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Enhancing Soil Nitrogen Retention Capacity by Biochar Incorporation in the Acidic Soil of Pomelo Orchards: The Crucial Role of pH

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Abstract: Biochar is commonly used to improve acidic soil and reduce nitrogen loss. However, the impact of biochar on soil nitrogen retention, especially at varying pH levels, is not fully understood. Soil samples were obtained from an acidic red soil citrus orchard. The soil pH was adjusted using CaO, with five levels (4.0, 5.1, 5.8, 6.6, and 7.2), and two biochar doses (0% and 1%) were applied. The study used ^{15}N-Tracer and *Ntrace* to investigate biochar's influence on soil nitrogen retention at different pH levels. The results showed that soil amendment with biochar improved gross mineralization rates (*TM*) and gross NH_4^+ immobilization rates (*TI*), except at pH 4.0 for TI. Biochar enhanced heterotrophic nitrification (O_{Nrec}) within pH 4.0–7.4, with a threshold for autotrophic nitrification (O_{NH4}) at pH 6.4. The findings revealed biochar's positive effect on soil nitrogen retention within pH 4.5–6.4. Biochar had a greater impact on *TI* than *TM* and inhibited O_{NH4}, potentially enhancing nitrogen retention in this pH range. These results highlight the significance of considering biochar incorporation for improving nitrogen use efficiency and reducing NO_3^--N loss in subtropical pomelo orchards.

Keywords: nitrogen retention capacity; soil pH; biochar incorporation; *TM*; *TI*; O_{NH4}

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1. Introduction

The soil nitrogen cycle is one of the main biogeochemical cycles in terrestrial ecosystems [1]. Understanding the soil nitrogen cycle process is of great significance for soil nitrogen management. Soil mineralization, nitrification, and assimilation are the main processes in the nitrogen cycle [1]. Soil mineralization is the process of changing organic nitrogen into inorganic nitrogen, which reflects the nitrogen supply capacity of soil [2]. Soil nitrification can generate nitrates, which are crucial nitrogen sources for plants, as well as N_2O gas, a powerful greenhouse gas [3]. Soil immobilization is the process by which inorganic nitrogen is absorbed into the soil organic nitrogen pool and is often used to measure the nitrogen retention capacity of soil [4]. In addition, the production and consumption of soil nitrate nitrogen is a dynamic process, and the decrease in production rate or the increase in consumption rate is another important index of soil nitrogen retention ability [5]. The ecological implications of soil nitrogen retention are significant, as it pertains to the ability of soil to maintain and protect the crucial nitrogen that supports plant growth and ecosystem operation [6]. However, soil acidification [7] and agricultural practices [8] can interfere with soil N retention mechanisms, leading to increased environmental risks. An oversupply of N fertilizer can result in various environmental problems, such as water eutrophication caused by nitrate leaching [9] and warming effects due to N_2O emissions [10]. Proper N management can help to improve soil nitrogen retention while reducing environmental pressure.

Soil pH plays a major role in N transformation, affecting gross N mineralization [11], gross immobilization of ammonium and nitrate [4,11], gross nitrification rates [12,13], and dissimilatory nitrate reduction to ammonium (*DNRA*) [13]. Using soil nitrification as an example, autotrophic ammonia-oxidizing bacteria (AOB) are highly sensitive to soil pH and struggle to grow below pH 5.0–5.5 [14]. According to a study conducted by Zhang et al. [15], the production of NO_3^- from heterotrophic nitrification increases with a decrease in soil pH. The regulation of nitrification activity relies heavily on soil pH, with higher pH levels promoting nitrate production [16]. As a result, soil pH significantly impacts soil nitrogen transformation and ultimately affects the retention capacity of soil for nitrogen.

Subtropical red soil (equivalent to Ultisols in US soil taxonomy) is widely distributed in southern China, and is known for its rich iron and aluminum oxides [17,18]. Orchards are a crucial agroecosystem in subtropical regions, occupying an area of more than 5 million ha [19]. Citrus is a widespread fruit in subtropical regions, with China's pomelo [*Citrus maxima* (Burm) Merr.] having the largest planting area in the world [20]. However, an oversupply of N fertilizers and artificial acid deposition have significantly aggravated soil acidification [21]. This has led to nutrient deficiency [22] and an increase in the availability of heavy metals [23], which have now become important limiting factors for agroecosystems in China. Soil acidification is an important feature of Chinese orchards [24], which reduces the soil nitrification rate [25]. Previous studies have indicated that citrus thrives on nitrate nitrogen and that excessive ammonium nitrogen can impede citrus growth [26]. Soil N transformation characteristics in strongly acidic soil may not support the optimal growth of citrus. From an environmental perspective, soil acidification can lead to a decrease in soil nitrification rates and an increase in the proportion of ammonium nitrogen in soil inorganic nitrogen. High levels of ammonium nitrogen accumulation in the soil can also promote N loss processes [27]. Therefore, it is important to implement agricultural management measures to mitigate the impact of soil acidification on soil nitrogen management in orchards and improve nitrogen use efficiency (NUE).

Biochar is a promising soil amendment that has recently been recommended as an agricultural management measure. It reduces soil acidification [28] and improves crop yield and quality [29–31]. In addition, biochar increases the biodiversity of soil microbes because biochar provides them with a carbon source [32]. The macroporosity and water permeability of biochar enhance *Gemmatimonadetes*, thereby enhancing rhizosphere function and promoting crop growth [18]. It has also been reported that biochar provides a suitable growth habitat for microorganisms, increasing microbial biomass [32]. A study on the stability of biochar has shown that physical and chemical degradation of biochar occurs; but for low-carbon soils, biochar application is an effective measure to increase organic carbon [33]. Nitrogen is a crucial nutrient for crop growth, and enhancing nitrogen consumption to minimize environmental risks is a key aspect of sustainable agriculture. Can the effects of biochar on nitrogen transformation improve the nitrogen mineralization and retention capacity of acidified soil, thereby facilitating nitrogen uptake and utilization by crops and reducing the risk of nitrate leaching? Biochar incorporation is not only beneficial to the improvement of soil quality but also seems to support nitrogen retention, as evidenced in studies by Bruun et al. (2012) [34,35], Ibrahim et al. (2020) [36], and Lehmann et al. (2003) [37]. Moreover, biochar has been found to enhance crop nitrogen use efficiency (NUE) in certain studies [38,39]. Nevertheless, there are instances where the liming effect of biochar has unexpectedly amplified the soil's nitrification capacity [40], leading to a possible escalation in the risk of soil NO_3^- leaching and blocking nitrogen retention in subtropical orchards. These previous reports did not provide a systematic explanation of how biochar affects nitrogen transformation in soils with varying pH levels, particularly in strongly acidic soil, and the underlying mechanism.

