

## **$^{15}\text{N}$ isotope tracing of nitrogen runoff loss on red soil sloping uplands under simulated rainfall conditions**

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### **ABSTRACT**

Stable isotope  $^{15}\text{N}$  tracer technique was used in combination with artificial rainfall simulation to study the influence of interflow and surface-flow on nitrogen (N) migration loss of soil-plant systems on typical red soil sloping uplands. This study also investigated the utilization efficiency of fertilizer N during different peanut plant growth stages. The results indicated that soil N loss was predominantly via interflow and erosive sediment. Fertilizer N loss during the initial growth stage was mainly through surface runoff, while that occurred as interflow increased from less than 5% to around 16% during the middle and late growth stages. The loss of fertilizer N through surface runoff, erosive sediment and interflow accounted for over 18% of the total N application. The utilization rate of fertilizer N by peanut plants was around 45% through its life cycle, and that 70% of N absorbed by this plant derived from the soil. This highlighted the importance of adopting effective methods to reduce nutrient loss through interflow and surface-flow, the need to increase the utilization rate of fertilizers, and the importance to maintain soil fertility at a relatively high level.

**Keywords:** soil erosion; vertical infiltration; leaching loss; nutrients; precipitation

Interflow refers to the movement of water in soil, including vertical infiltration (i.e., leaching) and horizontal flow, and it has an important influence on runoff generation, nutrient loss and soil erosion (Wilson et al. 2008). Under the action of rainfall and runoff, it is easy for nutrients on sloping land to enter water body through interflow mainly in liquid phase due to low sediment concentration and surface-flow mainly in both liquid and solid phases because of high sediment concentration, which may lead to environmental problems such as water eutrophication and groundwater pollution (Ye et al. 2011). The development of agriculture in the red soil sloping uplands has been restricted by the environment (low water availability and high temperatures), high levels of nitrogen loss, and low nitrogen fertilizer efficiency and utilization rates. In these regions, although there is sufficient and concentrated rainfall, strong soil eluviation and interflow,

coupled with serious soil erosion, has resulted in interflow and surface-flow to become the important pathways for nutrient migration (Zheng et al. 2014). Recently, researchers have studied the migration patterns of red soil nutrients through surface-flow (including surface runoff and its erosive sediment) and the impact factors arising from these migrations (Chen et al. 2015), as well as the generation of interflow and its influence on nutrient outputs (Chu et al. 2010). However, there are relatively few investigations that have comprehensively considered the influence of both interflow and surface-flow on the migration of red soil nutrients. Some studies have been undertaken under conditions without crops, thus they cannot comprehensively recognize the pathways of nutrient loss from soil-plant systems, or their influencing factors.

To investigate the ratio of nitrogen loss through runoff in the soil-plant system, and to identify

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the nitrogen (N) loss pathways on red soil sloping uplands, this work studies the influence of interflow and surface-flow on N migration loss and the utilization of exogenous N during different growth stages. The findings will provide a reference for the prevention and treatment of soil and water loss and non-point source pollution, and for the effective utilization rate of fertilizers on sloping field.

## MATERIAL AND METHODS

**Overview of the experimental site.** The experiments were conducted at the Jiangxi Research and Innovation Base for Soil and Water Conservation (115°42'38"~115°43'06"E, 29°16'37"~29°17'40"N), located in the suburb of De'an county in the Poyang Lake water system, Northern Jiangxi province. This area, having a semi-tropical monsoon climate, has annual average precipitation of 1469 mm, annual average evaporation of 1558 mm, annual average temperature of 16.7°C, annual average frost-free period of 249 days, and a yearly sunshine duration of 1700–2100 h. The landform is a shallow hillock area with an altitude of 30–100 m a.s.l. and a slope of 5–25°. The zonal vegetation is subtropical evergreen broad-leaved forest, and the soil is dominated by red soil developed from Quaternary red clay.

