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Original Research

Soil Net Organic Nitrogen Mineralization Kinetics in Response to Short- and Long-Term Land-Use Conversion of Woodland to Tea Fields

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Abstract

In recent years, with growing demand for tea consumption, tea cultivation area in China has been increasingly expanded, largely as a result of land-use conversion from woodland into tea field. Up to now, however, soil net nitrogen mineralization kinetics in response to short-and long-term land-use conversion of woodland to tea field remain unclear. Thus, a laboratory aerobic incubation test under constant temperature in combination with a double-pool exponential model were conducted to explore soil organic net nitrogen mineralization dynamics, and quantify soil net nitrogen mineralization kinetics from different planting ages of tea fields (1, 2, 3, 4, 5, and 30 years old, named T1, T2, T3, T4, T5, and T30 correspondingly) that converted from Masson pine woodland and adjacent woodland (W30, 30 years old). The results showed that land-use conversion exerted great influence on soil physicochemical properties. Though there were no differences in soil total nitrogen (TSN) and total phosphorus (TP) contents between W30 and TN, the TSN and TP were strongly affected by long-tern land-use conversion. The double-pool exponential model fitted soil net nitrogen mineralization data well (R^2_{adj} ranged from 0.75 to 0.99; RMSE ranged from 0.18 to 0.99). The fitted parameter of soil active mineralization organic nitrogen pool (N_0) and soil active nitrogen mineralization rate constant (k_1) for the woodland soil were 9.09±2.32 mg N kg^{-1} and 0.24±0.13 d^{-1}, respectively. The N_0 for the tea plantation within two years

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of the growth stage presented decreasing tendencies, which indicated that soil nitrogen mineralization was stimulated after land-use conversion, and then showed increasing trends with the increasing tea planting ages. Besides, two stepwise regression models developed in this study showed that the nitrogen mineralized kinetic parameters could be well predicted by soil physicochemical properties. In conclusion, soil net nitrogen mineralization is greatly influenced by agricultural land-use conversion and planting ages.

Keywords: land-use conversion, nitrogen mineralization potential, nitrogen mineralized models, parameter estimation, tea plantations with different stand ages

Introduction

Soil organic nitrogen mineralization, which is generally considered as the transformation of organic nitrogen to inorganic nitrogen, is an important part of soil nitrogen biogeochemical cycle [1]. Mineralization of organic nitrogen reflects the endogenous nitrogen supply capacity of the ecosystem, and represents the important aspect to judge soil fertility [2-4]. Soil ammonium (NH_4^+) released by mineralization of organic nitrogen not only can be directly absorbed by crops, but also promotes the production and emission of greenhouse gases as substrate [1, 5]. Accurate evaluation of soil organic nitrogen mineralization dynamics is crucial for us to understand the soil organic nitrogen mineralization potential and advise the optimized fertilization.

Soil organic nitrogen mineralization is influenced by various factors such as soil total nitrogen (TSN), soil organic carbon (SOC), soil pH, temperature, moisture and so on [5, 6]. For instance, soil organic nitrogen mineralization was found to increase with the temperature increasing [7]. The soil water availability affects soil organic nitrogen mineralization by changing the number of microorganisms [8]. The changes of land use pattern and management practices may result in the change of soil properties, and thus changes the organic nitrogen mineralization process [2, 9]. For instance, studies have shown that the net mineralization rate of soil organic nitrogen in cultivated soil was significantly higher than that in adjacent forestland and grassland [10]. Screening of key factors that influenced soil organic nitrogen mineralization, and revealing its mechanism have become hotspots in the study of the terrestrial ecosystem nitrogen cycle.

It is essential to evaluate soil organic nitrogen mineralization potential and determine the soil organic nitrogen mineralization kinetics by employing models for guiding scientific fertilization. Currently, models for estimating nitrogen mineralization can be divided into four kinds: hyperbolic model, one-pool exponential model, double-pool exponential model, and special model [11, 12]. A large number of studies have indicated that the double-pool exponential model, which contains active and resistant nitrogen pools considering heterogeneous components of soil organic matter, was usually used frequently and has been found worked better [12].

