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Soil gross nitrogen transformations in forestland and cropland of Regosols

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Soil gross nitrogen (N) transformations could be influenced by land use change, however, the differences in inherent N transformations between different land use soils are still not well understood under subtropical conditions. In this study, an ^{15}N tracing experiment was applied to determine the influence of land uses on gross N transformations in Regosols, widely distributed soils in Southwest China. Soil samples were taken from the dominant land use types of forestland and cropland. In the cropland soils, the gross autotrophic nitrification rates (mean 14.54 ± 1.66 mg N kg^{-1} day^{-1}) were significantly higher, while the gross NH_4^+ immobilization rates (mean 0.34 ± 0.10 mg N kg^{-1} day^{-1}) were significantly lower than those in the forestland soils (mean 1.99 ± 0.56 and 6.67 ± 0.74 mg N kg^{-1} day^{-1}, respectively). The gross NO_3^- immobilization and dissimilatory NO_3^- reduction to NH_4^+ (DNRA) rates were not significantly different between the forestland and cropland soils. In comparison to the forestland soils (mean 0.51 ± 0.24), the cropland soils had significantly lower NO_3^- retention capacities (mean 0.01 ± 0.01), indicating that the potential N losses in the cropland soils were higher. The correlation analysis demonstrated that soil gross autotrophic nitrification rate was negatively and gross NH_4^+ immobilization rate was positively related to the SOC content and C/N ratio. Therefore, effective measures should be taken to increase soil SOC content and C/N ratio to enhance soil N immobilization ability and NO_3^- retention capacity and thus reduce NO_3^- losses from the Regosols.

Nitrogen (N) is an essential element for plant growth, and its form and amount are mainly controlled by N production and consumption processes in natural soils[1–3]. In terrestrial ecosystems, if N cannot be efficiently conserved in soils, potential N losses will induce negative effects on the climate, the environment and even human health[4–6]. Therefore, it is essential to quantify the simultaneously occurring N production and consumption processes to identify whether soils can effectively conserve N.

Soil gross N transformations, driven by microorganisms, are greatly impacted by many soil properties and climatic conditions, such as soil temperature and moisture, soil pH, substrate concentration (NO_3^-, NH_4^+ and organic N), and the quality and quantity of organic materials[7–11]. For instance, soil pH affects microbial community composition and activities, and is therefore important for NO_3^- production and consumption processes[7,12,13]. Soil carbon (C) and N availability can influence gross mineralization and NH_4^+ immobilization processes, which are important for assessing the indigenous soil N supply[14,15]. In general, the above mentioned factors are significantly affected by different land uses[16–18], which thus induce differences in soil N transformations[10,19,20].

With the development of ^{15}N dilution technique and numerical model methods, studies on the gross N transformations in different land uses have been greatly increasing[4,9,10,20,21]. Generally, previous studies indicated that in natural forest ecosystems, most available N could be effectively conserved in soils through inherent soil N conservation mechanisms[6,14]. For instance, in subtropical forest soils, low gross mineralization rates combined with negligible gross nitrification rates were confirmed as an effective N conservation mechanism through reducing the production of inorganic N[9]. Furthermore, Zhang et al. found that the coexistence of high inorganic N production and immobilization rates was also an effective N conservation mechanism in subtropical forest soils[4]. However, after forestland conversion to cropland, the changes of vegetation cover and management practices (e.g. tillage and fertilization) could significantly influence soil N transformations[16,17,19,20]. In subtropical acid soils of Southwest China, Xu and Xu reported that compared to forestland soil, the cropland soils had significantly higher

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gross nitrification rates and lower immobilization rates, which resulted more NO_3^- losses through leaching or denitrification[9]. However, the mechanisms of different land uses influencing N transformation processes may be quite different according to soil types in subtropical regions. Therefore, more detailed studies are still needed to quantify the inherent N transformation processes under different land use soils in other important soil types in subtropical regions, which is beneficial for understanding whether those different soils can effectively conserve N.

The Sichuan Basin in Southwest China is a subtropical region characterized by numerous hills. Regosols (locally known as purple soil) are the most important and widely distributed cropland soils in this region, covering an area of more than 1.6×10^5 km^2. Unlike the normally occurring soils in subtropical regions, Regosols are weakly developed mineral soils and are characterized by a shallow soil layer, coarse-textured sandy loams and good soil aeration[22]. Consequently, Regosols are susceptible to erosion and leaching, and the large N losses via leaching and overland runoff may cause local and widespread non-point source N pollution[23]. A previous study reported that annual nitrate leaching losses from sloping croplands could be up to 53.4 kg N ha^{-1} $year^{-1}$ in this region, which was the dominant N loss pathway[24]. Mitigating this high loss rate needs to enhance the soil N retention capacity by understanding inherent soil N cycling mechanisms in Regosols. Many studies have indicated that soil N transformations predominantly regulate N forms and composition in soils[14,25]. In particular, Zhang et al. revealed that the NO_3^- proportion was mainly regulated by the process of nitrification[26]. However, the inherent soil N transformation processes are still not well understood in the Regosols of the Sichuan Basin. Wang et al. investigated the soil gross N transformations in cropland soils under different fertilization regimes in this region and found that the increased gross nitrification rates governed the increases in cumulative NO_3^- losses via interflow and overland runoff[18]. In the Sichuan Basin, the dominated land uses are forestland and sloping cropland. The difference in land uses can cause significant differences in soil properties[27], which thus may affect soil gross N transformations. However, to date, how different land use soils influence gross N transformations in Regosols is not fully understood in this region. Understanding N transformations in Regosols and the effects of different land uses would provide important information for assessing the risks of N losses, and further provide the scientific basis for how to regulate the N transformation process to mitigate N losses in the Sichuan Basin of Southwest China.