Our study is based on ^{15}N-Tracer and *Ntrace* to quantify gross N transformation rates, which has been applied to the research of orchards, paddy, vegetable, grassland, forest, and other soils [5]. The aim of our study was to verify the impact of biochar on nitrogen transformation in soils with varying pH levels, based on the following hypotheses: (1) The incorporation of biochar can promote soil microbial activity and improve soil mineralization and nitrification. (2) Biochar, composed mainly of recalcitrant organic carbon, cannot act as a substrate for ammonium immobilization, thereby not improving the soil's immobilization of ammonium. (3) The addition of biochar increases the rate of nitrification, possibly due a pH effect, leading to an increased risk of nitrate leaching as nitrogen retention in the soil decreases.

2. Materials and Methods

2.1. Soil and Biochar Sampling

The study was conducted in Dongkeng Village, located in Banzai Town, Pinghe County, Fujian Province, China. The precise coordinates of the area are 24.283360 N, 117.340555 E (Figure S1). The area is characterized by a typical subtropical climate, with an average yearly temperature of 23.4 °C and precipitation of 1677 mm. The land is mainly utilized for cultivating pomelo [*Citrus maxima* (Burm) Merr.] orchards. Samples of soil (0–20 cm depth) were gathered via the five-point approach, sifted through a 4 mm sieve, mixed, and then kept at 4 °C for storage. The biochar was produced at 600 °C via a slow pyrolysis of rice straw. Tt was purchased from Jiangsu Huafeng Agricultural Bioengineering Co., Ltd. (Xinyang Road, Yangzhong City, Zhenjiang, Jiangsu Province, China). The soil type is Ferrisol, which belongs to low activity and strong acid soil according to WRB classification, and the texture is clay. The properties of soil and biochar are shown in Table 1.

Table 1. Physicochemical properties of soil and biochar.

	Soil	Biochar
pH	3.7	9.3
SOC	15.3 g kg^{-1}	286 g kg^{-1}
TN	1.3 g kg^{-1}	4.6 g kg^{-1}
specific surface area (SSA)		54.0

2.2. pH Adjustment

The soil was adjusted to achieve five pH levels (4.0, 5.1, 5.8, 6.6, 7.2) by adding varying amounts of CaO (0 g kg^{-1},1.5 g kg^{-1}, 2.6 g kg^{-1}, 3.4 g kg^{-1}, 4.3 g kg^{-1}). Previous reports have demonstrated that soil microorganisms reached a stable state of respiration after one month of incubation [41]. The manipulated soil is incubated in a greenhouse for two months, where the average temperature ranges between 24 and 26 °C, and the air humidity is maintained at the same level as the surrounding humidity due to all-around ventilation in the greenhouse. After incubation, the soil was sieved through a 2 mm mesh and stored at 4 °C for further experiments.

2.3. ^{15}N Tracing Experiment

Ten treatments were conducted, including a control group (CK) without biochar, and another group (BC) with 1% (*w*/*w*) biochar. The treatment groups were named S1, S2, S3, S4, and S5, and their corresponding groups with biochar were named S1 + B, S2 + B, S3 + B, S4 + B, and S5 + B. For each treatment, 24 Erlenmeyer flasks were utilized. Approximately 30 g soil (dry weight equivalent) was mixed with varying amounts of biochar and placed into the flasks. The flasks were then stabilized in an incubator at 25 °C for 24 h. The soil samples were weighed bi-daily, and water was added when required to maintain moisture levels (30–40% of its water-holding capacity). To ensure proper soil aeration, the Erlenmeyer flasks were opened for an hour every two days. For 12 of the flasks in each treatment group, fertilizer was added as $^{15}NH_4NO_3$ (10.12 atom% excess), while the other 12 received $NH_4{}^{15}NO_3$ (10.25 atom% excess). Each flask contained 60 μg N g^{-1} dry soil for N application. Once NH_4NO_3 fertilizer was added, soil moisture was adjusted to 60% of its water holding capacity and placed in a 25 °C incubator. Destructive sampling was carried out at 0.5, 48, 96, and 144 hours after NH_4NO_3 application. To measure the concentrations of $NH_4{}^+$-N and $NO_3{}^-$-N and ^{15}N enrichment, six Erlenmeyer flasks were picked at random from each treatment group.

2.4. Laboratory Analyses

Soil moisture content was determined through a process of drying the soil at 105 °C for 8 h. Soil pH was measured at a soil to water ratio of 1:2.5 (*v:v*). Total nitrogen in soil was determined using the Kjeldahl method, while soil organic carbon was measured through the H_2SO_4-$K_2Cr_2O_7$ digestion method. Soil samples were then taken and extracted with a 2 mol L^{-1} KCl solution, using a 1:5 (*v:v*) soil-solution ratio, with subsequent analysis for ammonium nitrogen and nitrate nitrogen concentrations carried out using a flow analyzer (SkalarSAN++, SKALAR, Breda, The Netherlands). To ensure data accuracy, one reference material per 10 samples is used for quality control when testing with a flow analyzer. A remaining portion of the soil sample was allotted to measure dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) using a TOC instrument (ASI-L, Shimadzu, Japan). The isotopic compositions of $NH_4{}^+$ and $NO_3{}^-$ were measured using an improved diffusion method followed by measurement with an isotope mass spectrometer (IsoPrime 100, elementar, Hanover, Germany), according to Zhang et al. (2020) [8]. One quality control sample is included for every fifteen samples during the process of isotope determination to ensure data accuracy.

