

Rapid recuperation of soil nitrogen following agricultural abandonment in a karst area, southwest China

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Abstract Soil nitrogen (N) dynamics are crucial for ecosystem development and carbon sequestration. However, N dynamics during post-agriculture succession have not been well understood. Here soil N dynamics during post-agriculture succession were investigated in a subtropical karst area, southwest

China. The succession sequence includes grassland (~10 years), shrubland (~29 years), secondary forest (~59 years) and primary forest with cropland as reference. The soil is calcareous lithosols. Soil N stock (0–15 cm) in the cropland ($335.2 \pm 55.1 \text{ g N m}^{-2}$) was not significantly different from that in the grassland, but was elevated by 120 to 221 % in the later three succession stages. Across the sequence, soil N accumulated with an average rate of $12.4 \text{ g N m}^{-2} \text{ yr}^{-1}$. Soil N stock recovered to the primary forest level in about 67 years following agricultural abandonment. NO_3^- concentrations increased while NH_4^+ concentrations decreased with years following agricultural abandonment. High NO_3^- concentrations ($66.5\text{--}120.9 \text{ mg N kg}^{-1}$) may imply N saturation in the shrubland, secondary forest and primary forest. Rates of net N mineralization and nitrification increased with succession. $\delta^{15}\text{N}$ of soil, leaves and litter was significantly elevated in the later three succession stages relative to the grassland, indicating that the N cycle became more open with succession. N release from bedrock weathering was likely a potential N source in addition to atmospheric N deposition and biological N fixation. Our study suggests that N would not be the limiting nutrient for secondary succession and ecological restoration in the karst region of southwest China.

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Introduction

Nitrogen (N) is the major limiting nutrient for terrestrial ecosystems and is among the key limiting nutrients for the re-generation of forest communities (Macedo et al. 2008; Siddique et al. 2008). Failure in afforestation/reforestation has often been attributed to nutrients, particularly N limitation to tree growth, and spontaneous succession from abandoned cropland may be delayed or even trapped in grass-dominated vegetation due to insufficient availability of soil N (Mudge et al. 2014). Furthermore, N availability is a key parameter in determining long-term terrestrial carbon (C) sequestration (Luo et al. 2004). As a result, it is undoubtedly imperative to explore N dynamics following agricultural abandonment in order to predict the velocity of secondary succession and the capacity of ecosystem C sequestration.

Forest regrowth and plantation have been found to be the major drivers of global C sink in land ecosystems (Pan et al. 2011). As for China, the largest area of plantation forest in the world has been achieved by implementing several national-scale forestry projects, including the so-called “Grain for Green” project (GGP) since the end of the 20th century (Shi and Han 2014). GGP is one of the most ambitious ecological restoration programs aiming at transforming the low-yield slope cropland or barren land into woodland or grassland (Chang et al. 2011). Around 2.7×10^7 ha land has been restored under GGP by the end of 2012, of which 33 and 56 % were related to afforestation on cropland and barren land, respectively, while 10 % was undergone spontaneous regrowth after cropland abandonment (Shi and Han 2014). GGP has been proposed to substantially increase soil organic C (SOC) accumulation (Deng et al. 2014; Shi and Han 2014; Song et al. 2014; Zhang et al. 2010; Zhou et al. 2012). According to a recent meta-analysis, cumulative soil C sequestration due to GGP was 156 ± 108 Tg C (95 % confidence interval) over the period of 1999–2012 with a mean accumulation rate of 12 ± 8 Tg C yr⁻¹ (Shi and Han 2014). Nevertheless, whether SOC sequestration will be sustained is largely determined by whether soil N availability proportionately increases and soil C saturation is reached along with ecosystem development (Luo et al. 2004; Six et al. 2002). Due to the short period since GGP implementation, it is hard to predict long-term N dynamics based on investigation on restored sites. Instead, knowledge

obtained from spontaneous succession can be used to guide restoration as there is strong linkage between succession and restoration (Hobbs et al. 2007; Prach and Walker 2011; Walker et al. 2007).

N dynamics during post-agriculture succession have not been well understood in the tropical and subtropical zones due to limited data available. Most relevant studies only focused on soil bulk N. According to these studies, soil bulk N may increase (Feldpausch et al. 2004; Guggenberger and Zech 1999; Pellegrini et al. 2014), decrease (Johnson et al. 2001), or change negligibly (Bautista-Cruz and del Castillo 2005; Davidson et al. 2007; Feldpausch et al. 2007; Hughes et al. 1999; Marin-Spiotta et al. 2009). In the case of soil N increase during secondary succession, almost none of the relevant studies have well explored the underlying mechanisms. For example, soil N accrual following pasture abandonment was attributed to atmospheric N deposition, biological N fixation (BNF) and subsoil mining of leached nitrate, but none of the above sources were quantified (Feldpausch et al. 2004). Unlike soil bulk N, post-agriculture dynamics of other soil N cycling variables including dissolved inorganic N concentration and N transformation rates were rarely reported. In the tropical Amazonia, concentrations of soil NH₄⁺ decreased while those of NO₃⁻ increased, but soil bulk N levels changed insignificantly during post-agriculture succession (Davidson et al. 2007). Another study conducted in the coastal plain of the Gulf of Mexico revealed no change of soil NH₄⁺ while NO₃⁻ decreased linearly during post-agriculture succession (Markewitz et al. 2002). The limited and inconsistent studies impede us from generating general patterns and revealing the underlying mechanisms of N dynamics during post-agriculture succession.