In this study, we quantified gross N transformations in forestland and cropland of Regosols in the Sichuan Basin of Southwest China. This study aimed to (1) investigate the characteristics of gross N transformations in Regosols, (2) examine the differences in soil N transformations for different land uses in Regosols, and (3) evaluate the N conservation potential and N loss risks of Regosols under different land uses.

Materials and methods

Study region. This experiment was conducted at the Yanting Agro-Ecological Station of Purple Soil, Chinese Academy of Sciences, Yanting County of Sichuan Province (31° 16′ N, 105° 27′ E)[23]. It situates in the central Sichuan Basin and exhibits a moderate subtropical monsoon climate, with an average annual temperature and rainfall of 17.2 °C and 836 mm (30-year mean), respectively. The soil, classified as Eutric Regosols by the FAO soil classification[28], is locally termed "purple soil" due to its purplish colour[23]. It is typically non-zonal and weakly developed mineral soil, mostly characterizing by a neutral or alkaline reaction[18,23]. Currently, forestland and sloping cropland are the main land uses in this area.

Site description. To investigate the differences of inherent N transformations between different land use soils, sampling was conducted from the two main land uses of forestland and cropland. The forestland was initially planted with *Alnus cremastogyne* and *Cupressus funebris* in the 1970s to reforest cropland, and then, this site experienced natural succession without artificial management. It is now dominated by the representative forest type of *Cupressus funebris* in the study region, with a density of 1595 stems ha^{-1}. The selected forestland site was with an area of approximately 1.3 ha^{-1}. The cropland site was adjacent to the forestland site, with an area of 100 m × 100 m. It has been cultivated for more than 50 years, conventionally rotated with winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.). Fertilizers in the winter wheat and summer maize seasons were applied at the same amounts of K (36 kg K_2O ha^{-1}) and P (90 kg P_2O_5 ha^{-1}), but at different rates of N (130 and 150 kg N ha^{-1} as ammonium bicarbonate, respectively). All the fertilizers were manually applied and incorporated into the surface soil (0–20 cm) together with harrowing (approximately 20 cm deep). No irrigation was applied during either the wheat or maize season. The forestland and cropland sites had the same soil type (Regosols) and slope (5%).

Field soil sampling and N loss monitoring. For forestland and cropland sites, grids with an area of 10 m × 10 m were uniformly divided. Then eleven and eight grids were randomly selected for soil sampling from different slope positions (i.e. upslope, middle slope, and downslope) in forestland and cropland sites, respectively, to minimize the potential effect of spatial heterogeneity on the experimental results. Furthermore, to minimize the effects of fertilization on gross N transformations in cropland soils, soil samples were collected in April 2016 when closed to the wheat harvest. At each sampling grid, the organic layer was removed first if present and three soil cores were randomly taken from the surface 0–20 cm layer. Then, they were well mixed, passed through a 2-mm sieve, and ultimately separated into two sub-samples. One sample was air-dried for soil property analyses, and the other sample was stored for the incubation experiment at 4 °C for < 1 week.

To better identify how soil N transformations regulate the mechanisms involved in NO_3^- losses, field monitoring of NO_3^- loss was conducted following each rainfall event during 2016. At each site, NO_3^- concentration in surface soil (0–20 cm) was also continually monitored at least once a week. Taking the selected sloping forestland site as a whole drainage area (1.3 ha^{-1}), the discharge was monitored by the triangle weir installed at the outlet and the water samples were taken from the collecting tanks installed under the weir. At the cropland site, lysimeters

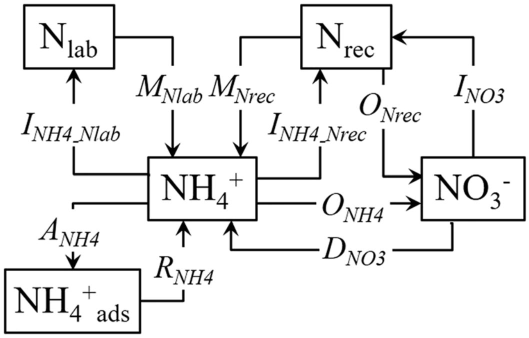

Figure 1. ^{15}N tracing model used for data analysis. (N_{lab} labile soil organic N, N_{rec} recalcitrant soil organic N, $NH_4^+{}_{ads}$ adsorbed NH_4^+).

were permanently established to take both interflow and overland runoff water samples[23]. Following each runoff event, the discharges of both interflow and overland runoff were determined. For both forestland and cropland sites, water samples were collected using 500-mL polyethylene bottles for assaying NO_3^- concentration. The annual NO_3^- loss flux (Q, kg N ha^{-1} year^{-1}) was estimated as follow:

$$Q = \sum_{i=1}^{n} \left(C_i \times q_i / 100 \right) \tag{1}$$

where C_i is the NO_3^- concentration in the interflow and overland runoff sample (mg L^{-1}), q_i is the interflow and overland runoff discharges (mm), and n is the number of runoff events during the monitoring period. During the experimental period, the daily precipitation was automatically monitored by a meteorological station located at a distance of approximately 3 km from the sampling sites.