2.5. Data and Statistical Analyses

Gross N transformations in soil were quantified by the ^{15}N tracing tool *Ntrace* [42]. The model included the following processes: M_{Nlab} and M_{Nrec}, which convert labile and recalcitrant organic nitrogen to $NH_4{}^+$, respectively; O_{NH4} and O_{Nrec}, which oxidize $NH_4{}^+$ and recalcitrant organic nitrogen to $NO_3{}^-$, respectively; *DNRA*, which reduces $NO_3{}^-$ to $NH_4{}^+$; I_{NO3}, which immobilizes $NO_3{}^-$ onto recalcitrant organic nitrogen; A_{NH4}, which adsorbs $NH_4{}^+$ onto cation exchange sites; R_{NH4}, which releases adsorbed $NH_4{}^+$; I_{NH4_Nlab}, which immobilizes $NH_4{}^+$ onto labile organic nitrogen; and I_{NH4_Nrec}, which immobilizes $NH_4{}^+$ onto recalcitrant organic nitrogen.

The Equations (1) and (2) were used to determine the gross mineralization rates (*TM*) and the gross $NH_4{}^+$ immobilization rates (*TI*), respectively, as shown in Zhang et al. (2020) [8]:

$$TM = M_{Nrec} + M_{Nlab} \tag{1}$$

$$TI = I_{NH4\_Nrec} + I_{NH4\_Nlab} \tag{2}$$

Furthermore, the gross rates of inorganic N production (*NP*), the gross rates of inorganic N consumption (*NI*), and the net rate of inorganic N supply (*NS*) were calculated using the Equations (3) and (4) from Zhang et al. (2020) [8]:

$$NP = M_{Nrec} + M_{Nlab} + O_{Nrec} \tag{3}$$

$$NI = I_{NH4\_Nrec} + I_{NH4\_Nlab} + I_{NO3} \tag{4}$$

$$NS = NP - NI \tag{5}$$

Curve-fitting and bivariate correlation analysis were employed to explore the relationships between N transformation rates, soil properties, and biochar incorporation.

N transformation rate differences were analyzed with standard deviation and 95% confidence intervals due to the high number of *Ntrace* iterations [43,44]. Multiple comparisons of treatments were conducted with Duncan's test, noting significant differences at $p < 0.05$. Statistical analysis was performed through Origin 2021 (Origin Lab, Los Angeles, CA, USA) and IBM SPSS Statistics 20 (IBM SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Soil Properties

As displayed in Table 2, biochar incorporation significantly increased the pH value ($p < 0.05$) by 18.1%, 15.3%, 12.7%, 6.7%, and 7.5% and increased the SOC content ($p < 0.05$) by 7.5%, 4.6%, 9.3%, 6. 9%, and 9.0%. With regards to the S1 treatment, the DOC content in soil supplemented with biochar was significantly reduced ($p < 0.05$) by 34.0%, while in other treatments, it exhibited an upward trend (7.5% to 44.4%). The incorporation of biochar increased the DON content.

Table 2. Effect of adding biochar on the chemical characteristics of soil at pH 4.0, pH 5.1, pH 5.8, pH 6.6, and pH 7.2.

Treatment	pH	SOC $g\ kg^{-1}$	TN $g\ kg^{-1}$	DOC $mg\ kg^{-1}$	DON $mg\ kg^{-1}$
S1	4.10 i	14.42 h	1.61 a	77.11 bc	27.07 e
S2	5.05 g	16.05 de	1.77 a	60.19 f	27.79 e
S3	5.65 f	14.77 gh	1.63 a	64.92 ef	32.93 d
S4	6.57 c	17.27 b	1.88 a	61.80 f	33.57 d
S5	7.03 b	15.07 fg	1.65 a	70.36 cde	35.34 abc
S1 + B	4.84 h	15.50 ef	1.59 a	50.89 g	27.85 e
S2 + B	5.82 e	16.79 bc	1.71 a	69.67 de	34.43 cd
S3 + B	6.37 d	16.14 d	1.67 a	82.60 b	37.59 abc
S4 + B	7.01 b	18.46 a	1.92 a	89.21 a	39.43 a
S5 + B	7.56 a	16.43 cd	1.73 a	75.62 cd	38.95 ab

Note: Different lowercase letters denote significant differences across various treatments at $p < 0.05$ (ANOVA, Duncan-test). The data are the averages of three replicates.

3.2. NH_4^+ and NO_3^- Concentrations and Enrichment during Incubation

Throughout the majority of the incubation period, NH_4^+ concentrations within the S1, S2, and S3 treatments remained consistently below those observed in S1 + B, S2 + B, and S3 + B, respectively (Figure 1a–c). Conversely, the NH_4^+ concentrations in the S4 and S5 treatments were higher than those in S4 + B and S5 + B, respectively (Figure 1d,e). Furthermore, the concentrations of NO_3^- in the S1, S2, and S3 treatments were higher than those in S1 + B, S2 + B, and S3 + B, respectively (Figure 1a–c). The ^{15}N enrichment of NH_4^+ following the addition of $^{15}NH_4NO_3$ decreased during the incubation period, and the slope of $^{15}NH_4^+$ enrichment increased as the soil pH increased (Figure 1f–j). These findings suggested that incorporating biochar and increasing pH can lead to an increase in the gross rate of mineralization. Over time, the $NH_4{}^{15}NO_3$ treatment demonstrated a decline in the ^{15}N enrichment of NO_3^-, along with a reduction in the rate of $^{15}NH_4^+$ as pH levels increased (Figure 1k–o). Additionally, an increase in soil pH was observed to correspond with a higher gross nitrification rate (Figure 1f–h). The gross nitrification rate in the S4 and S5 treatments was higher than that in the S4 + B and S5 + B treatments during most of the incubation period (Figure 1i,j).

3.3. Rates of Gross Transformation of N

The *Ntrace* analysis showed that biochar incorporation increased the *TM* of soil at different pH values by 16.1%, 39.8%, 98.1%, 44.2%, and 13.3% (Figure 2a). In the S1 treatment, biochar incorporation reduced *TI* and O_{Nrec} by 13.2% and 26.3%, respectively, while biochar was able to significantly ($p < 0.05$) increase *TI* and O_{Nrec} in the other treatments (Figure 2b,c). In the S1, S2, and S3 treatments, biochar significantly ($p < 0.05$) reduced

O_{NH4} by 16.6%, 59.9%, and 45.3%, respectively (Figure 2d). Biochar incorporation had varying effects on *DNRA* and I_{NO3}, with a decrease in S1, S2, S3, and S5 treatments but a significant ($p < 0.05$) increase of 11.3 times in the S4 treatment (Figure 2e). Similarly, biochar incorporation resulted in a decrease in I_{NO3} in the S2 and S3 treatments but a significant ($p < 0.05$) increase in the S1, S4, and S5 treatments (Figure 2f). Furthermore, biochar incorporation caused a significant ($p < 0.05$) increase in A_{NH4} and R_{NH4a} in the S1 treatment but a decrease in the other treatments (Figure 2g,h).