There is about 5.1×10^7 ha of contiguous exposed/outcropped carbonate rock area (or karst area), accounting for 5.8 % of the national land, in the southwest China (Jiang et al. 2014). This region is famous for the extremely fragile geological background and susceptibility to land degradation upon human disturbances, particularly agricultural activities. In the past, a large portion of the karst region in southwest China has been degraded due to soil erosion following destruction of natural vegetation and arable cultivation, but most of the degraded lands in the karst region have been transformed into woodland or

grassland over the last two decades. Since a large portion of soil N has been lost during the period of cultivation, the post-agricultural succession was supposed to be limited by N at least in the early stage (Zhang et al. 2015). In order to detect how N status change along with succession, in the present study soil N stock and N cycling indicators (e.g., soil dissolved inorganic N concentration, foliar N concentration, ^{15}N of foliar and soil etc.) were determined in cropland, grassland, shrubland, secondary forest and primary forest in this region with the grassland, shrubland and secondary forest developed spontaneously from abandoned cropland. We hypothesized that soil N cycling in the karst ecosystems would become more open along with succession based on our previous study, which showed that vegetation was limited by N only at the very early stage but limited by phosphorus (P) at the later successional stages in the karst area of southwest China according to the shift of foliar N:P mass ratio (Zhang et al. 2015). In addition, soil Ca^{2+} and Mg^{2+} levels in the cropland were decreased due to elevated erosion and biomass harvesting which moved Ca and Mg away from the system, but would be rapidly replenished following agricultural abandonment owing to the rapid weathering of Ca and Mg rich bedrock in the karst region. As stabilization by interaction with mineral surfaces via polyvalent cations (e.g., Ca^{2+} and Mg^{2+}) is a major mechanism underlying soil organic matter (including both C and N) retention (Kaiser et al. 2011; von Lützow et al. 2006), we hypothesized that Ca^{2+} and Mg^{2+} would play an important role in soil N recuperation as they are the two dominant polyvalent cations in the karst region.

Methods

Site description

This study was conducted at Mulun National Nature Reserve ($107^{\circ}53'–108^{\circ}05'$ E, $25^{\circ}06'–25^{\circ}12'$ N) in Guangxi Zhuang Autonomous Region, southwest China. This region is located in the subtropical humid forest life zone with a monsoon climate. Annual mean relative humidity is $>80\%$. Mean annual air temperature is $15.0–18.7^{\circ}\text{C}$, with the lowest monthly mean in January ($3.4–8.7^{\circ}\text{C}$) and the highest in July ($23.0–26.7^{\circ}\text{C}$). Mean annual precipitation ranges from 1530 to 1820 mm with a distinct seasonal

pattern. The period from April to August is wet season and that from September to March is dry season. The studied areas are characterized by a typical karst landscape with gentle valleys flanked by steep hills. The bedrock is mostly limestone nested with dolomite. The soil is calcareous lithosols (limestone soil) according to the FAO/UNESCO classification system (Anon 1974). Soil depth varies from 0 to 80 cm in the valley and ranges from 0 to 30 cm on the slopes.

Mulun National Nature Reserve has an area of 10829.7 ha and was established in 1991 in order to protect the remnant primary forests for the calcareous karst region, southwest China. The primary forests, usually evergreen and deciduous mixed forests, are located in the core zone of the reserve. In the outer zone of the reserve, many vegetation types in various successional stages are distributed.

Field sampling

Four vegetation types, i.e., grassland, shrubland, secondary forest and primary forest were selected and represented different stages of spontaneous succession following agricultural abandonment. These vegetation types were spontaneously developed from abandoned croplands distributed in the lower slopes or the valley at different periods, i.e., in the 2000s, 1980s and 1950s for the grassland, shrubland and secondary forest, respectively. The primary forest has not been disturbed over the last 150 years. Soil is calcareous lithosols for all the studied sites with soil texture being silty clay loam or silt loam. Land use history was determined by inquiring the native elder people, therefore uncertainty existed for the estimation of years following agricultural abandonment. The grassland, shrubland and secondary forest were estimated to be 10, 29 and 59 years following agricultural abandonment with ± 5 years in uncertainty. The croplands over the slope were typically planted with maize and were cultivated for over 50 years before abandonment. The croplands were fertilized with animal or human excreta in combination with urea or compound fertilizer with an annual fertilizer N input of about 150 kg ha^{-1} . Six plots of about $20 \times 20\text{ m}^2$ were established for each successional stage. The plots for shrubland, secondary forest and primary forest were distributed within the reserve, with the primary forest located in the core zone and the shrubland and secondary forest located in the outer zone of the

reserve. The plots for grassland and cropland were distributed out of, but close to the reserve. All the plots were selected within a distance <6 km. The selected sites were distributed over an elevation range from 300 to 550 m. The slope of the sites ranged between 10° and 15°. The dominant species for each successional stage are presented in Table S1. N-fixing species were not investigated for the studied sites. However, N-fixing species at nearby grassland, shrubland, secondary forest and primary forest were surveyed by Liu (2015) (Table S2). According to this survey, the abundance of N₂-fixing plants varied from 0.8 to 1.2 % for the grassland, from 0.5 to 1.7 % for the shrubland, from 1.2 to 10.7 % for the secondary forest and from 2.3 to 5.4 % for the primary forest (Table S2). Some soil properties are presented in Table 1.

The field sampling was conducted in July 2014. Foliar samples from all the dominant species in each vegetation type were sampled. Leaves of grasses and low shrubs were collected by scissors while leaves of canopy trees and tall shrubs were sampled with pole tree pruners from the upper canopy. The leaves from each plot were mixed by species. Only young and fully expanded leaves were selected for analysis since $\delta^{15}\text{N}$ might be sensitive to leaf age (Schimann et al. 2008). Following published sampling methodology (Doetterl

et al. 2015), soil samples were randomly collected with soil corers at 10 points (>1 m from the trunk of a tree) within each plot. At each sampling point, surface litter (Oi) was collected in an area of 10 × 10 cm². Considering the shallow and heterogeneous soil depth, mineral soil samples to a depth of 15 cm were collected after removal of organic layer. The ten soil samples in a plot were mixed as a composite sample.

In the laboratory, leaves and litter were dried at 60 °C to constant weight (~72 h), and then ground to uniform, fine powder using a ball mill. For soil samples, roots and stones were picked out using forceps and soils were passed through a 2-mm mesh sieve. The sieved soil samples were divided into two portions with one portion keeping fresh and the other being dried at 60 °C for analysis of N concentrations and $\delta^{15}\text{N}$ after ground to uniform, fine powder using a ball mill. Subsamples were further dried at 105 °C to determine water content.