^{15}N tracing experiment and model. The inherent gross N transformations in the forestland and cropland soils were determined using a ^{15}N tracing technique. Two ^{15}N sources, $^{15}NH_4NO_3$ (9.44 atom% excess) and $NH_4{}^{15}NO_3$ (9.75 atom% excess), were used in this study. For each soil sample (three replicates for each ^{15}N labelling treatment), we prepared a series of conical flasks (250-mL) using 20 g of fresh soil (oven-dry basis) and added $NH_4{}^{15}NO_3$ or $^{15}NH_4NO_3$ solutions to obtain the same NH_4^+-N and NO_3^--N concentrations (20 mg N kg^{-1}). Then they were adjusted to 60% WHC (water-holding capacity). In agricultural soils, the transformation of NH_4^+ to NO_3^- was generally fast[6,21]. To avoid the low NH_4^+ concentrations in soils after incubation and guarantee the ^{15}N detection requirements[29,30], therefore, relatively high application amounts of NH_4NO_3 were added compared to initial NH_4^+ and NO_3^- concentrations in this study. After 0.5, 12, 24, and 48 h of incubation at 25 °C, soils were extracted to measure the NH_4^+ and NO_3^- concentrations and isotopic composition. Distillation with MgO and Devarda's alloy were conducted to separate NH_4^+ and NO_3^-, strictly following the procedures described in previous studies[31,32]. Before separating, the recovery of NH_4^+ and NO_3^- in a standard solution (1 g NH_4^+-N/NO_3^--N L^{-1}) was determined. The results showed that the recovery of NH_4^+-N and NO_3^--N in the solution was more than 99% and 95%, respectively. Finally, the ^{15}N abundances of NH_4^+ and NO_3^- were analyzed by an automated C/N analyser and isotope ratio mass spectrometer (IRMS 20–22, SerCon, Crewe, UK).

The widely used numerical ^{15}N tracing model was employed to investigate the gross N transformations in this study. For the details of this model, numerous previous studies can be referenced[18,21,31,32]. Briefly, this model mainly involved the following ten simultaneously-occurring processes (Fig. 1): M_{Nlab} and M_{Nrec}, mineralization of labile organic N and recalcitrant organic N to NH_4^+, respectively; $I_{NH4\text{-}Nlab}$ and $I_{NH4\text{-}Nrec}$, immobilization of NH_4^+ to labile organic N and recalcitrant organic N, respectively; A_{NH4} and R_{NH4}, adsorption and release of adsorbed NH_4^+ on cation exchange sites, respectively; O_{NH4}, oxidation of NH_4^+ to NO_3^- (autotrophic nitrification); O_{Nrec}, oxidation of recalcitrant organic N to NO_3^- (heterotrophic nitrification); D_{NO3}, dissimilatory NO_3^- reduction to NH_4^+ (DNRA); and I_{NO3}, immobilization of NO_3^- to recalcitrant organic N[29,30]. The transformation rates were calculated by zero-order, first-order or Michaelis–Menten kinetics by minimizing misfits between the modelled and determined concentrations and ^{15}N enrichments of NH_4^+ and NO_3^- (averages ± standard deviations). Aikaike's Information Criterion (AIC) was utilized to select the best model, and the Markov chain Monte Carlo-Metropolis algorithm (MCMC-MA) was employed for optimizing the parameter[21,30,32]. The MCMC-MA routine was conducted using MATLAB software (Version 7.2, The MathWorks Inc.). Finally, average transformation rates over a 48 h period (mg N kg^{-1} day^{-1}) were calculated on the basis of the kinetic settings and the final parameters.

The total mineralization rates (M_N) were estimated as the sum of M_{Nlab} and M_{Nrec}, and the total NH_4^+ immobilization rates (I_{NH4}) were calculated as the sum of $I_{NH4\text{-}Nlab}$ and $I_{NH4\text{-}Nrec}$. Nitrification capacity was expressed as the ratio of O_{NH4} to M_N. The NO_3^- retention capacity was defined as the ratio of NO_3^- consumption ($I_{NO3} + D_{NO3}$) to total nitrification ($O_{NH4} + O_{Nrec}$).

Soil chemical property measurements. Soil property analyses strictly followed the procedures described in Soil Agro-Chemical Analysis[33]. Soil pH was measured in a 1:2.5 (soil-to-water) suspension using

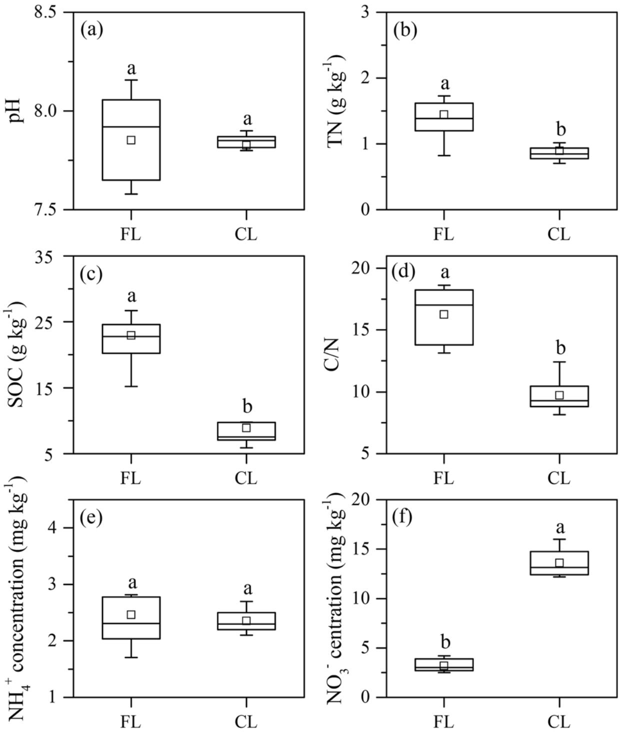

Figure 2. Soil properties of pH (**a**), TN (**b**), SOC (**c**), C/N (**d**), NH_4^+ concentration (**e**) and NO_3^- concentration (**f**) in the forestland and cropland. *FL* forestland, *CL* cropland. The different letters in each sub-figure indicate significant differences between the different land use soils ($P < 0.05$). The bottom/top of the box denote 25th/75th percentiles. Whiskers denote 5th/95th percentiles. The squares denote the mean values, and the black lines denote the mid-values.

a DMP-2 mV/pH detector (Quark Ltd, Nanjing, China). Soil organic carbon (SOC) and total N (TN) were measured by wet digestion with H_2SO_4–$K_2Cr_2O_7$, and by semi-micro Kjeldahl digestion using Se, $CuSO_4$ and K_2SO_4 as catalysts, respectively. The concentrations of NH_4^+ and NO_3^- were measured by extraction with 2 M KCl, filtration with filter paper, and analysis using an AA3 continuous-flow analyser (Bran + Lubbe, Norderstedt, Germany).