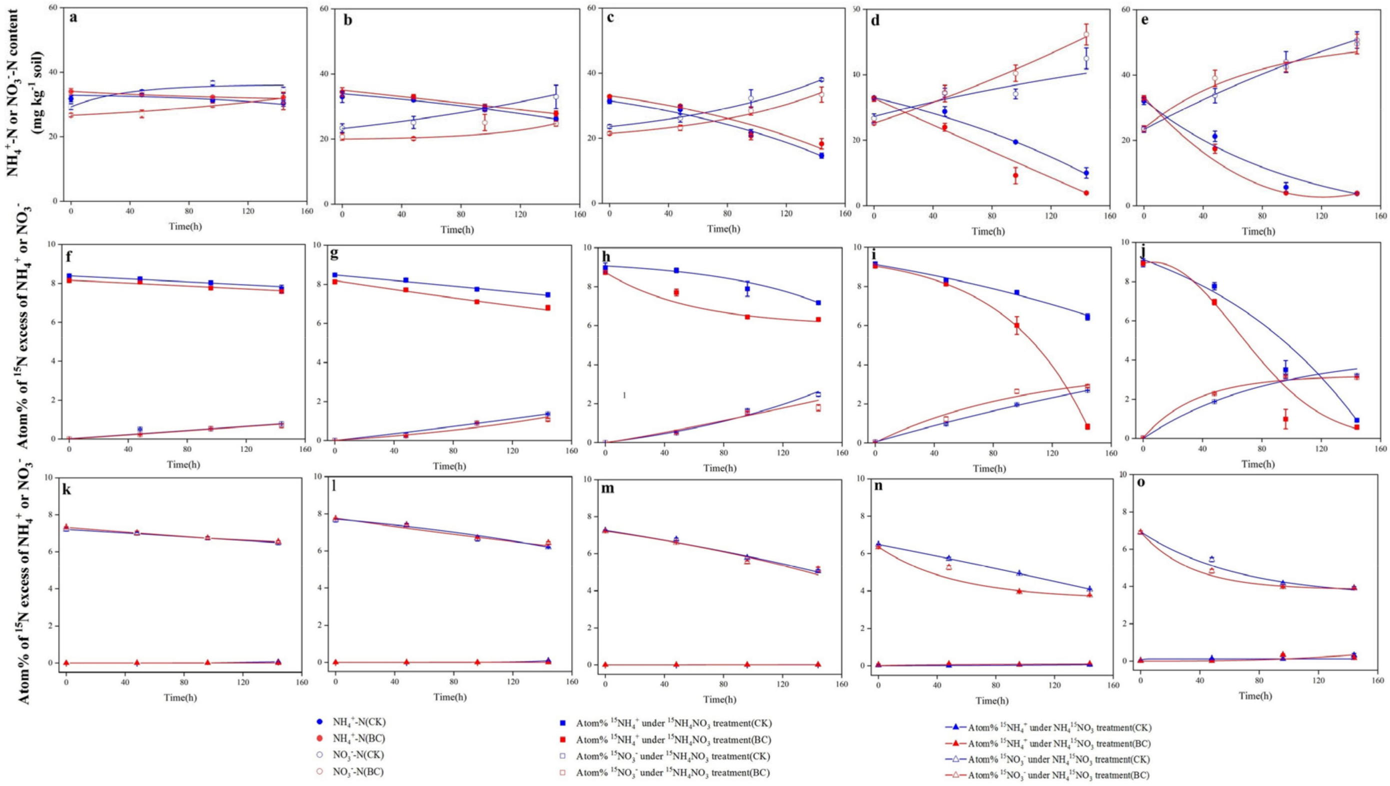

Figure 1. Measured concentrations and ^{15}N enrichments of NH_4^+-N and NO_3^--N pools of S1 (**a**,**f**,**k**), S2 (**b**,**g**,**l**), S3 (**c**,**h**,**m**), S4 (**d**,**i**,**n**), and S5 (**e**,**j**,**o**). Where, S1, S2, S3, S4, and S5 soil pH values are 4.0, 5.1, 5.8, 6.6, and 7.2, respectively. The concentrations of NH_4^+-N or NO_3^--N in different treatments were the average concentrations under $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ treatments ($n = 6$); the atom% of ^{15}N excesses were the average ^{15}N enrichments of NH_4^+-N and NO_3^--N in soils under $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$ treatments ($n = 3$).

3.4. Correlation between Soil Physicochemical Properties and Rates of Gross N Transformation

As *TM* increased, *TI* also showed a significant increase (Figure S2). The soil pH value had a positive correlation with *TM*, *TI*, and O_{NH4}, leading to a significant increase (Figures 3 and 4). However, O_{Nrec} showed a decrease (Figure 4). Biochar incorporation proved to be beneficial for increasing O_{Nrec} when the pH value was between 4.0 and 7.4 (Figure 4). When the pH value exceeded 6.4, biochar incorporation led to an increase in O_{NH4} (Figure 4). A significant increase was observed in *TM* and *TI* with an increase in DON content ($p < 0.001$; Figure S3), while O_{Nrec} and O_{NH4} were found to be related to the C/N ratio ($p < 0.05$; Figure S4). Biochar incorporation showed a significant promotion in *NP* when the soil pH increased from 4.2 to 7.1 ($p < 0.01$; Figure 5). Biochar incorporation promoted the increase in *NI* in soils at different pH values ($p < 0.01$; Figure 5). Moreover, biochar incorporation led to a decrease in *NS* when the soil pH exceeded 4.5 ($p < 0.05$; Figure 5).