In recent years, with growing demand for tea consumption, tea cultivation area in China has being increasingly expanded, which has reached nearly 3.05 million ha in 2017, and accounted for approximately 52% of the world' total acreage [13, 14]. The conversion from woodland into tea field is the most common agricultural practice to achieve faster benefits [15, 16]. The characteristics of soil organic nitrogen mineralization vary among different land-use types [12]. In contrast to woodland, tea plantations are characteristic with more intensively anthropogenic interference including periodical tillage and fertilization. Research shows that tillage could change soil moisture condition and soil aeration, and thus enhanced soil organic nitrogen mineralization [17]. Tea plant, as an acidophilous and perennial crop, need enough nitrogen fertilizers to satisfy nutrient requirements [18]. The nitrogen fertilizer application rates of tea field in China are usually 450 to 1200 kg N ha^{-1} yr^{-1} [13, 19] and even more in some cases [13]. Soil organic nitrogen mineralization response to nitrogen addition remains controversial that Luce [20] reported that net nitrogen mineralization may increase with the external nitrogen input, while Kowaljow and Mazzarino hold the opposite opinion [21]. The previous study shows that at the beginning of land use conversion from woodland to tea fields dramatically increased soil nitrogenous gas emissions possibly due to accelerating soil organic nitrogen mineralization [15]. Soil organic nitrogen pool and soil pH are bound to change with the increasing tea planting age. However, little is known about how planting ages affect the soil organic nitrogen mineralization [22]. In several previous studies, researchers have also measured soil net nitrogen mineralization rates in different agricultural fields and forests [2, 12, 23], but dynamics shifting of nitrogen mineralization kinetics in response to short-and long-term land-use conversion of woodland to tea fields are still lack of understanding.

We hypothesized that the newly converted tea field from woodland in the early stage was greatly disturbed by human activities compared with woodland, which may accelerate the mineralization of soil organic nitrogen; and that soil organic nitrogen mineralization kinetics were greatly affected by different ages of tea plantations. Therefore, we collected topsoil samples from a woodland (W30, aged 30 years), a newly established

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tea plantation (TN), and the adjacent old tea field (T30, aged 30 years). Both tea fields were converted from woodland. Six consecutive years (2013-2018) of soil samples from TN treatments (aged from 0 to 5 years, and named T0, T1, T2, T3, T4, and T5 correspondingly) were collected. Our aims were to: (1) elucidate the response of soil net organic nitrogen mineralization dynamics to land use conversion from woodland to tea field over short and long periods of time, (2) determine the soil organic nitrogen mineralization kinetics and obtain the important soil physicochemical properties that affected the dynamic parameters. This study will reveal soil nitrogen supply potential after conversion of woodland into tea field, and provide scientific references for applying nitrogen fertilizer into tea field.

Material and Methods

Experimental Site Description and Topsoil Sampling

Soil sampling site was located in Changsha county (28°32′50″N, 113°19′58″E), Hunan province, China, where belongs to typically subtropical monsoon climate. The average annual temperature in this region is 17.5ºC, and the mean annual precipitation is 1330 mm [24]. Woodland and tea field are the typical land-use types in this area.

Topsoil samples were collected from W30, TN, and T30 treatments, respectively. Soils from W30 and T30 treatments have been planted with secondary Masson pine woodland and tea for 30 years, respectively. The TN treatment was established after removal of woodland in 2013. For each treatment, one plot of ~3 ha was selected and divided into four subplots (600 m^2). No fertilizer was applied into woodland. The conventional nitrogen fertilizer application rate in old tea field is 450 kg N ha^{-1} yr^{1} [25]. The newly converted tea plantation from Masson pine woodland received 0-450 kg N ha^{-1} yr^{1} fertilizers depending on the tea ages [15]. The timing and amount of nitrogen fertilization application for the two tea field plots are listed in Supplementary Table S1.