Chemical analysis

A portion of each fresh soil sample was extracted with 2 M KCl solution (10 g soil to 100 ml KCl). The extracts were filtered using precombusted glass-fiber filters. Dissolved inorganic N (DIN = NH₄⁺ + NO₃⁻)

Table 1 Variation of bulk density (BD, g cm⁻³), pH, soil organic carbon (SOC, g C kg⁻¹), total P (g P kg⁻¹), exchangeable cations (cmol kg⁻¹) and soil texture (%) along with vegetation succession (with a soil depth of 0–15 cm)

	BD	pH	SOC	C:N	P	Ca	K	Mg	Na	Clay	Silt	Sand
CR												
Mean	1.32 ^b	6.31 ^b	20.86 ^b	12.14 ^a	0.52 ^c	8.61 ^{b,c}	0.44 ^{a,b}	1.95 ^b	0.37 ^a	32.66 ^a	50.58 ^b	16.77 ^b
SE	0.01	0.09	4.45	0.56	0.03	2.36	0.10	1.27	0.06	1.36	0.65	0.70
GR												
Mean	1.37 ^a	6.28 ^b	19.91 ^b	10.83 ^a	0.83 ^b	8.21 ^c	0.20 ^c	2.00 ^b	0.40 ^a	36.28 ^a	47.23 ^c	16.49 ^b
SE	0.01	0.10	1.21	0.54	0.09	0.61	0.02	0.17	0.03	1.43	0.62	0.95
SH												
Mean	1.16 ^c	6.88 ^{a,b}	42.97 ^a	10.26 ^a	1.57 ^a	25.58 ^{a,b}	0.31 ^b	9.42 ^a	0.52 ^a	23.48 ^b	58.54 ^a	17.99 ^{a,b}
SE	0.05	0.31	6.41	0.66	0.20	6.17	0.02	2.92	0.07	3.03	2.25	0.82
SF												
Mean	1.01 ^c	7.31 ^a	64.59 ^a	11.59 ^a	1.37 ^{a,b}	33.00 ^a	0.30 ^b	14.79 ^a	0.16 ^b	17.81 ^b	59.56 ^a	22.63 ^a
SE	0.05	0.15	7.72	2.97	0.34	3.96	0.03	2.42	0.02	2.36	1.11	2.29
PF												
Mean	1.05 ^c	6.91 ^a	57.88 ^a	10.34 ^a	1.73 ^a	28.45 ^a	0.53 ^a	11.65 ^a	0.46 ^a	22.61 ^b	57.65 ^a	19.74 ^{a,b}
SE	0.03	0.18	5.42	0.22	0.07	3.27	0.07	1.67	0.04	1.35	0.88	1.51

CR cropland, GR grassland, SH shrubland, SF secondary forest, PF primary forest, SE standard error

Different letters represent significant difference among succession stages at $p < 0.05$ level

concentrations were analyzed by an autoanalyzer (FIAstar 5000, FOSS, Sweden). Final values of N pools were reported as mg N kg⁻¹ dry soil, where dry soil refers to constant weight after drying a subsample at 105 °C. Net rates of N mineralization (NMR) and nitrification (NNR) were determined with a 7 days aerobic incubation method (Piccolo et al. 1994). Soil samples of ca. 10 g were incubated in the dark at room temperature (25 °C). NH₄⁺ and NO₃⁻ before and after incubation were determined as described above. Net N mineralization rates were determined from the difference between DIN pools before and after incubation, and the results were expressed on a basis of mean daily inorganic N production. Net nitrification rates were determined from the difference in NO₃⁻-N before and after incubation, and the results were expressed on a basis of mean daily NO₃⁻-N production. SOC was measured by wet oxidation with KCr₂O₇ + H₂SO₄ and titrate with FeSO₄ (Nelson and Sommers 1996). Soil pH (1:2.5 soil/water ratio) was measured with a pH meter (FE20 K, Mettler-Toledo, Switzerland). Soil phosphorus (P) was determined using acid digestion with an HClO₄ + H₂SO₄ solution (Carter and Gregorich 2006) while foliar P was determined by ICP-OES after oxidation under 500 °C (Kalra 1998). Exchangeable calcium (Ca) and magnesium (Mg) were displaced via compulsive exchange in 1 mol L⁻¹ ammonium acetate at pH 7.0 and analyzed by Agilent 720 ICP-OES (Agilent, Santa Clara, CA, USA) (Hendershot et al. 2006).

N isotope natural abundance and N concentration for ball-milled plant and soil samples were analyzed using a Thermo Scientific Flash 2000HT elemental analyzer coupled online to a ConFlo III interface connected to a MAT 253 isotope-ratio mass spectrometer (Thermo Finnigan MAT, Bremen, Germany). N isotope data are reported as δ¹⁵N value (δ¹⁵N in unit of per mil (‰) vs. atmospheric N₂ =

$$\left[\left(\frac{^{15}\text{N}/^{14}\text{N}}{\text{sample}} \right) / \left(\frac{^{15}\text{N}/^{14}\text{N}}{\text{air}} \right) - 1 \right] \times 1000.$$

Data analysis

Soil N stock (g N m⁻²) was estimated according to N concentration (g kg⁻¹), soil sampling depth (cm), bulk density (g cm⁻³) and fraction of >2 mm fragments (Schrumppf et al. 2011; Song et al. 2005). To facilitate

comparison with other studies, soil N stocks in 0–15 cm horizon were converted to values of the 0–30 cm depth using a methodology coupled with depth function (Li et al. 2012; Yang et al. 2011) derived from the global averaged depth distribution of soil N (Jobbágy and Jackson 2001) as shown below:

$$X_{30} = \frac{1 - \beta^{30}}{1 - \beta^{d_0}} \times X_{d_0} \quad (1)$$

where X₃₀ represents soil N stock in the 0–30 cm soil horizon (g N m⁻²); d₀ denotes the original soil depth (cm); β is the relative rate of decrease in soil N stock with soil depth. In the present study, the global average depth distribution for soil N was adopted to calculate the value of β (0.9831) (Li et al. 2012).