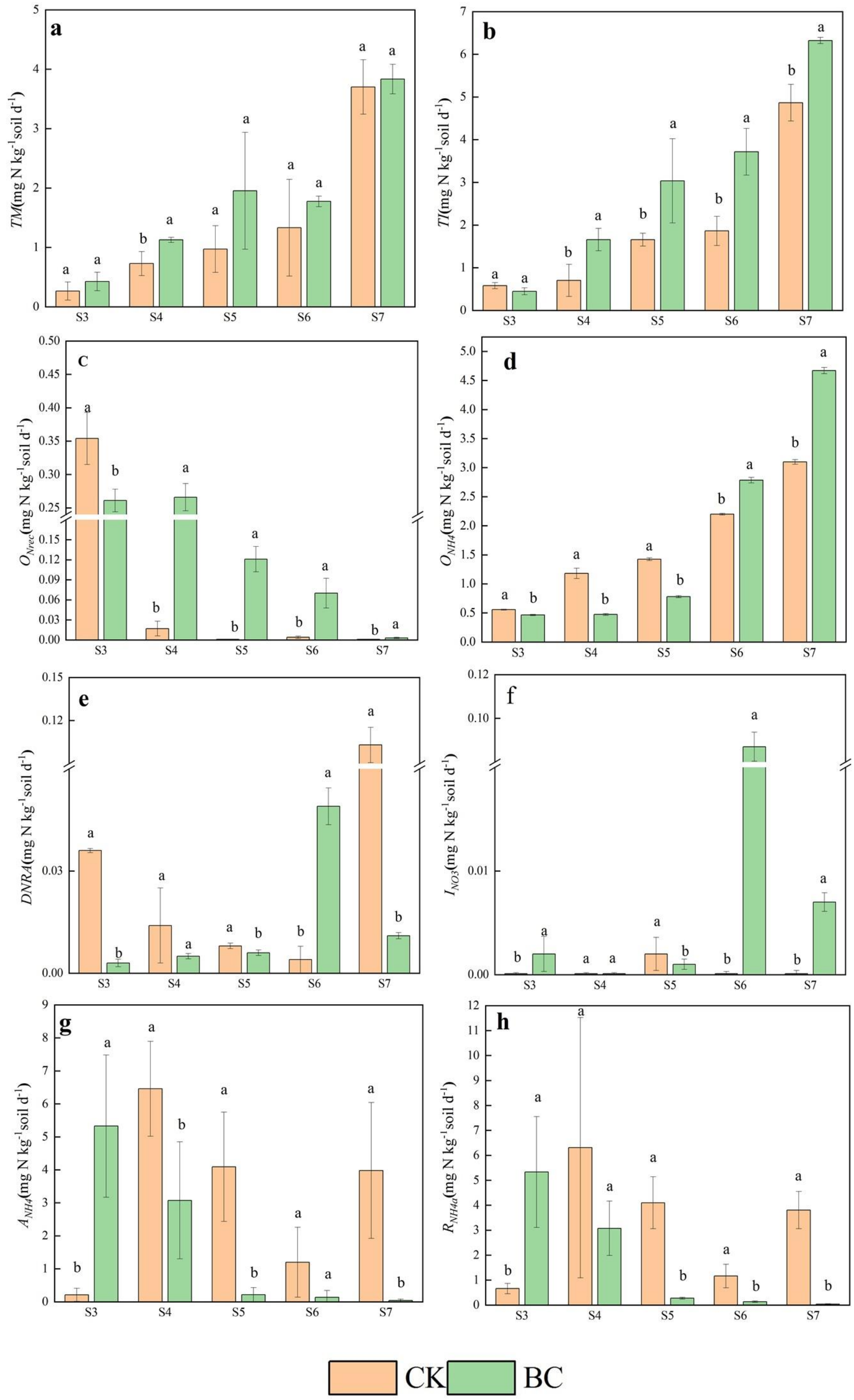

Figure 2. Temporal variation in gross N transformation rates in soil simulated by the ^{15}N trace model. Where, S1, S2, S3, S4, S5 soil pH values are 4.0, 5.1, 5.8, 6.6, 7.2, respectively. Where *TM* = the gross mineralization rates, *TI* = the gross NH_4^+ immobilization rates, I_{NO3} = immobilization of NO_3^- to recalcitrant organic N, O_{Nrec} = oxidation of recalcitrant organic N to NO_3^-, O_{NH4} = oxidation of NH_4^+ to NO_3^-, *DNRA* = dissimilatory NO_3^- reduction to NH_4^+, A_{NH4} = adsorption of NH_4^+, R_{NH4a} = release of NH_4^+ ads. The standard errors of the means ($n = 3$) are shown as vertical bars and letters represent significant differences between values within time ($p < 0.05$).