**Experiment equipment.** Using a typical hilly red soil as the soil substrate, peanut plants were planted as a local representative sloping upland crop. Stable isotope  $^{15}\text{N}$  tracer technique was used in combination with artificial rainfall simulation to study the influence of runoff on N migration loss. Rainfall simulations were conducted using the Fulljet rotating pendent nozzle manufactured by Spraying Systems Co., Ltd. (Illinois, USA). The size of the soil bins was 3 m (length)  $\times$  1.5 m (width)  $\times$  0.6 m (height) which allowed the observation of surface-flow and interflow. Detail information of the rainfall simulator and soil bins are presented

in Zheng et al. (2014). A 0.10 m layer of fine sand (silted by 5 mm meshes) was placed on bottom of each soil bin, and then a total of 0.40 m soil depth (silted by 10 mm meshes) was filled by four 0.10 m layers. Bulk density was controlled at  $1.35 (\pm 0.1) \text{ g/cm}^3$ . The experimental soil was red soil developed from Quaternary red clay, of which the total nitrogen content was 0.76 g/kg, the alkali-hydrolyzable nitrogen content was 93.28 mg/kg and the  $^{15}\text{N}$  abundance was 0.449 atom%.

**Experiment design.** The slope of the soil bin was adjusted to 10°, representing the typical slope of the red soil sloping uplands in Jiangxi province. Peanut seeds were planted as the main crop and the cultivar was Baisha Harvest King. The hold seeding in shallow furrows method was used. On May 8, 2015, all of the soil bins were seeded by 8000 holes/ha, three peanut seeds/hole, and two plants/hole after seedling. Due to the minimum evaporation loss of urea, as well as to eliminate the influence of peanut nitrogen fixation, the study had two treatments:  $^{15}\text{N}$  urea and ordinary urea, each of which was repeated three times. The  $^{15}\text{N}$  urea, provided by the Shanghai Research Institute of Chemicals, had a nitrogen content of 46.8%, and its abundance was 10.15%. 100 g of urea, 293 g of single superphosphate and 90 g of potassium sulfate were evenly sprayed in each soil bin as the base fertilizer. The fertilization level and other management methods were the same as the local farmers. The artificial rainfall simulations were conducted at the peanut seedling stage (June 11<sup>th</sup>), pegging stage (July 30<sup>th</sup>) and ripening stage (September 11<sup>th</sup>). Each rainfall simulation was controlled at 60 mm/h. The rainfall control and runoff sediment collection were conducted according to previous investigations (Zheng et al. 2014) and the rainfall-generated runoff and sediment yields were calculated, as detailed in Table 1.

**Sample collection and its analysis.** Collected surface runoff was left undisturbed for about 4 h before the supernatant was collected, and the erosive sediment was collected as well. These were

Table 1. Surface runoff, erosive sediment and interflow under simulated rainfalls

Item	Seedling stage	Pegging stage	Ripening stage
Surface runoff ( $\text{m}^3$ )	$0.13 \pm 0.05$	$0.14 \pm 0.07$	$0.14 \pm 0.04$
Sediment yield (kg/ha)	$3669 \pm 797$	$2645 \pm 431$	$3749 \pm 824$
Interflow ( $\text{m}^3$ )	$0.14 \pm 0.04$	$0.10 \pm 0.01$	$0.10 \pm 0.01$

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used to measure the total nitrogen content and  $^{15}\text{N}$  abundance of surface runoff in the water and eroded phase. As the amount of interflow sediment was limited, this was only used for measuring the nitrogen content of interflow in the water phase. After each rainfall simulation one full plant was collected from the top, middle and bottom slopes, respectively. The plant samples were used to measure the total nitrogen content and  $^{15}\text{N}$  abundance. The runoff samples were distilled under basic conditions using nitrogen analysis alloy and were absorbed by 2% boric acid solution. The nitrogen determination by hydrochloric acid standard solution was conducted on the samples. An adequate amount of volumetric solution from the nitrogen analysis was collected, acidified and concentrated by heat (the temperature was controlled at 65–70°C) into  $^{15}\text{N}$  samples for testing. The total nitrogen content was determined by the semi-micro Kjeldahl method. The total nitrogen content of the peanut plant was tested by the distillation method after digestion by concentrated sulfuric acid and hydrogen peroxide. The  $^{15}\text{N}$  abundance of the runoff, sediment and plant samples was tested at the Shanghai Research Institute of Chemicals using a MAT-271 isotope mass spectrometer (Finnigan MAT, California).

**Calculation of relevant parameters of runoff nitrogen.** The nitrogen content and ratio of the runoff and its sediment were calculated based on the method of Zanetti et al. (1997) using the following equations: Percentage of nitrogen in the surface runoff (interflow) from the nitrogen fertilizer:

$$(\%) = \frac{(^{15}\text{N} \text{ abundance of runoff} - C_w)}{(^{15}\text{N} \text{ abundance of fertilizer} - C_0)} \times 100 \quad (1)$$

Where:  $C_w$  –  $^{15}\text{N}$  abundance of the experimental water;  $C_0$  –  $^{15}\text{N}$  natural abundance.