Composite soil samples (0-20 cm) of each subplot from TN treatments were randomly collected with a stainless auger at 10 points in an ‘S’ shape on typical days in late January every year from 2013 to 2018 before nitrogen fertilizer application, while composite topsoil samples from W30 and T30 treatments were collected only in late January 2013. The collected soils were naturally air-dried for determining N mineralization and soil basic physicochemical properties [e.g., SOC, TSN, pH, bulk density (BD), clay content, total P (TP), and total K (TK) content]. SOC was measured by wet oxidation with H_2SO_4-K_2CrO_7. TSN was detected using an automated continuous-flow injection analyzer (Seal AA3, Germany) after digested by H_2SO_4 and catalysts. Soil pH was detected with soil and water mixture (1:2.5) by a pH meter (Metro-pH320, China). BD was measured by cylinders. Clay content (<0.002 mm) was measured by hydrometer method. Soil TP and TK contents were determined with a spectrophotometer (Lambda 25 UV/VIS spectrophotometer, USA) and atomic absorption spectroscopy (novAA 350, Germany) after fusing with NaOH.

Before measuring nitrogen mineralization, 20-g dry soil was adjusted to 40% water holding capacity (WHC) by distilled water, weighted into 120-mL glass bottle, and then was dark-pre-incubated in an incubator chamber at 25ºC for a week for the purpose of activating microorganism. After pre-incubation, soil moisture content was readjusted to 60% WHC, and the bottles were wrapped in perforated plastic film to exchange gas. Subsequently, soils were incubated for 40 days at 35ºC and at 95% relative humidity. In total, twenty-four samples of each composite soil samples were incubated. The samples (three replicates) were extracted with 0.5 mol L^{-1} K_2SO_4 (soil: water = 1:5) at 0, 3, 6, 9, 12, 21, 28, and 40 day for measuring the NH_4^+-N and nitrite nitrogen (NO_3^--N) concentrations. Soil moisture content is always maintained 60% WHC during the whole incubation period by adding distilled water according to the weight changes. Net accumulated soil nitrogen mineralization content was calculated by measuring the inorganic nitrogen (NH_4^+-N and NO_3^--N) at each extraction time, then subtracting the inorganic nitrogen at day 0. An automated flow-injection analyzer (Fiastar 5000, FOSS, Sweden) was used to determine the mineral nitrogen (NH_4^+-N and NO_3^--N) contents of the soil via extraction with 0.5 mol L^{-1} K_2SO_4 (soil : water = 1:5), respectively.

Calculation and Statistical Analyses

To determine the soil net organic nitrogen mineralization kinetics, the double-pool exponential model was used to fit the observed mineral N vs. incubation days:

$$N_t = N_0 * (1 - e^{-k_1 t}) + N_1 * (1 - e^{-k_2 t}) \quad (1)$$

...where N_t presents the net accumulated soil nitrogen mineralization content during time t (mg N kg^{-1}). N_0 and k_1 represent nitrogen mineralization potential, and soil nitrogen mineralization rate constant of the active nitrogen pool, respectively. N_2 and k_2 are the corresponding N mineralization potential and soil nitrogen mineralization rate constant of the resistant nitrogen pool, respectively [12]. Definite values of 2.5×10^{-4} and 7.5×10^{-5} d^{-1} were assigned to the parameter of k_2 for the woodland and tea plantation, respectively, as they resulted a better performance of the model. Microsoft Excel 2013 software with Solver was used to perform the fitting procedure for soil nitrogen mineralization kinetics. The fraction of soil active nitrogen pool (f, %) was calculated as follows:

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$$f = \frac{N_0}{TSN} * 10^{-1} \quad (2)$$

All statistical analyses were performed by R statistical software. One-way ANOVA, followed by LSD test at the 5% level, was used to compare soil physicochemical properties among treatments. Redundancy analyses (RDA) was performed to test the potential correlation between soil nitrogen mineralization kinetics (indicator of soil nitrogen supply potential) and soil basic physicochemical properties. The relationship between the observed and predicted f and k_1 were examined by the multiple linear regression models. Two diagnostic indicators including the adjusted determination coefficient of the model (R^2_{adj}) and the root mean squared error (RMSE) were used to evaluate the performance of the models.