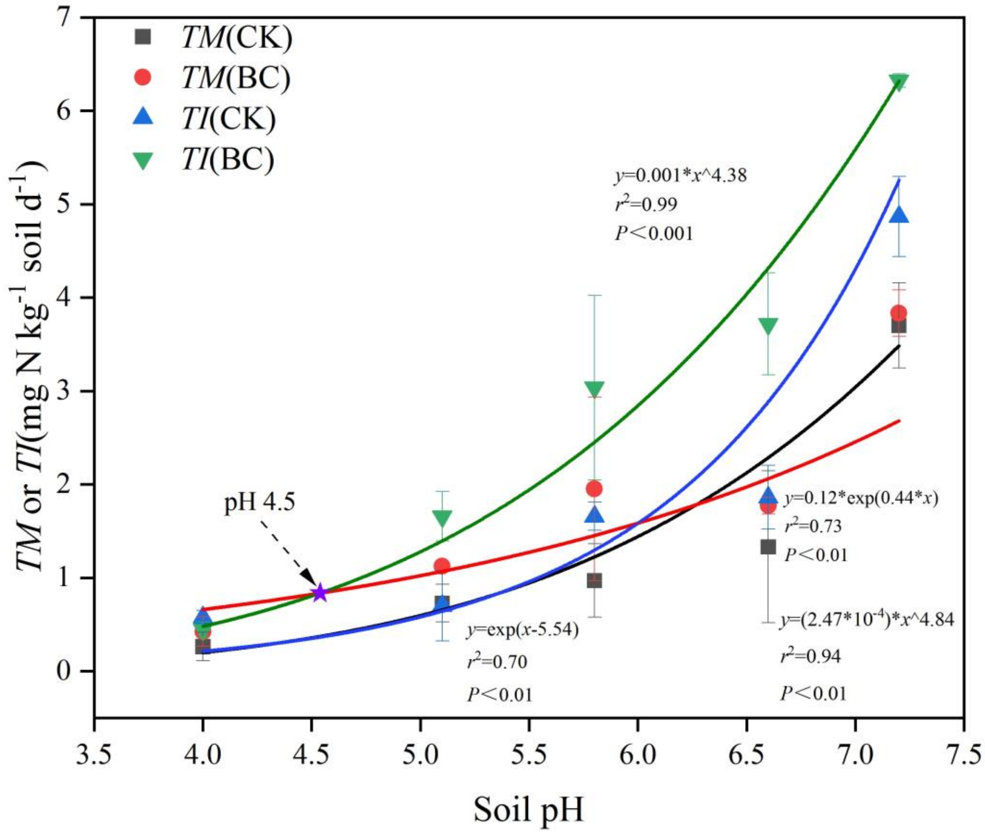

Figure 3. The relationship between soil pH and *TM* (*TI*). The standard errors of the means ($n = 3$) are shown as vertical bars. Where *TM* = the gross mineralization rates; *TI* = the gross NH_4^+ immobilization rates.

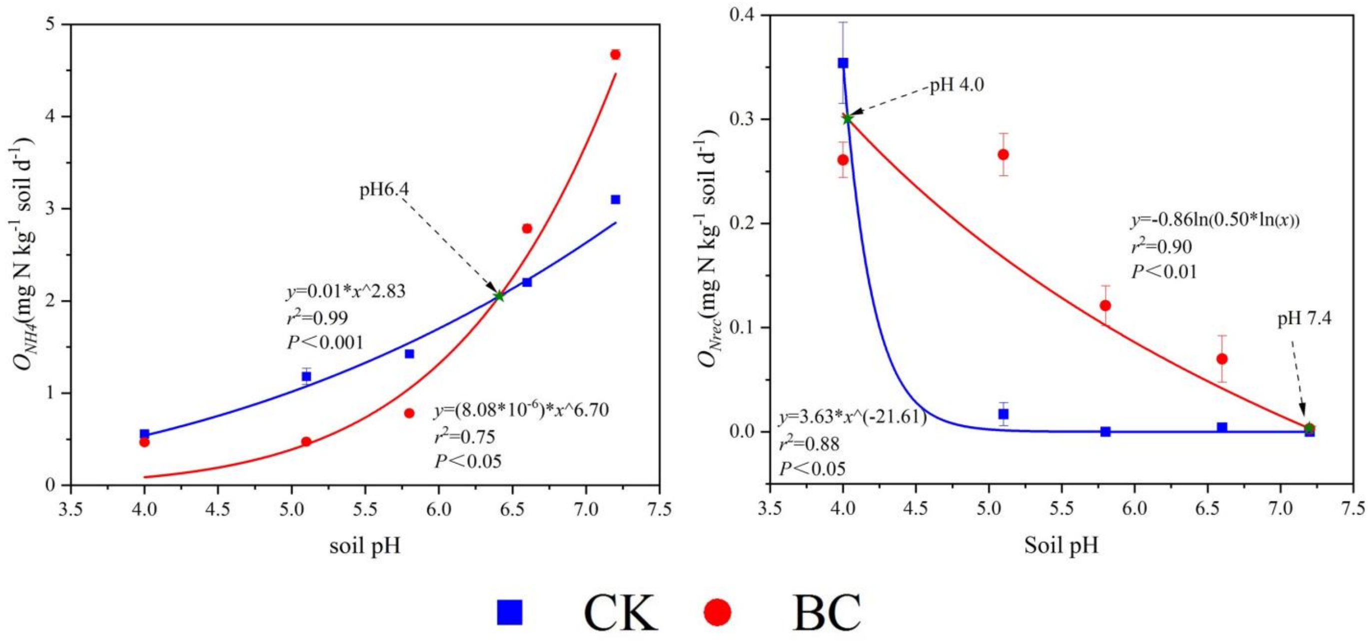

Figure 4. The relationship between soil pH and O_{NH4} (O_{Nrec}). The standard errors of the means ($n = 3$) are shown as vertical bars. Where O_{Nrec} = oxidation of recalcitrant organic N to NO_3^-; O_{NH4} = oxidation of NH_4^+ to NO_3^-.