Percentage of nitrogen in the erosive sediment from the nitrogen fertilizer:

$$(\%) = \frac{(^{15}\text{N} \text{ abundance of the sediment} - C_s)}{(^{15}\text{N} \text{ abundance of the fertilizer} - C_0)} \times 100; \quad (2)$$

Where:  $C_s$  –  $^{15}\text{N}$  abundance background value of the experimental soil. Output nitrogen in the runoff and sediment from the nitrogen fertilizer = the runoff (sediment) nitrogen loss  $\times$  the percentage of nitrogen in runoff (sediment) from the nitrogen fertilizer (3).

**Calculation of the relevant parameters of plant nitrogen.** The  $^{15}\text{N}$  abundance difference

between the plants treated by  $^{15}\text{N}$  urea and those treated by ordinary urea was set as the  $^{15}\text{N}$  atom % excess (A% E), as per the following equations: Plant nitrogen accumulation amount = the dry weight of the plant  $\times$  the plant nitrogen content (4); percentage of the plant accumulated nitrogen from the nitrogen fertilizer (NDFF) (%) = A%E of the plant sample/A%E of the fertilizer sample  $\times$  100 (5). Amount of plant accumulated nitrogen from the nitrogen fertilizer = the plant nitrogen accumulation amount  $\times$  NDFF (6); utilization rate of the nitrogen fertilizer (%) = the amount of plant accumulated nitrogen from the nitrogen fertilizer / the total nitrogen content of the soil bin  $\times$  100 (7).

**$^{15}\text{N}$  soil residual and gaseous loss calculation.** According to the nitrogen balance principle, the  $^{15}\text{N}$  soil residual and gaseous losses during different growth stages were calculated using the differential method.

**Data analysis.** The measurements of all of the preceding equations were repeated three times and the average values were calculated. Microsoft 2010 (Microsoft, Beijing, China) was used to generate the figures and tables.

## RESULTS AND DISCUSSION

**Influence of interflow and surface-flow on N loss.** Most of previous investigations (Bronson et al. 2000, Ebid et al. 2008) focused on plains where N loss occurred in runoff through interflow leaching and few studies have looked into sloping lands where N loss was reported in runoff through interflow, surface runoff and its erosive sediment. This investigation took interflow and surface-flow into consideration, which may lead to an adequacy in comprehensively studying the nitrogen losses of farmlands. Results for N runoff loss at different growth stages under the same rainfall simulation conditions are shown in Figure 1. During the seedling stage, N loss through surface runoff was mainly from fertilizer N (72.6%), while losses through interflow and erosive sediment were predominantly from soil N (95.1% and 90.9%, respectively). Fertilizer N loss through interflow was very small (< 5%) during this stage, which were mainly due to the fact that fertilizer N was applied to the soil surface and its leaching was limited over a short time period. Compared to the seedling stage, fertilizer N loss through surface runoff

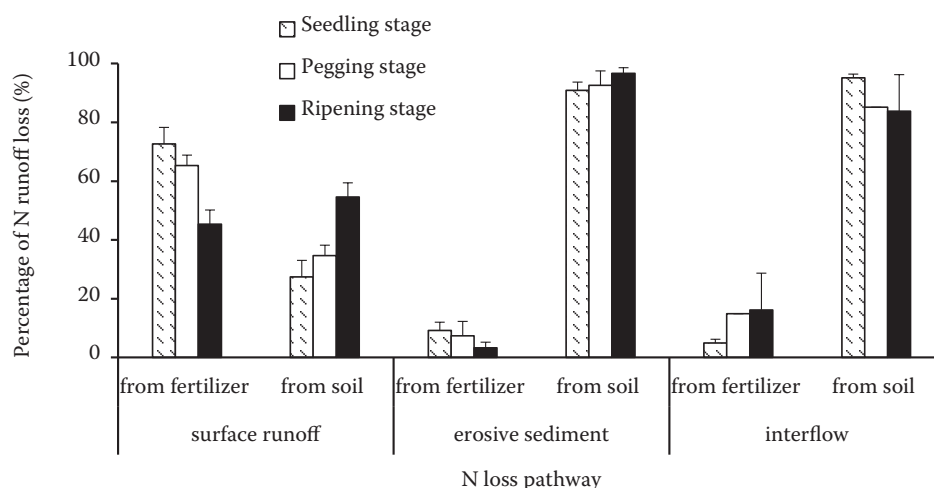


Figure 1. Nitrogen (N) loss pathways and its percentage in runoff at different growth stages