Results

Soil Physicochemical Properties

Soil physicochemical properties are summarized in Fig. 1. At the start of the land use conversion experiment, the T0 treatment had the similar soil physicochemical properties with W30 treatment (p>0.05, Fig. 1). Land-use conversion from woodland soil to tea plantation significantly affected SOC, C:N ratio, BD, and pH (Fig. 1). SOC ranged from 6.27 to 12.65 g C kg^{-1} across all treatments. SOC content decreased when converting from woodland into tea plantation, and then increased with the tea age increasing. Soil from all treatments were acidic with pH 4.01-4.61 (Fig. 1). Soil pH firstly increased and then decreased with the increase of the tea ages. Soil BD was

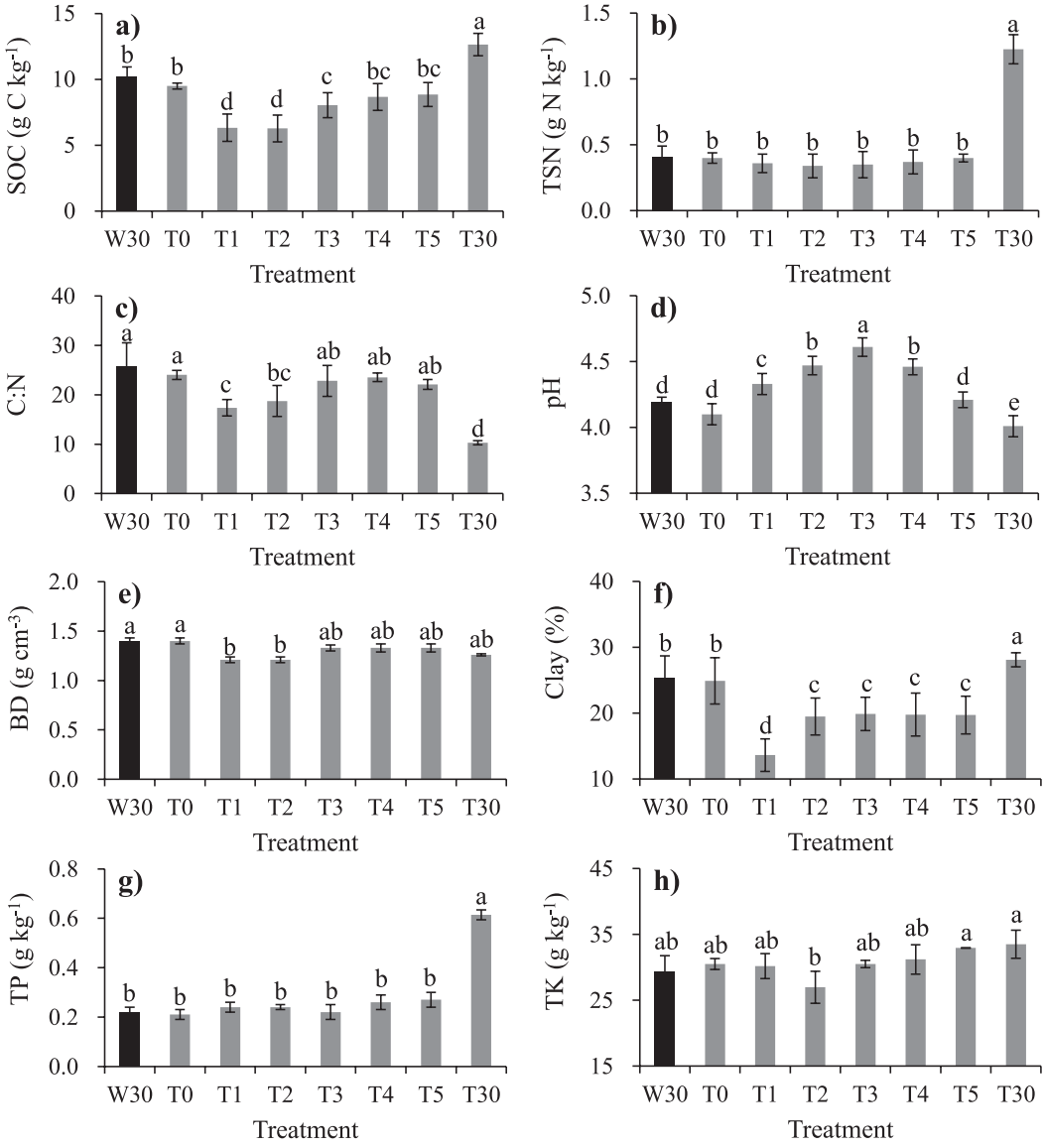

Fig. 1 Comparison of topsoil physicochemical characteristics among the eight treatments. *Abbreviation*: (a) SOC – total organic carbon, (b) TSN – total nitrogen content, (c) C:N – C:N ratio, (d) pH – soil pH value, (e) BD – bulk density, (f) clay – clay content, (g) TP – total phosphorus, and (h) TK – total potassium. Different letters indicate significant differences at the 0.05 level.

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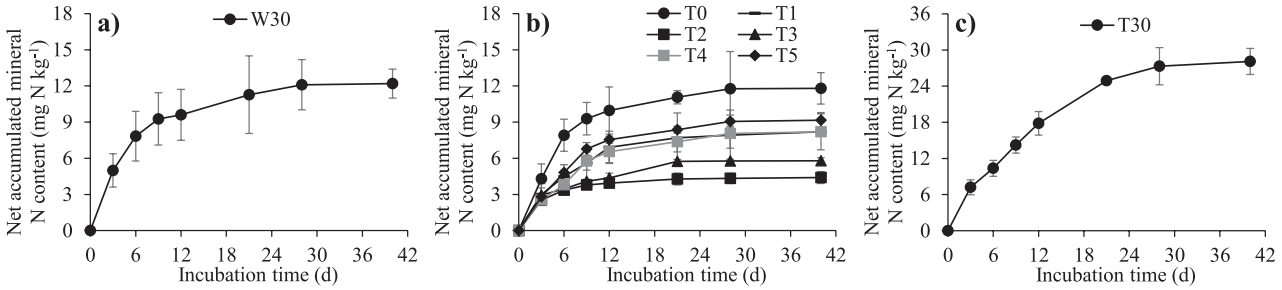

Fig. 2. The dynamics of net accumulated mineral nitrogen concentration in surface (0-20 cm) soils for a) woodland (W30, aged 30 years), b) newly converted tea field (aged from 0 to 5 years, and named T0, T1, T2, T3, T4, and T5 correspondingly), and c) old tea field (T30, aged 30 years) after 40 days of laboratory incubation at 35ºC.