One-way analysis of variance (ANOVA) with LSD test was conducted to examine the significant difference in target variables among different succession stages. Variation of soil N stocks with years following agricultural abandonment was fitted with a Chapman function (Eq. 1):

$$\text{Stock}_t = \text{Stock}_{t_0} + A(1 - \exp(-B \times \text{years}))^C \quad (2)$$

where stock_t denotes N stock after t years following agricultural abandonment; stock_{t₀} is N stock before agricultural abandonment, i.e., N stock in the cropland; A is the difference between steady state N stock and stock before agricultural abandonment, therefore N stock at steady state (stock_{st}) after agricultural abandonment is the sum of stock_{t₀} and A, B is the growth constant, and C is the shaping parameter (Sun et al. 2004). We assumed that soil N stock reached the steady state level if it was 95 % of the steady state N stock. Accordingly, years (T) needed to reach steady state N stock level can be estimated by Eq. 2:

$$T = - \frac{\ln\left(1 - \frac{C \sqrt{95\% \text{Stock}_{st} - \text{Stock}_{t_0}}}{A}\right)}{B} \quad (3)$$

Since the rate of N accumulation can't be directly derived from the above nonlinear fitting, the average rate of N accumulation was estimated by dividing A by T. Linear regression was conducted to explore the relationship between exchangeable Ca or Mg and soil N stock. Significant difference was presented as *p* < 0.05. The statistical analyses were performed using SPSS 16 (SPSS Inc., Chicago, IL, USA).

Results

Soil N stock

Soil N stock at 0–15 cm depth was 335.2 ± 55.1 g N m⁻² in the cropland (Fig. 1a). N stock in the grassland was not significantly increased relative to the cropland, but that in the shrubland was significantly increased by 120.0 % on average relative to the cropland ($p < 0.05$) (Fig. 1a). In the secondary forest, N stock was 1074.9 ± 157.7 g N m⁻², which represents an increase of 221 % in comparison to the cropland. There was no significant difference in soil N stock between the primary forest and the secondary

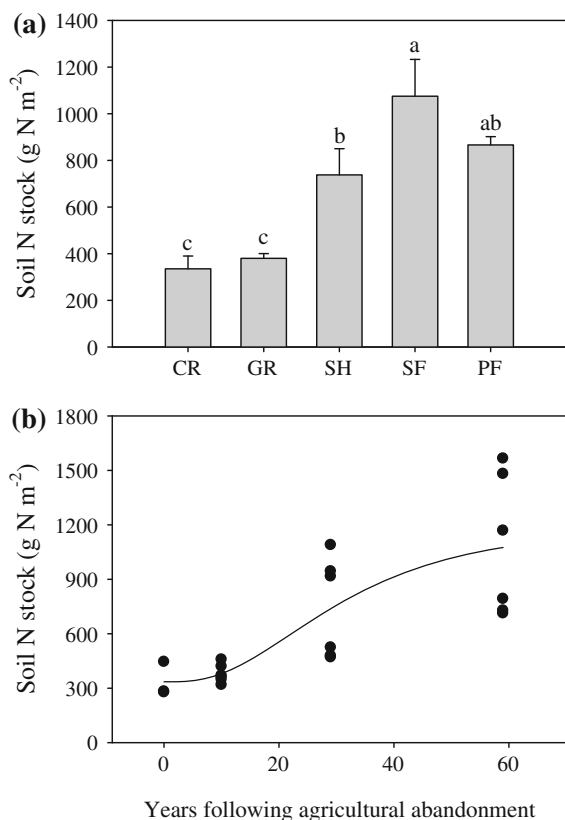


Fig. 1 Variation of soil N stock (0–15 cm) along post-agriculture succession. **a** N stock in different successional stages (*CR* cropland; *GR* grassland; *SH* shrubland; *SF* secondary forest; *PF* primary forest). Bars represent mean \pm standard error. Different letters represent significant difference among successional stages at $p < 0.05$ level. **b** Relationship between soil N stock and years following agricultural abandonment ($y = 335.22 + 829.31(1 - \exp(-0.06x))^{3.60}$, $r^2 = 0.62$, $p < 0.0001$, $n = 21$). Each point denotes the mixture of 10 soil samples in a plot

forest or the shrubland. Variation of N stocks with years following agricultural abandonment could be well fitted with a Chapman function (Fig. 1b). According to the fitting, the time needed for N stock to reach 95 % of steady state level was about 67 years. The average rate of N accumulation over the course of N recovery was 12.4 g N m⁻² yr⁻¹ (0–15 cm). Both exchangeable Ca and Mg levels were lowest in the cropland and grassland while there was no significant difference among shrubland, secondary forest and primary forest (Table 1). Significant correlation was found between soil N stock and exchangeable Ca ($y = 26.98x + 118.32$, $R^2 = 0.97$, $p = 0.002$, $n = 5$) or Mg ($y = 54.70x + 243.16$, $R^2 = 0.99$, $p = 0.0002$, $n = 5$) (Fig. 2).

Dissolved inorganic N pools and net N transformation rates

Soil NH₄⁺ concentrations ranged from 0.4 to 39.7 mg N kg⁻¹ across the succession stages with concentration in the cropland or secondary forest significantly lower than that in the grassland (Fig. 3a). Soil NO₃⁻ concentration in the cropland was 24.4 ± 1.1 mg N kg⁻¹ and was not significantly different from that in the grassland (Fig. 3a). Nevertheless, soil NO₃⁻ pools were greatly elevated in the later three succession stages, where NO₃⁻ concentrations ranged between 66.5 and 120.9 mg N kg⁻¹ (Fig. 3a). Soil NH₄⁺ concentration was significantly greater than

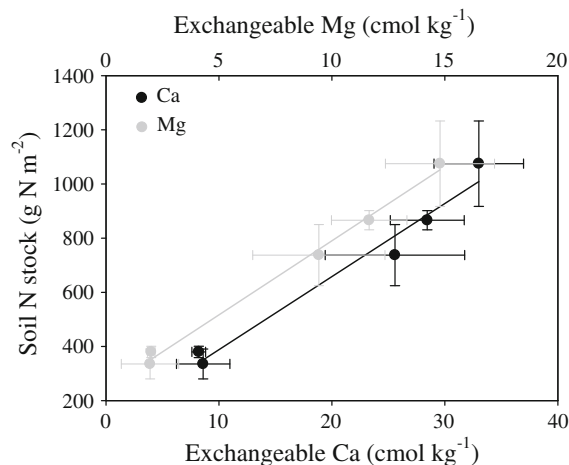


Fig. 2 Relationships between soil N stocks and exchangeable Ca ($y = 26.98x + 118.32$, $r^2 = 0.97$, $p = 0.002$, $n = 5$) and Mg ($y = 54.70x + 243.16$, $r^2 = 0.99$, $p = 0.0002$, $n = 5$). Each point represents a succession stage

