil organic carbon, leading to reductions in both the slow carbon pool and the total SOC pool, while increasing the active carbon pool [84]. Our results indicate a reduction in LLB_C (i.e., higher stability and slower decomposition), which resembles a recalcitrant soil carbon pool, particularly in the FF under conditions of lower nitrogen input and decreased sufficient phosphorus availability (i.e., P(M). The deficiency of phosphorus in soil may result in microbes more efficiently utilizing recalcitrant phenol, thereby influencing soil organic matter priming and $N_2O$ emissions [42]. Adding phosphorus (P) accelerated SOC mineralization by forcing microbes to work harder and speeding up the rate at which

C substrates from outside the body went through mineralization [41,42]. The combined effects of labile carbon and phosphorus are substantial, and the interaction between labile C and P can significantly influence SOC mineralization. For instance, adding P can intensify the priming effect of labile C, resulting in increased SOC loss [43,44]. A P addition can also inhibit the decomposition of SOC, especially in scenarios of heightened nitrogen deposition, an interaction that influences microbial community composition and enzyme activities, resulting in alterations in SOC stability [85].

Optimal N to K ratios are crucial for high-yielding crops. Both deficiency and excess of potassium can inhibit plant growth and nutrient uptake [86,87]. Nitrate ($NO_3^-$) tends to enhance potassium uptake and distribution [43,86]. A lack of nitrogen supply, coupled with low nitrate in variant FZ, cannot support better K uptake and overall plant health. In FZ, high SOC levels and possible clay mineral composition significantly influenced high K retention and availability [80]. The soil in the FZ treatment had a higher residual K content compared to both FP and FS variants. Even though continuous cropping without K fertilization can deplete soil K, the presence of certain clay minerals can mitigate this depletion to some extent [88]. K availability has a significant impact on the arable soil's SOC. This study showed that the positive correlation is between the LLB_C/K ratio and SOC and the highest availability of K in the FZ treatment, as K availability can limit humus-C sequestration [89,90]. SOC sequestration strongly correlates with the availability of N and P, whereas the role of K is less clear [50,91,92]. Furthermore, K could impact both the labile forms, like hot water-extractable carbon (HWEC), and the stable forms, like soil organic carbon. Voltr et al. [93] reported that labile forms such as HWEC were negatively affected by K (−7%). Furthermore, in arable soil, a high concentration of K reduced the soil's ability to sequester carbon by altering its physical structure [94] and led to the loss of SOC. K fertilization alone did not significantly affect organic carbon levels in upland and paddy soils [95]. It may decrease nitrate-induced soil $N_2O$ emissions [96]. The addition of K can enhance $CO_2$ sequestration in cropland soils, with the extent of sequestration influenced by soil pH, $Ca^{2+}$ concentration, and crop growth conditions [46].

In future research, we plan to investigate a long-term replacement plan with less nitrogen input while switching labile carbon to recalcitrant carbon with certain crops. Additionally, we have identified this issue as one of the limitations of this study. We now explicitly state that each plant's cropping history, farming practices, and related factors should ideally be addressed individually in future research to gain more accurate insights. We also aim to model the optimal N input for crop yield, soil fertility, and soil carbon storage.

We would like to summarize that short-term reduced-nitrogen fertilizers can swiftly lead to a decrease in the availability of nutrients, particularly N. This, in turn, results in a decrease in carbon fractions, especially stable ones like the FF and LLB_C, which decompose more slowly, while simultaneously increasing some labile carbons like POXC. This outcome ultimately leads to a promising SOC in arable soil (Figure 9).