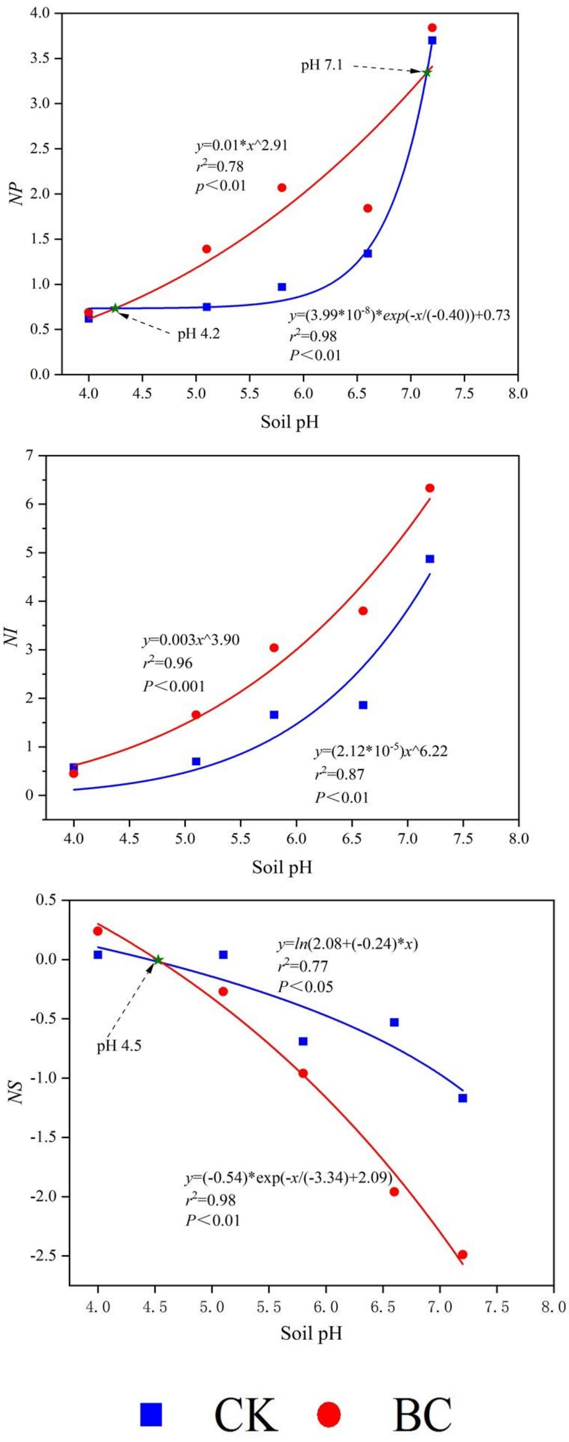

Figure 5. The relationship between soil pH and *NP*, *NI*, *NS*. The standard errors of the means (n = 3) are shown as vertical bars. Where *NP* = the gross rates of inorganic N production; *NI* = the gross rates of inorganic N consumption; *NS* = the net rate of inorganic N supply; $NP = M_{Nrec} + M_{Nlab} + O_{Nrec}$; $NI = I_{NH4\_Nrec} + I_{NH4\_Nlab} + I_{NO3}$; $NS = NP - NI$.

4. Discussion

4.1. Effects of Biochar Incorporation on TM and TI in Soils at Different pH Values

The incorporation of biochar into soil was found to increase *TM* at different pH values (Figure 2). This finding is consistent with Nelissen et al. [45], who reported that biochar had a positive effect on soil mineralization in acidic soils. The alkaline nature of biochar can improve soil pH values (Table 2), which can further enhance *TM* (Figure 3). The increase in soil pH values can promote microbial activity [46], which facilitates soil nitrogen mineralization [47]. Moreover, an increase in soil pH values can improve the substrate decomposition ability of microorganisms and promote microbial mineralization [48]. Mineralization was positively correlated with TOC and TN content [49], and our results showed that biochar incorporation can increase soil carbon and nitrogen contents (Table 1). The unstable organic carbon in biochar can stimulate rapid microbial responses, resulting in a soil priming effect and promoting soil mineralization [50]. The addition of biochar in acidic soil increases the pH, regulating soil microorganisms and enhancing microbial activity, ultimately facilitating mineralization [51]. It has been reported that an increase in soil organic N promotes net N mineralization [52]. Our results found a good correlation between DON and *TM* ($p < 0.001$; Figure S3). Additionally, biochar can provide important microbial available nitrogen, alleviate restrictions on the growth and activity of soil microorganisms under low pH conditions, and contribute to soil nitrogen mineralization [53]. Our results confirmed the hypothesis that biochar incorporation was conducive to gross mineralization rates (*TM*). Certainly, aside from soil conditions, climate factors also exert an influence on soil nitrogen mineralization. To further enhance our understanding, future studies will focus on strengthening research in this aspect.

Biochar incorporation had different effects on *TI* in soils at various pH values. While biochar incorporation reduced *TI* in soil at pH 4.0 (Figure 2b), it increased *TI* in soils at other pH values, leading to improved soil nitrogen retention (Figure 2b). Biochar also enhanced NH_4^+-N concentrations in soil at pH 4.0, when nitrification rates were low (Figure 2c,d). However, excess NH_4^+-N in soil was not immobilized [54]. The incorporation of biochar was observed to be beneficial to A_{NH4} in soil at pH 4.0 value (Figure 2g), which was competitive with *TI* and O_{NH4} [49,54]. Furthermore, the positive effect of biochar incorporation on *TI* was found to be stronger than that on *TM* (Figure 3). The C:N ratio of biochar was identified as a key factor in determining nitrogen mineralization and immobilization, with C:N > 25 being favorable for nitrogen immobilization [55]. The incorporation of biochar promoted the retention of organic nitrogen (*TI* > *TM*), thereby enhancing the availability of plant nitrogen [56]. Additionally, the results showed a linear relationship ($r^2 = 0.93$; $p < 0.001$) between *TM* and *TI* (Figure S2), with previous studies highlighting their similarity [57,58]. Our findings suggested that gross NH_4^+ immobilization rates (*TI*) are affected by soil pH after biochar incorporation, supporting the hypothesis partly.

4.2. Effects of Biochar Incorporation on O_{Nrec} and O_{NH4} in Soils at Different pH Values

Biochar incorporation enhanced heterotrophic nitrification (O_{Nrec}) within the pH range of 4.0–7.4. While heterotrophic nitrification is commonly associated with low pH soils [59], recent studies have revealed that it can occur under a wide pH range and is prevalent in natural and artificial ecosystems, playing a crucial role in maintaining the inorganic nitrogen balance of soil [60]. Our findings indicated that heterotrophic nitrification has a positive impact on strongly acidic soil (Figure 2), and the rate of heterotrophic nitrification decreases as pH value increases ($p < 0.05$; Figure 4). Furthermore, biochar has a distinct threshold for heterotrophic nitrification in soils with different pH values. In soils with a pH < 4.0 or pH > 7.4, the incorporation of biochar was observed to inhibit the process of heterotrophic nitrification. Previous studies have indicated that substrate properties and availability have a significant impact on heterotrophic nitrification. In low soil pH levels, metals such as iron and aluminum tend to fix biochar's organic matter, leading to a decrease in DOC content added by the biochar (Table 1) and subsequently lowering heterotrophic nitrification [50]. Conversely, when the pH value was between 4.0 and 7.4, incorporating biochar promotes heterotrophic nitrification. Furthermore, when soil pH levels are high, DOC content increases with biochar incorporation (Table 1), leading to a higher heterotrophic nitrification rate (Figure 2), which supports Zhang et al.'s [60] findings. The results of our study indicate that heterotrophic nitrification (O_{Nrec}) varies with soil Ph levels after biochar incorporation, and some of these findings support our hypothesis.