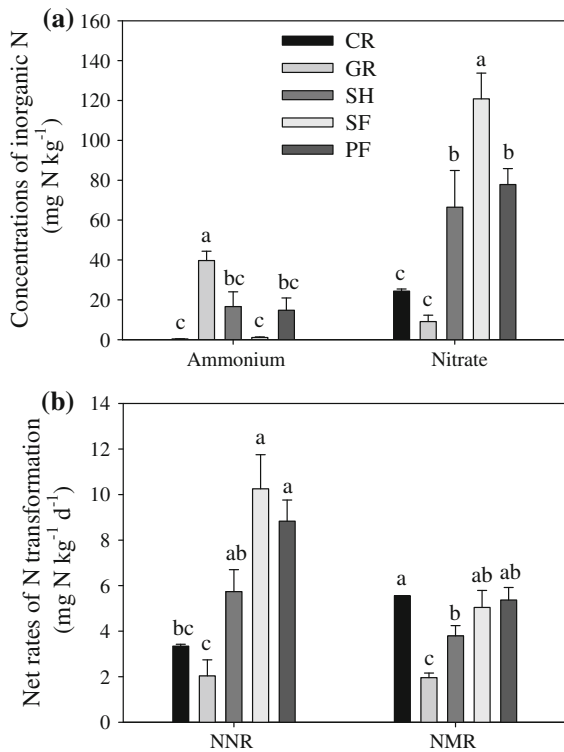


Fig. 3 Variation of soil inorganic N concentrations and net rates of nitrification (NNR) and mineralization (NMR) along post-agriculture succession. CR cropland; GR grassland; SH shrubland; SF secondary forest; PF primary forest. Bars represent mean \pm standard error. Different letters represent significant difference among successional stages at $p < 0.05$ level

NO_3^- concentration only in the grassland, while NO_3^- concentrations were greater in the cropland and the later three stages.

The rates of net mineralization (NMR) were similar in the cropland and grassland, but rate of net nitrification (NNR) in the cropland was significantly greater than that in the grassland (Fig. 3b). Following agricultural abandonment, NMR and NNR changed in a similar way with the values higher in the later successional stages than in the grassland (Fig. 3b). NMR in the secondary forest and primary forest were significantly greater than in the cropland, but NNR were similar for the cropland, secondary forest and primary forest.

Concentrations and $\delta^{15}\text{N}$ of total N in different ecosystem compartments

Averaged soil N concentration (0–15 cm) was $1.69 \pm 0.28 \text{ g N kg}^{-1}$ in the cropland (Table 2). There

was no significant difference in soil N concentrations between the grassland and cropland. Soil N concentrations in the later three successional stages were significantly elevated relative to the cropland. Similar to the dynamics of soil N, the concentrations of foliar and litter N were significantly elevated for the later successional stages relative to the grassland (Table 2). The $\delta^{15}\text{N}$ values of soil N were similar in the cropland and grassland, but were significantly increased in the later three stages (Table 2). The $\delta^{15}\text{N}$ values of foliar and litter N changed in a similar way as soil N (Table 2).

Discussion

Soil N stock in comparison to other regions

Soil N stock in the present study was at a relatively high level compared to other regions of China or the world. Averaged soil N stock (0–30 cm) was $405 (70\text{--}1660) \text{ g N m}^{-2}$ across different soil types according to China's Second National Soil Survey (CSNSS) with 134 limestone soil samples (all from cropland) included (Yang et al. 2007). The averaged soil N stock (0–30 cm) for limestone soils was 560 g N m^{-2} (Yang et al. 2007). In the current study, N stocks were only measured for the soil horizon of 0–15 cm but were converted to values of the 0–30 cm depth (Fig. S1) to facilitate comparison with other studies. It seems that the mean N stock for limestone soil from CSNSS was similar to the averaged value for cropland in our study ($594.8 \pm 97.8 \text{ g N m}^{-2}$). Since N stocks in cropland are relatively low compared to other land use types (Guo and Gifford 2002), the values based on CSNSS should represent the low N stock level for the karst region with limestone soil. Averaged soil N stock (0–30 cm) across the world were $670 (150\text{--}2180) \text{ g N m}^{-2}$ (Batjes 1996). It seems that the global averaged value was similar to the level of cropland or grassland in the present study, while N stocks of shrubland ($1308.5 \pm 200.3 \text{ g N m}^{-2}$) and forest ($1722.2 \pm 147.7 \text{ g N m}^{-2}$) in the present study were much greater than the global average. Similarly, high N levels have been reported for some karst areas. For example, soil N concentrations (0–20 cm) in the Bohemian Karst of Czech Republic varied from 2.9 to 9.4 g kg^{-1} (Hofmeister et al. 2002), which are a bit greater than the values ($1.9\text{--}6.7 \text{ g kg}^{-1}$) observed in our study (Table 2).