during the pegging stage decreased by 7.3% and that through sediment erosion decreased by 1.8%; fertilizer N loss through interflow at this period increased by 10.0%. These results were mainly due to fertilizer N leaching deeper into the soil over time. Fertilizer N loss through surface runoff and erosive sediment during the ripening stage decreased compared to that during the pegging stage, to 45.4% and 3.3%, respectively. Correspondingly, soil N loss through surface runoff and erosive sediment continuously increased to 54.6% and 96.7%, respectively. Fertilizer N loss through interflow accounted for 16.2%, higher than the fertilizer N loss ratio during the seedling stage, but similar to that during the pegging stage. This result may be due to the fast growth of the plants at this stage which led to increased absorption and utilization of fertilizer N. Influenced by the continuous migration of fertilizer N into deeper soils and the absorption and utilization by the plants, fertilizer N loss through surface runoff and erosive sediment during the ripening stage decreased compared to that during the pegging stage.

**Influence of interflow and surface-flow on fertilizer N loss.** Due to the separation of research on interflow loss (Matsushima et al. 2009) and surface-flow erosion loss (Våje et al. 1999) for nitrogen fertilizer loss, the incomprehensive evaluation of nitrogen fertilizer loss and its influence on water pollution has arisen. In this study, the fate of fertilizer N at different growth stages is shown in Table 2. The rainfall amount during the seedling stage was 60 mm. N losses through surface runoff, erosive sediment and interflow accounted for 1.1, 3.1, and 0.1% of the total fertilizer N, respectively. Due to the influence of the small peanut plants, the absorbed nitrogen content during the seedling stage only accounted for 1.5%. Most of the applied N became soil residuals or evaporated in gas forms, accounting for 94.2%. Another 60 mm of rainfall was replenished during the pegging stage. The N losses through surface runoff, erosive sediment and interflow all increased and the accumulated fertilizer N losses accounted for 3.4, 4.6, and 0.9% of the total applied N, respectively. During this period, the growth of the peanut plants increased

Table 2. Tracking the fate of fertilizer nitrogen (N) at different growth stages (kg/ha)

Loss pathways of fertilizer N	Seedling stage	Pegging stage	Ripening stage
Loss through surface runoff	0.11 ± 0.02	0.35 ± 0.03*	0.63 ± 0.01**
Loss through sediment	0.32 ± 0.19	0.48 ± 0.10*	0.57 ± 0.06**
Loss through interflow	0.01 ± 0.00	0.09 ± 0.01*	0.69 ± 0.35**
Uptake by crop	0.16 ± 0.11	0.84 ± 0.06	4.73 ± 2.94
Soil residue and gaseous volatilization	9.80 ± 0.10	8.63 ± 0.12	3.78 ± 2.55

\*indicates the total loss of two simulated rainfalls; \*\*indicates the total loss of three simulated rainfalls