This study's findings highlight apprehensions about the exclusive dependence on SSNM for fertilizer application. Although we must evaluate the fertilization recommendations of this policy and contemplate decreasing the use of synthetic fertilizers, if only to mitigate GHG emissions, this action would not intentionally contribute to climate change within agricultural practices. It is essential to sustain target crop yields while reducing fertilizer costs for farmers, and we must also consider fertilizer application for soil fertility and quality to combat climate change by sequestering carbon in the soil. It is critical to use quick and accurate soil analysis methods, as well as nutrient requirement models for each plant species, and to consider the soil quality and long-term health of an ecosystem. We can then implement these recommendations through training programs and use the results of this study, along with other similar knowledge patterns, to make recommendations to local farmers. Our study on reduced nitrogen inputs in Northern Thailand's soil organic carbon dynamics may not fully capture its long-term effects, so expanding our research to

different regions and exploring interactions with other soil amendments could therefore provide deeper insights.

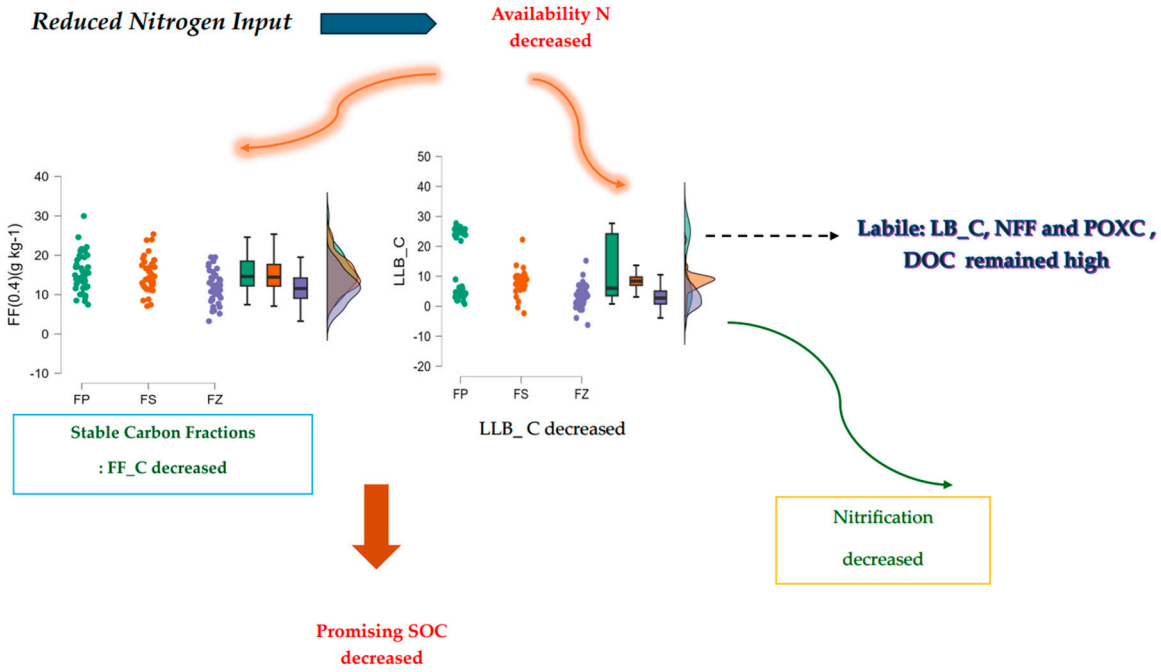



**Figure 9.** Schematic representation of reduced nitrogen input decreasing N availability, leading to a reduction in recalcitrant carbon content (i.e., FF on soil organic carbon in arable soils).

## 5. Conclusions

The SSNM concept of reduced N addition resulted in a 2% decrease in SOC in long-term arable soils that were transitioned to a reduced nitrogen fertilizer. Conversely, treatment with no fertilization resulted in a 6% increase in SOC, indicating a decrease in LLB_C and FF, which were the stable carbon fractions compared to FP, while, in contrast, the labile carbon fractions, such as POXC and LB_C, experienced an increase. The availability of $NH_4^+$ and $NO_3^-$ thus influences the availability of reduced N additions.

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