Table 2 Variation of N concentration and $\delta^{15}\text{N}$ of soil, leaves and litter with succession

	N concentration (g N kg^{-1})			$\delta^{15}\text{N}$ (‰)		
	Soil N	Foliar N	Litter N	Soil N	Foliar N	Litter N
CR						
Mean	1.69 ^c			3.48 ^b		
SE	0.28			0.18		
GR						
Mean	1.85 ^c	18.52 ^b	10.92 ^b	3.88 ^b	-0.93 ^c	-2.57 ^b
SE	0.11	2.64	1.56	0.11	0.40	0.54
SH						
Mean	4.40 ^b	31.54 ^a	20.39 ^a	5.32 ^a	1.80 ^a	0.96 ^a
SE	0.83	2.37	1.80	0.37	0.37	0.49
SF						
Mean	6.65 ^a	30.00 ^a	21.49 ^a	5.07 ^a	0.72 ^b	0.52 ^a
SE	0.90	2.17	0.58	0.13	0.24	0.15
PF						
Mean	5.57 ^{a,b}	23.03 ^b	22.69 ^a	5.12 ^a	0.46 ^b	0.80 ^a
SE	0.42	1.87	1.36	0.14	0.27	0.24

CR cropland, GR grassland, SH shrubland, SF secondary forest, PF primary forest, SE standard error. Different letters represent significant difference among successional stages at $p < 0.05$ level

Rapid accumulation of soil N

We found that soil N was rapidly accumulated during the post-agriculture succession. Significant N accumulation was found after about 29 years (i.e., the shrubland stage) of agricultural abandonment. The soil N accumulation in the current study was more rapid relative to the global pattern. According to a meta-analysis, significant increase of mineral soil N stock was not found until 50 years after afforestation largely due to low increase of soil N in temperate and boreal zones (Li et al. 2012). Consistent with our study, significant increase of soil N shortly after afforestation or agricultural abandonment was observed by some studies in subtropical/tropical zones. For example, soil N level was significantly elevated after 18 years of reforestation with *pinus massoniana* in south China (Dou et al. 2013). Similarly, soil N was found to increase significantly 12 years after reforestation in southeastern China (Zhang et al. 2004). In contrast, some studies reported no change or decrease in soil N after afforestation in subtropical China (Li et al. 2014).

The rapid accumulation of soil N was also supported by the short duration (ca. 67 years) needed to reach 95 % of the steady state level (Fig. 1). Similarly, rapid recovery of soil N stock has been found in a few tropical/subtropical forests regenerated from cropland or pastures (Brown and Lugo 1990; Feldpausch et al. 2004), or from deforested sites (Macedo et al. 2008).

For example, the time for full recovery of soil N was about 15–20 years since agricultural abandonment in three subtropical life zones of Puerto Rico and US Virgin Islands (Brown and Lugo 1990), and was 13 years after planted with leguminous N-fixing trees on degraded site in Brazil (Macedo et al. 2008). Nevertheless, several studies conducted in the tropics reported negligible change of soil N levels during secondary succession from cropland or pasture (Davidson et al. 2007; Hughes et al. 1999; Johnson et al. 2001), even though indicators of N cycling recovered (Davidson et al. 2007). In contrast, recovery of soil N was relatively slow in temperate zone. For example, a study conducted in Illinois, USA showed that more than 400 years were required for soil N stock to reach pre-agriculture levels (Matamala et al. 2008). Soil N recovery to the pre-agriculture levels was predicted to be 180 years on a Minnesota sand plain (Knops and Tilman 2000).

Consistent with rapid soil N recovery, we found high rates of soil N accumulation ($12.4 \text{ N m}^{-2} \text{ yr}^{-1}$). So far, there were very limited studies which directly presented soil N accumulation rates or the rates could be estimated for post-agriculture succession (Davidson et al. 2007; Feldpausch et al. 2004; Lemenih et al. 2004; Liao et al. 2006; Resh et al. 2002; Zou and Bashkin 1998). According to these studies, soil N accumulation rates ranged from nearly zero to as high as $16 \text{ g N m}^{-2} \text{ yr}^{-1}$ for the soil depths from 0–10 to

0–45 cm. Compared to the these studies, soil N accumulation rates observed in the current study were at the upper end of the rate range observed in the tropical/subtropical regions.

It should be noted that only N accrual in the 0–15 cm soil depth was considered in the present study. According to several studies which determined N dynamics for different soil depths after agricultural abandonment, soil N stock change mostly occurred in the top soil (0–10 to 0–20 cm) layers (Hernandez-Ramirez et al. 2011; Hooker and Compton 2003; Knops and Tilman 2000). Therefore, it is reasonable for our study to consider the N stock change in the top soil layer only.

Possible mechanisms underlying the rapid accumulation of soil N

Soil N stock change is a balance between N inputs and outputs and is mediated by the capacity of a soil to stabilize N. The underlying mechanisms of soil N stock change during secondary succession are not well understood. To our knowledge, none of the relevant studies have clearly quantified N inputs and outputs which explain N stock change during secondary succession. Atmospheric N deposition and BNF are regarded as the two sources of external N inputs for natural ecosystems. Houlton et al. (2008) reported that symbiotic BNF ranged from 2 to 6 g N m⁻² yr⁻¹ for lowland tropical forest and savannas. Meanwhile asymbiotic BNF is ubiquitously distributed on the surface of plants, leaf litter and decaying wood, in soil and on moss (Reed et al. 2011). Recent evidence suggested that symbiotic BNF only accounted for 20–50 % of total BNF (Sullivan et al. 2014), which means that total BNF would be much greater than 2–6 g N m⁻² yr⁻¹ for lowland tropical forest and savannas. As for our studied area, the abundance of N₂-fixing plants (Table S2) are within the range of abundance of N₂-fixing trees (from non-existing to 19.3 % based on % trees) in various tropical forests (Hedin et al. 2009). In addition, mosses and lichen are widely distributed in all the successional stages occurring on the outcrop, tree stem, decaying wood and soil surface (Fig. S2). However, rates of BNF have not been measured in these sites to date. We assumed that the level of symbiotic BNF in these sites was similar to the lowland tropical forest and savannas, then total BNF inputs would be greater than

2–6 g N m⁻² yr⁻¹ for these sites. Total atmospheric N deposition in this area was about 3 g N m⁻² yr⁻¹ (Zhu et al. 2015). Therefore, the two recognized sources (5–9 g N m⁻² yr⁻¹) could explain 40–76 % of the rates of soil N accumulation (12.4 g N m⁻² yr⁻¹) in the depth of 0–15 cm in our study.