Statistical analyses. All statistical analyses were carried out in SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was conducted first to test the normality of the data. The differences in the gross N transformation rates and soil properties between the cropland and forestland soils were detected using an independent sample *t* test. The significance level was conventionally set at 0.05. The relationships between gross N transformation rates and soil properties were tested by Pearson correlation analysis.

Results

Soil properties. The average pH was 7.85 ± 0.06 and 7.83 ± 0.03 in the forestland and cropland soils, respectively, with no significant difference (Fig. 2a). However, the average SOC content (22.91 ± 1.37 g kg^{-1}), TN content (1.45 ± 0.13 g kg^{-1}) and C/N ratio (16.26 ± 0.65) in the forestland soils were significantly greater than those in the cropland soils (mean 8.90 ± 1.21 g kg^{-1}, 0.90 ± 0.07 g kg^{-1} and 9.72 ± 0.51 for SOC, TN and C/N, respectively, $P < 0.05$) (Fig. 2b–d). The average soil NH_4^+ concentrations were 2.46 ± 0.18 mg N kg^{-1} and 2.35 ± 0.07 mg N kg^{-1} in the cropland and forestland soils, respectively, with no significant difference (Fig. 2e). The average soil NO_3^- concentration in the cropland soils (13.60 ± 0.51 mg N kg^{-1}) was significantly greater ($P < 0.05$) than that in the forestland soils (3.02 ± 0.08 mg N kg^{-1}) (Fig. 2f).

Soil gross N transformation rates. The gross N mineralization (M_N) rates averaged 3.95 ± 0.51 mg N kg^{-1} day^{-1} and 4.84 ± 0.69 mg N kg^{-1} day^{-1} in the forestland and cropland soils, respectively, with no significant difference (Fig. 3a). No significant relationships were detected between the mineralization rates and the measured soil properties.

The gross NH_4^+ immobilization (I_{NH4}) rates in the cropland soils (0.11–0.92 mg N kg^{-1} day^{-1}, mean 0.34 ± 0.10 mg N kg^{-1} day^{-1}) were significantly lower than those in the forestland soils (3.56–10.31 mg N kg^{-1} day^{-1}, mean 6.67 ± 0.74 mg N kg^{-1} day^{-1}) ($P < 0.05$) (Fig. 3b). The soil gross NH_4^+ immobilization rates were positively related to the SOC content ($r = 0.81$) and the C/N ratio ($r = 0.84$) (Fig. 4).

Significantly higher gross autotrophic nitrification (O_{NH4}) rates occurred in the cropland soils (7.91–20.02 mg N kg^{-1} day^{-1}, mean 14.54 ± 1.66 mg N kg^{-1} day^{-1}) than in the forestland soils (0.25–6.29 mg N kg^{-1}

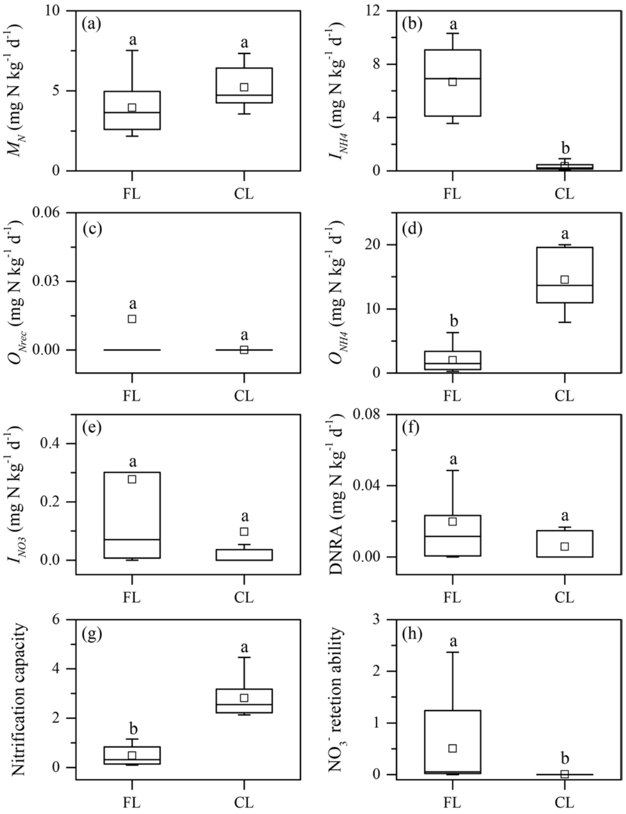

Figure 3. Gross N transformation rates in the forestland and cropland soils estimated using the ^{15}N tracing model. Abbreviations: M_N total N mineralization (**a**); I_{NH4} total NH_4^+ immobilization (**b**); O_{Nrec} heterotrophic nitrification (**c**); O_{NH4} autotrophic nitrification (**d**); I_{NO3} NO_3^- immobilization (**e**); DNRA: dissimilatory NO_3^- reduction to NH_4^+ (**f**); nitrification capacity: ratio of O_{NH4} to M_N (**g**); NO_3^- retention capacity ratio of total NO_3^- consumption rates (I_{NO3} + DNRA) to the total NO_3^- production rates (O_{NH4} + O_{Nrec}) (**h**); *FL* forestland, *CL* cropland. The different letters in each sub-figure indicate significant differences between different land use soils ($P < 0.05$). The bottom/top of the box denote 25th/75th percentiles. Whiskers denote 5th/95th percentiles. The squares denote the mean values, and the black lines denote the mid-values.