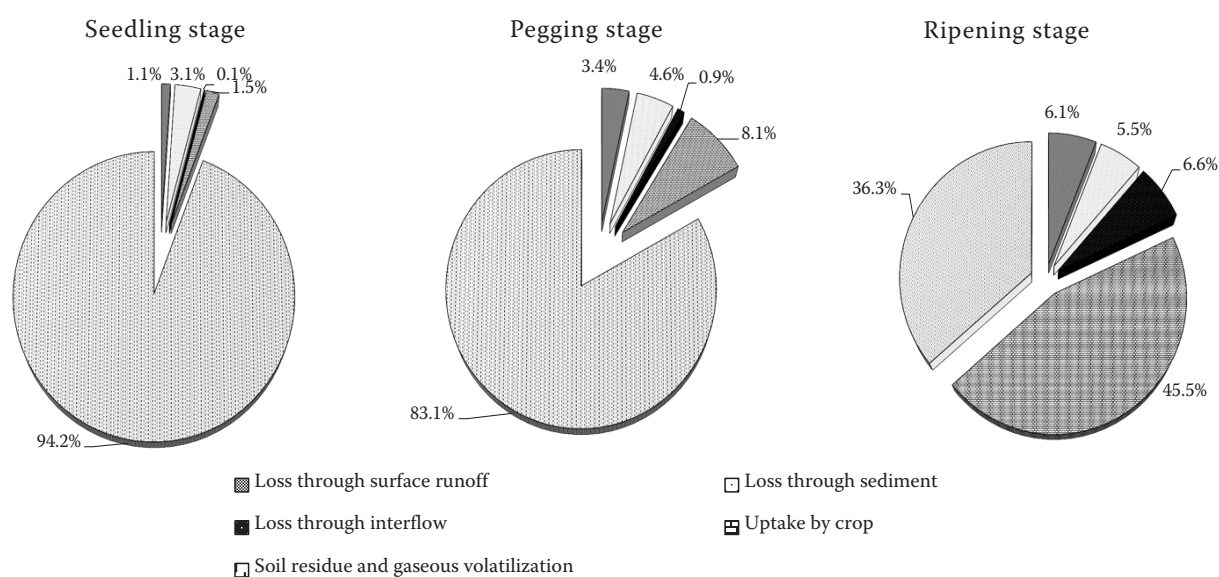


Figure 2. Distribution proportion of fertilizer nitrogen (N) at different growth stages

and the plant biomass significantly increased. N absorbed and utilized by the plants increased to 8.1%. Correspondingly, fertilizer N in the soil and gas evaporation decreased to 83.1%. With rainfall being replenished with further 60 mm during the ripening stage, N losses through surface runoff, erosive sediment and interflow significantly increased and the accumulated fertilizer N losses accounted for 6.1, 5.5, and 6.6%, respectively; influenced by the maximum plant biomass, N absorbed and utilized by the plants increased to 45.5% and fertilizer N in the soil and gas evaporation decreased to 36.3%.

During the whole peanut growth cycle in the experiment, fertilizer N loss through surface runoff, erosive sediment and interflow accounted for over 18% of the total applied N, which fell within the range of 16.2–39.0% in previous experiment results (Zhou et al. 2006). However, the rainfall simulations were relatively limited (a total precipitation of 180 mm) while rainfall in the ex-

periment region was substantially greater with an annual average of 1469 mm. Therefore, the actual nitrogen fertilizer losses through surface runoff, erosive sediment and interflow would be significantly higher than 18%. So, it was identified that interflow and surface-flow had a significant impact on N loss in the soil-plant system of the red soil sloping uplands.

**N absorption at different growth stages.** Absorbing nitrogen from soil and fertilizers is one of the main pathways for plants to obtain nitrogen (Reddy 1982). The total absorbed nitrogen contents and the absorptions from soil and fertilizer during each growth stage were calculated (Table 3). N accumulation in the peanut plants from the fertilizer increased from 15.3% at the seedling stage to 22.6% at the pegging stage before further increasing to 26.9% at the ripening stage. Correspondingly, soil N decreased from 84.7–77.4% (seedling to pegging stage), and then to 73.1% at the ripening stage. Therefore, during

Table 3. Uptake of nitrogen (N) by peanut plants at different growth stages

Item	Seedling stage	Pegging stage	Ripening stage
N accumulation in plant (kg/ha)	1.03 ± 0.14	3.96 ± 1.21	16.93 ± 2.753
Where from fertilizer (kg/ha)	0.16 ± 0.11	0.84 ± 0.06	4.73 ± 2.94
from soil (kg/ha)	0.87 ± 0.03	3.12 ± 1.28	12.21 ± 0.19
Utilization rate of fertilizer N (%)	1.6 ± 1.1	8.1 ± 0.6	45.5 ± 28.3

the whole peanut growth cycle, N absorbed by the peanut plants was mainly sourced from the soil, accounting for over 70%. This indicates that the nitrogen absorption from soil of peanuts is still higher than that from fertilizers. So, it is critical to adopt effective fertilization practices to maintain soil fertility at a relatively high level. At the seedling stage, due to the small size of the peanut plants, only a limited amount of fertilizer N was utilized by the plants. With the growth of the plants, the accumulated absorption of fertilizer N gradually increased and the absorption peak of fertilizer N emerged at the ripening stage. However, the overall utilization rate of nitrogen fertilizer during the whole growth cycle of the peanut plants was only 45.5%, indicating that the majority of fertilizers N were lost through runoff, sediment or gaseous evaporation, or it remained in the soil.

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