The Incorporation of biochar was found to trigger autotrophic nitrification, with a PH threshold of 6.4. Autotrophic nitrification was found to be the primary NO_3^- producing process in the soil. A curve-fitting analysis revealed that the incorporation of biochar could reduce the rate of autotrophic nitrification when soil pH is below 6.4 (Figure 4). Previous research has indicated that high ammonium nitrogen concentrations can inhibit nitrification [61]. In the S1, S2, and S3 treatments, biochar incorporation increased ammonium nitrogen concentrations to high levels, which can inhibit the nitrification process (Figure 2). However, when the soil pH was above 6.4, the addition of biochar increased the rate of autotrophic nitrification (Figure 4). Previous studies have shown that nitrifying bacteria and archaea are more abundant in soils with pH above 6 [62]. Therefore, when the soil pH is above 6.4, biochar incorporation can provide a suitable soil environment (including suitable pH and soluble organic carbon) that is more conducive to the growth and proliferation of nitrifying bacteria and archaea. Moreover, there have been indications that soil nitrification variables, namely NH_4^+-N availability, nitrifying activity, and soil pH, may interact with each other. Low nitrifying bacterial activity led to no increase in nitrification despite the addition of NH_4^+-N and pH elevation. Conversely, in situations of high nitrification potential, both soil pH and NH_4^+-N were found to encourage nitrification [53]. Immobilization of microbial NH_4^+ and adsorption of NH_4^+ can also influence autotrophic nitrification, indirectly affecting NO_3^- generation [49,54]. Our study revealed that biochar was beneficial for increasing the adsorption of NH_4^+ at soil pH 4.0 (Figure 2), which in turn affected NO_3^- production. At pH > 5.0, biochar promoted the conversion of NH_4^+ into organic N and affected autotrophic nitrification. When pH was near neutral, biochar was more conducive to autotrophic nitrification. Our findings partly supported the hypothesis that autotrophic nitrification (O_{NH4}) varies with soil pH after biochar incorporation.

4.3. Nitrogen Retention Mechanism after Biochar Incorporation in Soils at Different pH Values

One potential mechanism for improving nitrogen retention after incorporating biochar is a higher gross NH_4^+ immobilization rate (*TI*) compared to gross mineralization rates (*TM*). In subtropical regions characterized by high precipitation and warm conditions, improving soil nitrogen retention capacity is essential for enhancing agricultural soil NUE and reducing environmental risks. *NS* is one of the main indexes of soil nitrogen retention capacity [8]. Our study found that biochar incorporation can enhance the nitrogen retention performance of subtropical citrus orchard soil (*NS*; Figure 5). When soil pH is above 4.5, biochar promotes inorganic nitrogen consumption (*NI*; Figure 5), particularly the assimilation rate of ammonium nitrogen (*TI*; Figure 2), which is the primary mechanism by which biochar improves soil nitrogen retention performance. Further analysis revealed that the threshold of both *TM* and *TI* after biochar incorporation was pH 4.5 (Figure 3). When the pH was above 4.5, *TI* > *TM* led to enhanced inorganic nitrogen consumption (*NI*), ultimately resulting in improved soil nitrogen retention performance (*NS*).

The key mechanism behind the biochar-driven reduction in the risk of nitrate loss was the inhibition of autotrophic nitrification. This effect is crucial for enhancing the nitrogen retention ability of soil. In regions with high temperatures and rainfall, high levels of nitrate nitrogen can elevate the risk of nitrate entering the soil and nearby water bodies [5]. Therefore, reducing nitrate production and increasing nitrate consumption is desirable. Our study demonstrated that biochar primarily influenced nitrate production processes, namely autotrophic and heterotrophic nitrification (Figure 2). However, the consumption process of nitrate can be disregarded due to low rates of dissimilatory nitrate reduction (*DNRA*) and nitrate nitrogen assimilation (I_{NO3}) (Figure 2). A curve-fitting analysis showed that biochar incorporation could reduce NO_3^- production when the pH is below 6.4 (Figure 4).

Therefore, our findings suggest that the addition of biochar to subtropical orchard soils can enhance the soil's nitrogen retention ability when the pH falls between 4.5 and 6.4. Similar to our findings, the introduction of biochar into the soil enhances nitrogen fixation, resulting in the formation of a temporary organic nitrogen reservoir and reducing the likelihood of inorganic nitrogen leaching [37]. Furthermore, biochar possesses a porous structure and abundant functional groups, which contribute to enhancing the soil's capacity to retain nitrogen [63]. Our study highlights the importance of soil pH in nitrogen transformation processes, although other factors may also play a role. Future research efforts should focus on investigating the effectiveness of incorporating biochar to optimize nitrogen retention in subtropical orchard soils.

5. Conclusions

The impact of biochar on soil nitrogen transformation characteristics varied according to soil pH. Biochar addition was found to enhance nitrogen mineralization in acidic soils across different pH levels, but its effects on nitrogen assimilation and nitrification varied. Biochar incorporation enhanced heterotrophic nitrification (O_{Nrec}) within the pH range of 4.0–7.4, whereas it had a threshold for autotrophic nitrification (O_{NH4}) at pH 6.4. Biochar incorporation resulted in higher gross NH_4^+ immobilization rates (*TI*) than gross mineralization rates (*TM*) at pH > 4.5. Our correlation analysis revealed that soil pH was a critical factor in determining the impact of biochar on soil nitrogen transformation in subtropical citrus orchards. Our findings suggest that biochar incorporation could improve soil nitrogen retention when soil pH levels ranged from 4.5 to 6.4. The stimulation effect of biochar on gross NH_4^+ immobilization was found to be stronger than that on gross mineralization rates (*TM*), and its inhibition effect on autotrophic nitrification (O_{NH4}) may be the main potential mechanism for improving soil nitrogen retention capacity. Therefore, soil pH conditions should be taken into account when applying biochar in subtropical orchards, as appropriate soil pH levels are crucial for enhancing soil nitrogen retention capacity. For future research in subtropical orchards, it is crucial to investigate the potential variations in the nitrogen retention capabilities of different biochar preparation conditions and materials, specifically in subtropical pomelo orchards.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13082110/s1, Figure S1: Blue point is the location of the soil sampling area; Figure S2: The relationship between *TM* and *TI*; Figure S3: The relationship between DON and *TM* (*TI*); Figure S4: The relationship between C/N and O_{Nrec}.

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