significantly decreased for the first two years after converting from woodland, and then showed no distinct differences compared with W30 treatment. Compared with W30 and TN treatments, the old tea field soil has higher contents of SOC, TSN, TP and TK, while it has lower C:N ratio and soil pH (Fig. 1).

Soil Nitrogen Mineralization Kinetics

The change curves of the net accumulated mineral nitrogen content with the incubation days are showed in Fig. 2. The net accumulated mineral nitrogen content dramatically increased before 12 days of incubation, and then increased smoothly among the eight treatments (Fig. 2). The double-pool exponential model fitted soil nitrogen mineralization data well (R^2_{adj} ranged from 0.75 to 0.99, RMSE ranged from 0.18 to 0.98, Table 1). The fitted parameter of N_0 and k_1 for the W30 treatment were 9.09±2.32 mg N kg^{-1} and 0.24±0.13 d^{-1}, respectively. The N_0 for the TN treatments significantly decreased for the first three years after converting from Masson pine woodland, and then showed an increasing trend with the increasing tea planting age (Table 1). The active nitrogen mineralization potential among the different ages of tea plantations were in the order of T30>T5>T4 >T1>T3>T2, and the N_0 ranged from 4.94 to 28.07 mg N kg^{-1} (Table 1). The N_0 of the T30 treatment was significantly higher than that of the W30 treatment, but there was no significant difference on the fraction of soil active nitrogen pool of the two treatments (Table 1). The values of *f* among the different treatments were in the order of T30≈W30>T5≈T1≈T4>T3>T2, and the *f* ranged from 1.06% to 2.29 % (Table 1). The T2 treatment had the maximum k_1 (0.56±0.03 d^{-1}), while the T30 treatment had the minimum k_1 (0.08±0.01 d^{-1}) among the eight treatments (Table 1).