In addition to the above two N sources, mining of deeper soil nitrogen has been found to contribute to soil N accumulation in the top soil layer (Knops and Tilman 2000; McKinley et al. 2009; Mobley et al. 2015; Norby and Iversen 2006). The surficial gains and subsoil losses of soil N could result in no change or decrease of soil N in the whole soil profile (Mobley et al. 2015). In the present study, only surficial soil layer (0–15 cm) was sampled due to the generally shallow soil depth (normally <30 cm) over the slope. Considering the shallow soil depth, mining of N from deeper soil was impossible to sustain the observed soil N accumulation. Accordingly, the contribution from this source should be mild.

Recently, weathering of N-rich bedrocks has been suggested as a potentially important N source and used to resolved “missing N inputs” in ecosystems (Houlton and Morford 2015). It was proposed that weathering of N-rich bedrocks could enhance N availability and facilitate terrestrial succession even without BNF (Houlton and Morford 2015). These geological N sources have found to result in soil acidification and N saturation (Dahlgren 1994), high nitrate concentration in stream water (Holloway et al. 1998) and increased ecosystem C and N storage (Morford et al. 2011). Sedimentary rocks are found to be N rich with N concentration in limestone being 0.02–0.30 g kg⁻¹ (Holloway and Dahlgren 2002). The bedrock is limestone in our study area. Several lines of evidence suggested that the bedrock likely contributed to soil N accumulation in our study area. The bedrock was found to contain N concentrations ranging from 0.21 to 0.28 g N kg⁻¹ according to a preliminary survey covering our study area (Dr Kongcao Xiao, unpublished data). N release was reported to be 0.4–3.7 g N m⁻² yr⁻¹ for rocks containing N of 0.48–1.37 g N kg⁻¹ based on a laboratory leaching study with 2 M KCl solution and deionized water (Holloway et al. 2001). Although the rock N levels in our study were lower than those reported by Holloway et al. (2001), we still assumed that N release from rock weathering in the studied sites was about 0.4–3.7 g N m⁻² yr⁻¹ due to enhanced N release

under field conditions. To support for rock N release, it was found that N concentration in soils distributed on rocks ($7\text{--}19\text{ g N kg}^{-1}$) was found to be much higher than in continuous soils ($5.77 \pm 0.42\text{ g N kg}^{-1}$) in a nearby karst primary forest (Luo et al. 2010). Similarly, soil N level was significantly related to distribution of rock outcrops (Zhang et al. 2007). Additional evidence comes from soil $\delta^{15}\text{N}$, which was found to increase with soil N level (Table 2). Among the potential N sources, $\delta^{15}\text{N}$ of the input N from atmospheric deposition is often negative, e.g., from -5.5 to -1.4 ‰ for rural areas in the southwest China (Liu et al. 2012b); while $\delta^{15}\text{N}$ of N from BNF is around 0 ‰ in theory but varies from -2 to 0 ‰ in practice (Vitousek et al. 2013). Due to their negative or neutral $\delta^{15}\text{N}$ values, N inputs from atmospheric deposition and BNF are supposed to dilute or deplete the bulk soil ^{15}N pool. N released from rock was found to be positive in $\delta^{15}\text{N}$, e.g., $1.45\text{--}5.72\text{ ‰}$ for limestone (Holloway and Dahlgren 2002), which likely contributed to the positive soil $\delta^{15}\text{N}$ values ranging from 3.74 to 6.08 ‰ in the current study (Table 2).

The combination of the above N sources ($5.4\text{--}12.7\text{ g N m}^{-2}\text{ yr}^{-1}$) could explain $44\text{--}102\%$ of the rate of soil N accumulation in the depth of $0\text{--}15\text{ cm}$ in our study. Nevertheless, the rate of N accrual considered here is a balance of N input and loss. The actual rates of N input should be larger than the observed ones. Anyway, uncertainties existed largely in the estimation of N inputs from BNF and rock release. Further studies are necessary to quantify the contributions from these potential N sources, which in turn will make it possible to unravel the underlying mechanisms of N stock accrual during secondary succession.

However, external N inputs alone can't explain the rapid accumulation of soil N stock since the input N may be lost to the environment via multiple pathways if the N retention capacity of a soil is low. The capacity of N retention for calcareous soils have often been found to be higher than adjacent non-calcareous soils (Chen et al. 2012a; Fornara et al. 2011; Poulton et al. 2003). For example, Chen et al. (2012a) reported that both soil organic matter (SOM) and N concentrations were much greater in calcareous lithosols than in the adjacent rhodic ferralsols (red soil) in an area close to our studied sites. At Rothamsted Research station, a site heavily chalked in the 19th century accumulated SOC and N with rates 1.4 and 2.4 times greater relative to the adjacent site free of CaCO_3 (Poulton et al. 2003).

Since most soil N is in the form of SOM, the mechanisms that enhance SOM stabilization will in turn increase soil N retention. The regulation of soil properties on SOM stability can be roughly categorized into two major mechanisms, i.e., (i) occlusion in aggregates, and (ii) stabilization by interaction of SOM with mineral surfaces via polyvalent cations (Kaiser et al. 2011; von Lützow et al. 2006). The major polyvalent cations occurred in soil are Ca^{2+} and Mg^{2+} in neutral and alkaline soils (von Lützow et al. 2006). In the current study, both Ca^{2+} and Mg^{2+} concentrations were higher in the shrubland, secondary forest and primary than in the cropland and grassland. Lower level of soil Ca^{2+} and Mg^{2+} concentrations in the cropland was largely due to biomass harvesting which moved Ca and Mg away from the system as well. Nevertheless, owing to the rapid weathering of Ca and Mg rich bedrock, i.e., limestone nested with dolomite, soil Ca and Mg were rapidly replenished and recovered to primary forest levels in the shrubland. In addition, significant correlation between exchangeable Ca or Mg and soil N was found, supporting that interaction of SOM with mineral surfaces via Ca^{2+} and Mg^{2+} is likely one of the major mechanisms of SOM stabilization, and the roles of Ca^{2+} and Mg^{2+} in SOM stabilization were greater in the later three succession stages due to the higher levels of Ca^{2+} and Mg^{2+} .