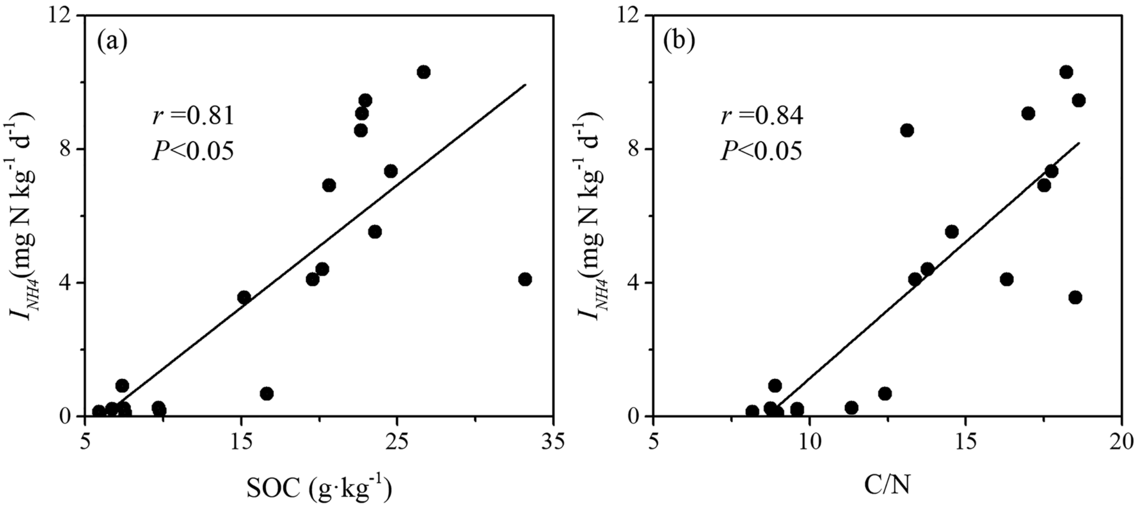

Figure 4. Relationships between soil gross NH_4^+ immobilization rates and SOC content (**a**) and C/N ratio (**b**).

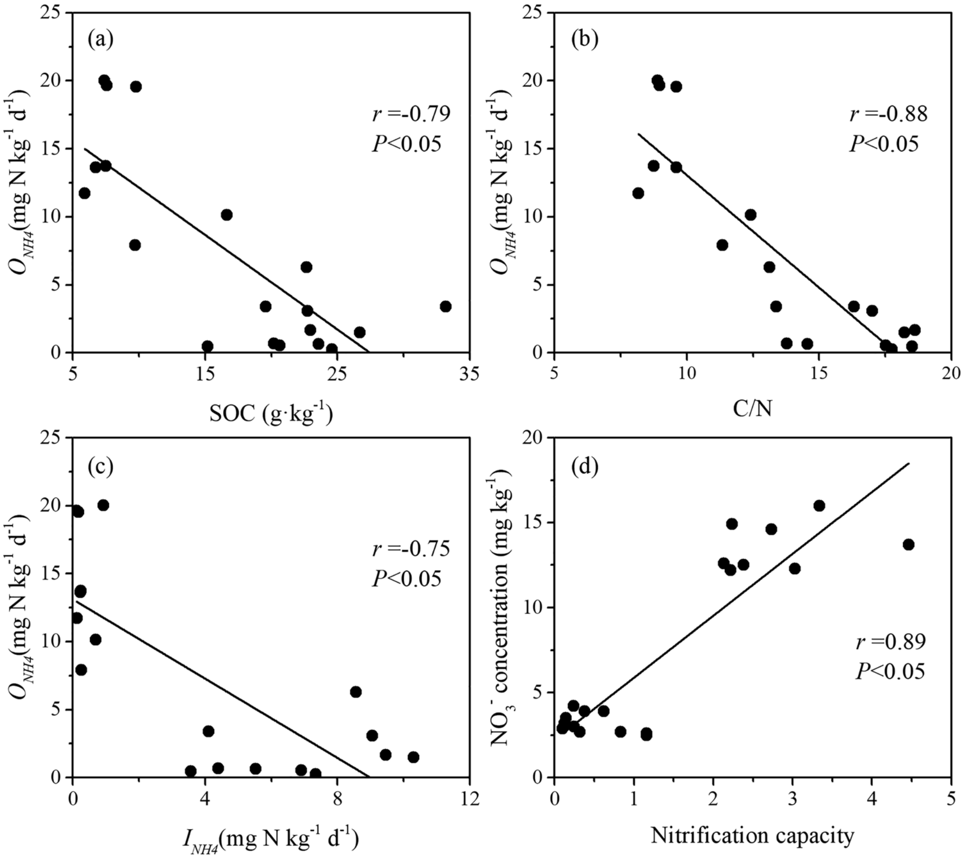

Figure 5. Relationships between soil gross nitrification rates and SOC content (**a**), C/N ratio (**b**) and gross NH_4^+ immobilization rates (**c**), and between nitrification capacity and NO_3^- concentration (**d**). Nitrification capacity: ratio of O_{NH4} to M_N.

day^{-1}, mean 1.99 ± 0.56 mg N kg^{-1} day^{-1}) ($P < 0.05$) (Fig. 3d). However, gross heterotrophic nitrification (O_{Nrec}) rates were negligible in both the forestland and cropland soils (Fig. 3c). The soil gross autotrophic nitrification rates were negatively related to the SOC content ($r = -0.79$) (Fig. 5a) and the C/N ratio ($r = -0.88$) (Fig. 5b). Negative correlations also existed between soil gross NH_4^+ immobilization and autotrophic nitrification rates ($r = -0.75$) (Fig. 5c). Nitrification capacity (i.e., O_{NH4}/M_N) in the forestland soils (mean 0.48 ± 0.12) was lower than that in the cropland soils (mean 3.94 ± 1.23) ($P < 0.05$) (Fig. 3g). A positive correlation was detected between the NO_3^- concentration and nitrification capacity ($r = 0.89$) (Fig. 5d).