Correlation of Soil N Mineralization Kinetics with Soil Physicochemical Properties

The correlation analysis showed that the *f* value was positively correlated with SOC, TSN, BD, and TK, and negatively related to soil pH (Fig. 3). The k_1 was negatively related to SOC, TSN, TP, TK and clay content (Fig. 3). The RDA explained approximately 66.4% of the total variations in the *f* and k_1 (Fig. 3).

Table 1. Fitted parameters and statistical indicators for the soil nitrogen mineralization kinetic model for the treatments of woodland (W30, aged 30 years), newly converted tea field (aged from 0 to 5 years, and named T0, T1, T2, T3, T4, and T5 correspondingly), and old tea field (T30, aged 30 years).

Treatment		(mg N kg^{-1})	(%)	(d^{-1})	R^2_{adj}	RMSE
W30		9.09±2.32b	2.24±0.13a	0.24±0.01b	0.98	0.34
TN	T0	9.03±2.22b	2.20±0.23a	0.25±0.01b	0.98	0.32
	T1	7.38±1.24c	2.04±0.15b	0.16±0.01c	0.99	0.18
	T2	3.61±1.40d	1.06±0.32d	0.56±0.03a	0.75	0.24
	T3	4.94±0.98d	1.40±0.24c	0.20±0.02b	0.87	0.40
	T4	7.46±0.87c	2.03±0.16b	0.13±0.02c	0.97	0.26
	T5	8.37±0.14b	2.10±0.34b	0.15±0.02c	0.99	0.25
T30		28.07±3.21a	2.29±0.74a	0.08±0.01d	0.98	0.98

N_0, *f,* and k_1 represent the active nitrogen mineralization potential, the fraction of soil active nitrogen pool, and soil active nitrogen mineralization rate constant, respectively. R^2_{adj}: adjusted determination coefficient, RMSE: root mean squared error.
Different letters indicate significant differences at the 0.05 level.

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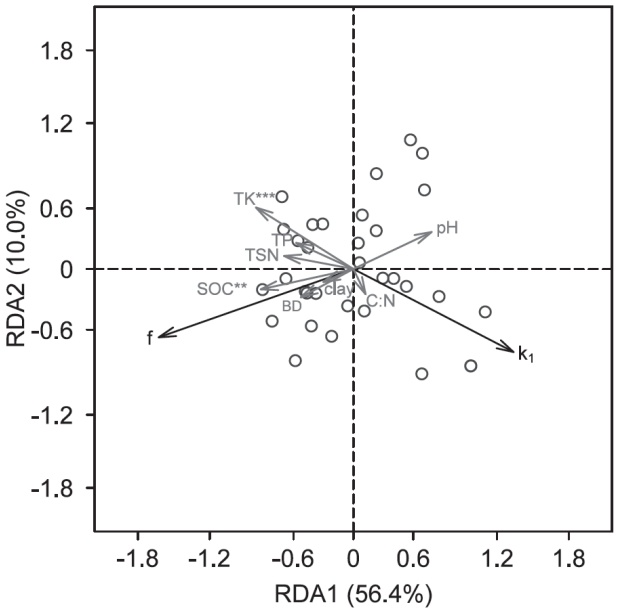

Fig. 3. Redundancy analysis (RDA) tested the associations among the and with soil physicochemical properties. *Abbreviation*: SOC – total organic carbon, TSN – total nitrogen content, C:N – C:N ratio, pH – soil pH value, BD – bulk density, clay – clay content, TP – total phosphorus, TK – total potassium.