Evidence of open soil N cycle with succession

Along with increase of bulk soil N stock, soil N availability was found to increase as well with succession in our study. Increase of soil N availability was confirmed by changes of the relative abundance of soil NO_3^- and NH_4^+ (Fig. 3). NH_4^+ is often the dominant form of inorganic N in N-limited systems, but NO_3^- level increases disproportionately as NH_4^+ becomes more available due to nitrification (Davidson et al. 2007; Pellegrini et al. 2014; Schimel and Bennett 2004). Consequently, $\text{NO}_3^-/\text{NH}_4^+ > 1$ can be used as indicative of abundant soil N (Davidson et al. 2007; Pellegrini et al. 2014). In the current study, $\text{NO}_3^-/\text{NH}_4^+$ ratio was >1 in the later three succession stages, indicating abundant soil N availability (Fig. 3a). With the increase of soil N availability, the N demands by both microbial and plant communities are met. In this case, the inorganic N produced by gross mineralization and nitrification will theoretically be greater than that consumed by microbes and plants. Subsequently,

the net rates of N mineralization and nitrification, which is the balance between gross production and consumption, will increase as soil N availability increase. This was confirmed by our results, which showed increase of both rates of N mineralization and nitrification with succession (Fig. 3b), implying more open of soil N cycling with succession. Our results also affirmed the findings of a previous study which reported transition of nutrient limitation along with succession, i.e., limited by soil N in the grassland, but by soil P in the secondary forest and primary forest based on analysis of foliar N:P ratio (Zhang et al. 2015).

The fact that soil N cycling became more open with succession was also supported by change of soil $\delta^{15}\text{N}$ value. As the known N inputs have $\delta^{15}\text{N}$ values lower or equal to soil $\delta^{15}\text{N}$ values, which were significantly greater in the later three succession stages than in the cropland and grassland, there should be substantial soil N losses which resulted in enrichment in soil $\delta^{15}\text{N}$. Soil erosion rates in cropland and grassland are much greater than forests in our studied region (Chen et al. 2012b), therefore, N losses via erosion likely accounted for a large portion of N loss in the cropland and grassland. Instead, N loss via erosion decreased and that via microbe-involved N cycling processes becomes dominant in N losses, including gaseous N loss during nitrification and denitrification or loss of the product of nitrification, NO_3^- . Microbes prefer using ^{14}N over ^{15}N , which leads to ^{15}N enrichment in soil N pool (Craine et al. 2015). In contrast, soil erosion does not result in N isotope fractionation (Craine et al. 2015). This is supported by the lower soil $\delta^{15}\text{N}$ in the cropland and grassland while higher soil $\delta^{15}\text{N}$ in the later three stages (Table 2; Fig. S4). Furthermore, soil $\delta^{15}\text{N}$ did not change with soil N level in the later three succession stages, most likely due to balanced N inputs and losses. Large N loss in the forest was indirectly supported by high water concentrations of NO_3^- , a product of nitrification, in the nearby forests as discussed below. Large inputs and outputs of N is an evidence of open soil N cycling (Craine et al. 2015).

It is out of our expectation that soil NO_3^- concentrations (66.5–120.8 mg N kg⁻¹) in the later succession stages were significantly greater than NO_3^- level (24.4 mg N kg⁻¹) in the cropland (Fig. 3a). These high levels of NO_3^- concentrations observed in our study have rarely been encountered in natural

ecosystems. For example, soil NO_3^- concentrations were lower than 10 mg N kg⁻¹ in forests impacted by atmospheric N deposition from about 15 to nearly 70 kg N ha⁻¹ yr⁻¹ across a urban–rural transect in southern China (Fang et al. 2011). Soil NO_3^- concentrations were <20 mg N kg⁻¹ in mature tropical forests (Davidson et al. 2007). Nevertheless, similar pattern of soil NO_3^- variation was found in a succession sequence from shrub-grass mixture field to primary forest in a nearby karst area, where soil NO_3^- concentration was 140.9 ± 67.4 in the primary forest (Liu et al. 2012a). This high level of soil NO_3^- concentration was accompanied by high NO_3^- concentrations (413 ± 17 to 2187 ± 165 $\mu\text{g N L}^{-1}$) in the stream and spring water over the nearby forests (Fig. S3, Dr Kongcao Xiao, unpublished data). The water concentrations of NO_3^- over the nearby forests were greater than those found in the tropical watersheds (8–878 $\mu\text{g N L}^{-1}$ with a mean of 212 ± 236 $\mu\text{g N L}^{-1}$) where the forests were proposed to be in a state of N saturation (Brookshire et al. 2012a, b). These evidences likely implied that the forests were N saturated in the studied karst area of the current study.

Conclusions and implications

In the current study, N dynamics along spontaneous succession was investigated in a karst area of southwest China. We found that soil N stock recovered to the primary forest level in about 45 years following agricultural abandonment with an average rate of N accumulation of 13.5 g N m⁻² yr⁻¹. Although much uncertainty existed in terms of external N inputs, bedrock weathering was proposed as a potential N sources besides atmospheric N deposition and biological N fixation. Abundant soil N availability was found in the shrubland, secondary forest and primary forest, supporting N was not a limiting nutrient element in these successional stages.

Due to the strong linkage between succession and ecological restoration (Hobbs et al. 2007; Walker et al. 2007), knowledge obtained from succession is critical in guiding restoration (Hobbs et al. 2007; Prach and Walker 2011; Walker et al. 2007). The information shared by succession and restoration includes energy flow, C accumulation and storage, nutrient dynamics, soil properties, water cycle (Walker et al. 2007). In

terms of southwest China, there is a karst area of about 5.1×10^7 ha, accounting for 5.8 % of the national land (Jiang et al. 2014). A large portion of the karst region have been degraded due to soil erosion following destruction of natural vegetation and arable cultivation in the past, but most of the degraded lands have been transformed into woodland or grassland over the last two decades. According to the present study, plant productivity may be limited by soil N in the early restoration stage, but not in the later stages. It is suggested to include N-fixing plants or to apply N fertilizer in the early stage in order to accelerate restoration. Additionally, soil C accumulation is often limited by N availability before soil C saturation is reached (Luo et al. 2004; Six et al. 2002). Our study suggests that soil C accumulation following agricultural abandonment in the karst areas of southwest China may not be limited by soil N except the early stage.

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