The gross NO_3^- immobilization (I_{NO3}) and DNRA rates were not significantly different between the forestland and cropland soils (Fig. 3e,f). The cropland soils had a significantly lower NO_3^- retention capacity (mean value of 0.01 ± 0.01) than the forestland soils (mean value of 0.51 ± 0.24) in this study (Fig. 3h).

Soil NO_3^- loss. During the whole year of 2016, six and seven runoff events were monitored in forestland and cropland sites, respectively (Fig. 6c,d). As shown, most NO_3^- losses mainly occurred in summer season (from July to August), during which the rainfall was usually heavy and contributed 51% of the annual precipitation (Fig. 6a). In particular, the heaviest rainfall event was observed at 2016/7/18 with precipitation of 162 mm (Fig. 6a). Then, the highest NO_3^- losses were monitored at 2016/7/18 in forestland and 2016/7/23 in cropland, respectively (Fig. 6c,d). The NO_3^- losses in each runoff event were all significantly higher in cropland than in forestland ($P < 0.05$). Significantly higher soil NO_3^- concentrations were also observed in cropland than that in forestland ($P < 0.05$), especially after the period of fertilization (Fig. 6b). The total NO_3^- losses were 0.25 ± 0.01 kg N ha^{-1} year^{-1} and 27.10 ± 2.54 kg N ha^{-1} year^{-1} for the forestland and cropland, respectively.

Discussion

Patterns of gross N transformations in Regosols. Different with the generally acidic and highly weathered soils in humid subtropical regions[29], the studied Regosols inherit most properties of parent materials and characterize by coarse texture and a neutral or alkaline reaction[18,22,23]. The specific soil properties of Regosols therefore may cause different N transformation processes compared to the normally occurring subtropical acidic soils.

In current study, gross N mineralization rates in the forestland soils (mean 3.95 ± 0.51 mg N kg^{-1} day^{-1}) were similar to those observed in subtropical zonal soils of Orthic Acrisols and Humic Planosols (FAO soil

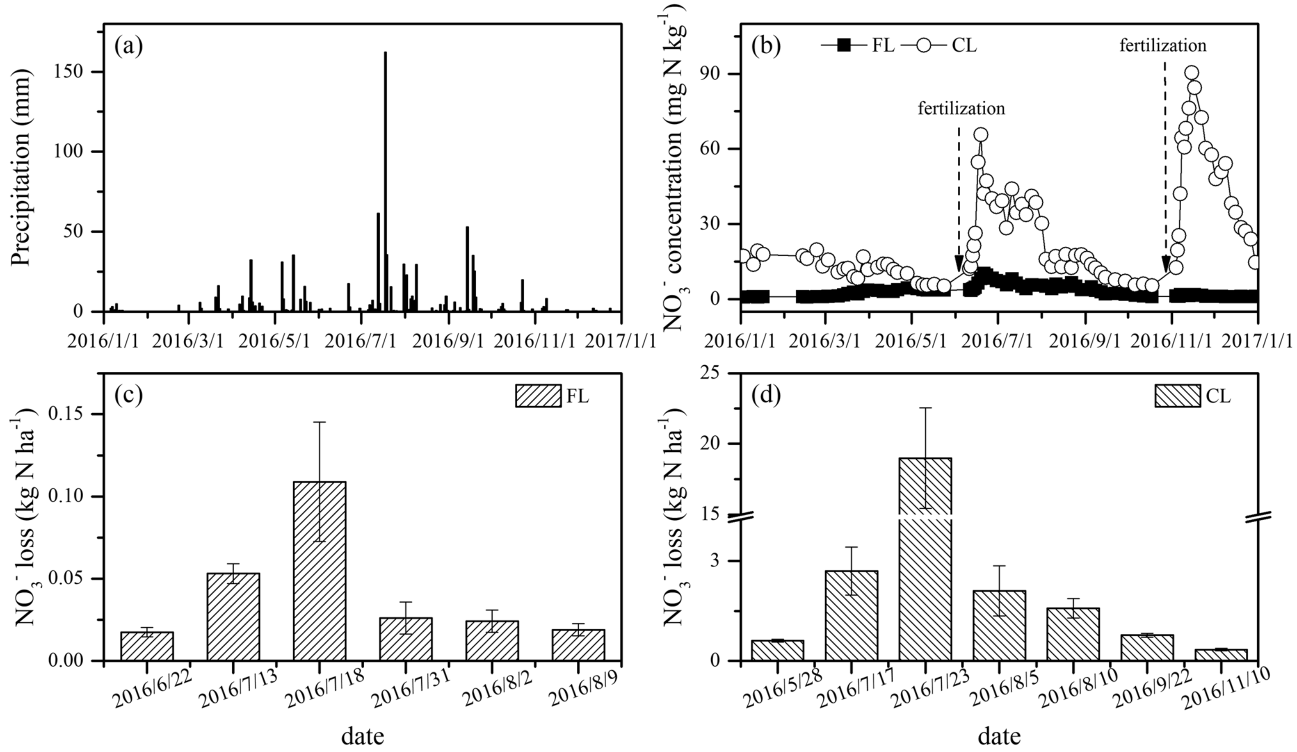

Figure 6. Dynamics of daily precipitation (**a**) and soil NO_3^- concentration (**b**), and soil NO_3^- losses at each runoff event from forestland (**c**) and cropland (**d**) during 2016. *FL* forestland, *CL* cropland.

classification) (mean 3.67 and 3.52 mg N kg^{-1} day^{-1}, respectively)[4,34]. But they were higher than those measured in subtropical acidic Regosols of southwest China (mean 1.23 mg N kg^{-1} day^{-1})[35]. This difference is most likely related to the differences in soil organic matter and pH between these two Regosols[13,14]. Moreover, the measured gross NH_4^+ immobilization rates in the forestland soils (mean 6.72 ± 0.74 mg N kg^{-1} day^{-1}) were higher than those observed in above mentioned subtropical soil types (0.82 mg N kg^{-1} day^{-1} to 2.25 mg N kg^{-1} day^{-1})[4,34,35]. These results indicated that the alkaline Regosols might have a faster NH_4^+ mineralization-immobilization turnover than other soil types in the subtropical regions.