SOC and TK were significantly correlated with f and k_1, accounting for 34.0% and 17.4% of the total variations, respectively (Fig. 3). Two stepwise regression models developed in this study showed that the nitrogen mineralized kinetic parameters (f and k_1) could be well described by a combination of soil physicochemical properties as follows (Fig. 4):

$$f = 5.85 - 1.21\text{pH} + 0.63\text{SOC} + 0.43\text{TSN} + 0.55\text{TK} + 0.25\text{BD} \quad R^2_{adj}=0.86, \text{RMSE}=0.17$$

$$k_1 = 1.24 - 0.37\text{SOC} - 0.39\text{TSN} - 0.38\text{TP} - 0.34\text{TK} - 0.57\text{clay} \quad R^2_{adj}=0.75, \text{RMSE}=0.09$$

Discussion

Soil Features

Soil physicochemical properties, including SOC, TSN, soil BD, pH, and texture and so on, are strongly affected by land-use change [15, 18, 26, 27]. Woodland soils that compound with rich organic matter, inevitably change soil physicochemical properties when converted into other agricultural land [15, 28], which is the same as the case of land-use conversion from woodland soil to tea field in this study. SOC, BD, and clay content in T1 and T2 treatments were significantly lower compared with other treatments due to frequent human activities, such as tillage and ditching, which will favor increase in leaching, soil erosion and so

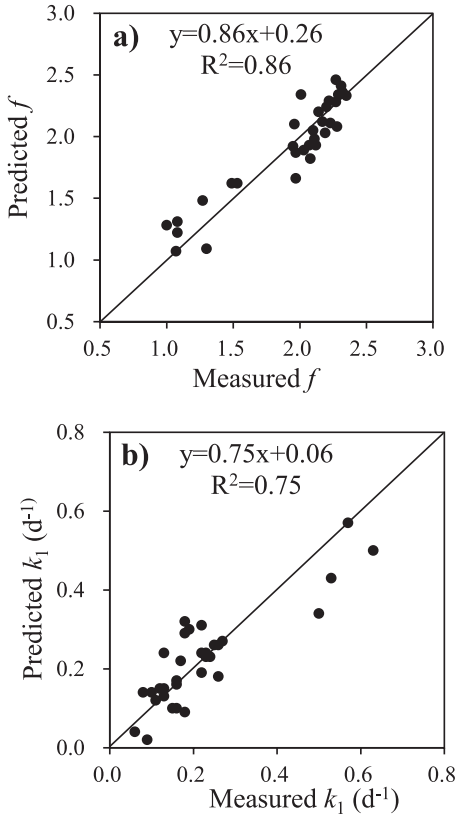

Fig. 4. Relationships between the observed and predicted f a) and k_1 b) by the multiple linear regression models.

on [28]. Previous study showed that soil particle size fractionation was significantly affected by land leveling [29]. Liu mentioned that soil anti-erosion capacity in the newly converted tea field from forest is low, and topsoil was easily removed by surface flow [30]. Generally, soil with low clay content has a low level of SOC; Tillage for the tea cultivation increased soil organic matter mineralization [15, 31]; which are the main reasons causing the decreasing SOC in T1 and T2 treatments. The application of N fertilizer and accumulation of litters help increase SOC after the tea plantation age ≥3 years [32], which was with agreement with our study. Soil pH firstly increased and then decreased with the increase of the tea ages after converted from woodland. Exchangeable base cations retained in Massion pine tree biomass were transferred into soil when converted into tea field, which attributed to the increase in acidity during the early stages of land use conversion [33]. Subsequently, tea gardens are received large amounts of nitrogen fertilization and root exudates, which lead to decline in soil pH [34].