The average total nitrification rates in forestland soils were 2.00 ± 0.57 mg N kg^{-1} day^{-1} in this study, and autotrophic nitrification contributed approximately 99.6%. This result indicated that autotrophic nitrification was the dominated NO_3^- production process in the studied Regosols. However, significantly lower total nitrification rates were reported in subtropical acid soils, and heterotrophic nitrification was their dominant process[4,34,35]. Previous studies have showed that the microbiological autotrophic nitrification would be inhibited in soil at pH values lower than 5[36], but be stimulated at pH values higher than 7.5[37]. Moreover, high soil pH might also inhibit the existence of fungi and their activities, which were related to heterotrophic nitrification[7]. Therefore, the differences in nitrification processes were likely related to the differences in soil pH between the alkaline Regosols and subtropical acid soils. Furthermore, the nitrification capacity (i.e., O_{NH4}/M_N ratio, mean 0.48 ± 0.12) in present forestland soils was significantly greater than that in acidic Regosols (0.02) and Orthic Acrisols (0.05)[4,35], which may therefore promote soil NO_3^- accumulation and leaching risk in the study region[22–24]. This result could be verified by the high NO_3^-/NH_4^+ ratio in forestland soils in this study.

Gross NO_3^- immobilization and DNRA were the important NO_3^- consumption and retaining processes in soils[38,39]. DNRA generally occurred in anaerobic conditions[40–42], however, it was negligible in this study due to the good aeration of Regosols. Gross NO_3^- immobilization rates in this study were also significantly lower than those in other subtropical soils[4,34]. Previous studies have indicated that NO_3^- immobilization generally needed high carbon availability[43]. The forestland soils in this study had lower soil organic C content (22.91 ± 1.37 g kg^{-1}) compared to those subtropical forest soils[4,34], which thus likely resulted the lower NO_3^- immobilization. In addition, the inhibition of fungal activities by the high soil pH might also control the NO_3^- immobilization in the studied Regosols[44]. Consequently, NO_3^- retention capacity was significantly lower in alkaline Regosols (mean 0.51 ± 0.24) than that in subtropical zonal soils of Orthic Acrisols and Humic Planosols (0.98 and 0.81, respectively) under forestland[4,34].

As discussed above, due to the specific soil properties, the non-zonal Regosols in the Sichuan Basin of Southwest China showed greatly different N transformation processes compared to the normally occurring soils in other subtropical regions. Overall, the alkaline Regosols had a faster NH_4^+ mineralization-immobilization turnover, higher nitrification rates, and lower NO_3^- immobilization rates compared to other reported subtropical soils.

Gross N transformations under different land use soils. The different land uses significantly affected the NH_4^+ immobilization and autotrophic nitrification in Regosols, while no significant differences were observed in gross mineralization, NO_3^- immobilization and DNRA between the forestland and cropland soils (Fig. 3). In the cropland soils, the gross NH_4^+ immobilization rates were significantly lower than those in the forestland soils (Fig. 3b). This result agrees with most previous findings obtained from other subtropical soils in China[9,45]. However, Zhang et al. observed similar gross NH_4^+ immobilization rates between forestland and agricultural soils[4]. Soil gross NH_4^+ immobilization rates were positively related to the SOC and C/N in this study (Fig. 4). Previous studies indicated that relatively higher SOC content and C/N ratio in the soils could stimulate the increase of N immobilization potentiality[9,46]. In the cropland soils, due to long-term mineral N fertilizer application and very few crop residual retention, organic matter sources are mainly dependent on crop roots[9]. Therefore, the SOC content and C/N ratio in the cropland soils significantly decreased compared to those in the forestland soils (Fig. 2c,d), which might be an important factor that significantly reduced NH_4^+ immobilization. Furthermore, the gross NH_4^+ immobilization rates in the forestland soils were greater than the gross mineralization rates (Fig. 3a,b), indicating that a large proportion of the NH_4^+ produced from mineralization could be effectively immobilized. This rapid NH_4^+ turnover in forestland soils likely reduced the accumulation of NH_4^+, thus left little available substrate for nitrifiers[19,25]. However, in the cropland soils, the gross mineralization rates were significantly greater than the NH_4^+ immobilization rates (Fig. 3a,b), which might leave more available NH_4^+ substrates in soils for autotrophic nitrification.

The gross autotrophic nitrification rates were significantly greater in the cropland soils than in the forestland soils (Fig. 3d). This finding is in accordance with the results of some previous studies[4,9]. Soil pH is generally viewed as the key factor influencing nitrification[4,12,13]. However, soil pH was not significantly different between the forestland and cropland in this study (Fig. 2a). Nitrogen fertilization has been considered as another important factor stimulating nitrification in the cropland soils[4,9]. Numerous studies have indicated that long-term N fertilizer applications could stimulate autotrophic nitrification rates[18,30,47,48]. Applying mineral N fertilizer could induce the rapid increases in NH_4^+ concentrations for several weeks in cropland soils, thus providing sufficient available substrates for nitrification[49–51]. However, in this study, soil samples were collected once in April when several months had passed since the last fertilizer application. Thus, the extremely high nitrification rates following fertilization might not been considered in this study. Previous studies have shown that long-term N fertilizer application could affect the ammonia-oxidizing microbe population size and activity, thus stimulate autotrophic nitrification[18,30]. Previous studies in the same study area have revealed that application of mineral N fertilizer significantly increased soil ammonia-oxidizing bacteria (AOB) population size and changed AOB composition[49,50]. Consequently, long-term applying mineral N fertilizer might be the main factor inducing the differences in nitrification rates between the different land use soils in this study.

The ratio of soil gross autotrophic nitrification to gross NH_4^+ immobilization (O_{NH4}/I_{NH4}) can effectively indicate their relative importance in NH_4^+ consumption[5,9,10]. In this study, a negative correlation was observed between the gross autotrophic nitrification rates and NH_4^+ immobilization rates (Fig. 5c). In the forestland soils, the average O_{NH4}/I_{NH4} ratio was 0.32 ± 0.09, indicating that NH_4^+ immobilization dominated NH_4^+ consumption. Conversely, the average O_{NH4}/I_{NH4} ratio was 69.99 ± 18.91 in the cropland soils, indicating that autotrophic nitrification was the dominant NH_4^+-consuming process. These results also implied that autotrophic nitrification was greatly enhanced in the cropland soils than in the forestland soils in the study region.

Overall, in comparison to the forestland soils, gross autotrophic nitrification rates were significantly increased, while gross NH_4^+ immobilization rates were significantly decreased in the cropland soils. The significant differences in soil N transformations were closely related to the long-term mineral N fertilizer application, and the significant SOC content and C/N ratio decreases in the cropland soils.

NO_3^- loss and retention driven by soil N transformations. During the whole monitoring period, the cropland soils had significantly higher NO_3^- concentrations than the forestland soils (Fig. 6b). The large amounts of NO_3^- accumulation in the cropland soils could be easily diluted and lost during the heavy rainfall events[22–24]. The field monitoring results showed that the NO_3^- losses in each runoff event were all significantly greater in the cropland soils than those in forestland soils (Fig. 6c,d). Previous studies indicated that the inorganic N form and amount in soils, especially the NO_3^- accumulation, were controlled by N transformation processes[25,26]. In the cropland soils, the gross NH_4^+ immobilization significantly decreased but the gross autotrophic nitrification significantly increased compared to the forestland soils, resulting in the NO_3^-/NH_4^+ ratio (mean 5.79 ± 0.16) being 4 times greater than that in the forestland soils (mean 1.24 ± 0.05). This result confirmed that NO_3^- not only was the dominant inorganic N form, but also has a higher concentration in the cropland soils.

The nitrification capacity (i.e., O_{NH4}/M_N ratio) and O_{NH4}/I_{NH4} ratio were two key indicators for the NO_3^- loss potential from the soils[52,53]. In this study, compared to the forestland soils, the cropland soils had much higher nitrification capacity and O_{NH4}/I_{NH4} ratio. The correlation analysis showed that the nitrification capacity and O_{NH4}/I_{NH4} ratio were positively correlated with the NO_3^- concentration and NO_3^-/NH_4^+ ratio ($P < 0.05$; Fig. 5d). Moreover, the low NO_3^- retention capacity was observed in both the forestland and cropland soils (mean 0.51 ± 0.24 and 0.01 ± 0.01). Overall, the NO_3^- production rates were greater than the NO_3^- consumption rates, resulting in huge NO_3^- accumulation in Regosols (mean 3.02 ± 0.18 mg N kg^{-1} and 13.60 ± 0.51 mg N kg^{-1} for forestland and cropland), which thus caused great risks of NO_3^- losses, especially from the cropland soils[9,18,54].

NO_3^- losses caused nutrient loss and threatened the environment and human health[1,55,56]. In this study, the NO_3^- losses occurring in the cropland soils were approximately 10% of the annual N fertilization. Consequently, NO_3^- losses to the environment should be minimized by retaining NO_3^- efficiently in the soils. As discussed above, the SOC content and C/N ratio significantly influenced soil N immobilization and nitrification. Thus, increasing the SOC content and C/N ratio would be effective strategies for NO_3^- retention in the alkaline Regosols, which can potentially reduce autotrophic nitrification and enhance NH_4^+ immobilization.

Conclusions

Compared to the typical zonal acidic soils in the subtropical regions, the non-zonal soils of alkaline Regosols in this study showed specifically inherent gross N transformations, i.e. the faster NH_4^+ mineralization-immobilization turnover, the higher nitrification rates, and the lower NO_3^- immobilization rates. Different land use significantly affected the gross N transformation processes of autotrophic nitrification and NH_4^+ immobilization in Regosols. In the cropland soils, the rates of gross autotrophic nitrification were significantly greater, but the rates of gross NH_4^+ immobilization were significantly lower than those in the forestland soils. The specific soil gross N transformations resulted in low NO_3^- retention capacity and thus high NO_3^- loss risks in the Regosol croplands. The total NO_3^- losses from the cropland soils were substantial (27.10 ± 2.54 kg N ha^{-1} $year^{-1}$) and much greater than those from the forestland soils (0.25 ± 0.01 kg N ha^{-1} $year^{-1}$). The great differences in the N transformations between the different land use soils may be attributed to the changes of the SOC content and C/N ratio and the application of mineral N fertilizer after long-term cultivation in the cropland.

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Author contributions

X.R., J.Z. and B.Z. designed the experiments. X.R., J.Z. and H.B. participated in acquisition and analysis of data for the work. X.R. wrote the manuscript. J.Z., C.M., Z.C. and B.Z. revised it critically for important intellectual content. All authors approved the submission.

Competing interests

The authors declare no competing interests.

Additional information

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