Effect of Land Use Conversion on Soil Nitrogen Mineralization

Soil nitrogen mineralization is greatly affected by agricultural land-use types and managements [2, 12]. The present study showed that the N_0 for the newly

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converted tea plantation within two years of the growth stage presented decreasing tendencies, indicating that soil nitrogen mineralization was stimulated after land-use conversion, and thus, resulted in a reduced soil labile mineralization organic nitrogen pool. Tillage for tea cultivation increased soil temperature and soil aeration, and thus enhanced soil organic matter mineralization [35]. In addition, the amount of nitrogen input via nitrogen fertilizer application were relative small for the young tea tree, which may also contribute to the decrease in N_0. The soil active organic nitrogen pool began to increase for the newly converted tea plantation with planting ages ≥3 years, but was lower than that of the adjacent 30-year old tea field (N_0 = 28.07 mg N kg^{-1}, in this study). This phenomenon could be attributed to the following causes: First, organic nitrogen fertilizer application in tea plantations could increase the soil active nitrogen pool [36]. Second, plant residues and metabolic secretions were accumulated with the increasing years of tea planting ages, which then replenished the pool of soil organic nitrogen [37]. Third, tea plant litter, which contains substances, are difficult to decompose; this delays the mineralization of soil organic nitrogen [38]. Therefore, a relative low soil active nitrogen mineralization rate was observed in the old tea plantation.

Previous studies have shown that soil physicochemical properties strongly affected nitrogen mineralization kinetics [1, 12]. The positive correlation between the fraction of soil active nitrogen pool with SOC and TSN in the present study is similar with Zou's study [12]. Generally, soil organic nitrogen mineralization is identified as the decomposition of organic substances into inorganic materials by microbes using soil organic matter as substrates and energy [1], which means that the contents of SOC and TSN largely determine the degree of mineralization. A negative correlation between the fraction of soil active nitrogen pool and soil pH was found in this study (Fig. 3), which echoed previous study by [39]. Tea soils are often acidic and Al-rich with the increasing tea ages, which makes the number of microorganisms decrease [40]. Consequently, soil nitrogen mineralization intensity weakens as the pH descends, and thus increase the soil active nitrogen pool. Soil nitrogen mineralization rate constant was negatively related to SOC, TSN, TP, TK, and clay content (Fig. 3). Research shows that soil organic nitrogen was not easily decomposed by microbes as clay particle content increased [12]. Previous study indicated that the available mineralized organic nitrogen was highest and the mineralization time was the longest (nitrogen mineralization rate constant was the lowest) for upland soil with high SOC, TSN, TP, TK, and clay content due to enhancing microbial immobilization [41, 42]. In addition, soil C:N ratio and nitrogen mineralization rate constant in T30 treatment were lower than other treatments. SOC content in T30 treatment may be insufficient to provide energy for maintaining the microbes' metabolism, and therefore the nitrogen mineralization rate constant decreased.

Conclusions

Soil net nitrogen mineralization is greatly influenced by agricultural land-use conversion and planting ages. The incubation of soil net organic nitrogen mineralization combined with double-pool exponential model indicated that the newly converted tea field within 2-year planting ages enhanced soil organic matter mineralization possibly due to big anthropogenic disturbance. Soil active organic nitrogen pool began to increase for the tea field with planting age ≥3 years. Land-use conversion exerted great influence on soil physicochemical properties, and soil physicochemical properties strongly affected net nitrogen mineralization kinetics.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Supplementary Information

Table S1. Cultivation and fertilization management practices for the newly converted tea field (T_N) during the studying period.

Treatment	Fertilizer type	Date	Rates (kg N ha^{-1})
TN	Urea	26 Feb 2013	300
	Oilseed residues*	18 Dec 2013	150
TO	Oilseed residues	20 Apr 2013	100
	Rice straw	04 Aug 2013	20
	Urea	11 Feb 2015	150
	Oilseed residues	26 Dec 2015	150
	Urea	03 Mar 2016	300
	Oilseed residues	16 Dec 2016	150
	Urea	14 Feb 2017	300
	Oilseed residues	31 Dec 2017	150

Oilseed residues contained 5.9% of N and had a C: N ratio of 5.1