國立臺灣大學生命科學學院生態學與演化生物學研究所

碩士論文

Institute of Ecology and Evolution Biology
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Master Thesis

合歡山地區台灣高山田鼠(*Microtus kikuchii*)排遺對土壤氮的影響

Effects of Taiwan vole (*Microtus kikuchii*) feces on soil nitrogen at the He-huan area

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摘要

草食性動物取食植物，排出氮含量高且分解快的排遺，可以快速的將養分回歸到土壤，提供氮一個快速循環的途徑(fast cycle)，進而影響植物的生長與群聚的組成。大型草食性動物在生態系營養循環的作用已經有相當多的研究，但小型哺乳動物所扮演的角色相關研究較少。台灣高山田鼠(*Microtus kikuchii*)為草食性小型嚙齒動物，在玉山箭竹草原中，族群數量多而穩定，並有排遺集中形成「公廁」的習性。本研究想了田鼠排遺對高山草原土壤氮含量的影響，探討以下問題：(1)高山田鼠的氮輸出量；(2)高山田鼠公廁的分佈及動態；(3)高山田鼠公廁對土壤氮含量的影響；(4)田鼠排遺對植物凋落物分解的影響。我於 2007 年起在合歡山的玉山箭竹草原中進行田鼠族群及野外公廁調查，透過飼養來計算田鼠排遺量，並在實驗室與野外進行田鼠公廁的孵育實驗。結果顯示，田鼠的氮輸出量範圍在 0.33 ~ 0.41 kg N ha^{-1} $year^{-1}$；高山田鼠族群及公廁分佈具空間異質性，公廁數目大抵隨田鼠族群數目波動，對土壤氮時空分佈影響很大；公廁會增加土壤中可萃取性氮，尤其是植物可利用的無機態氮；公廁易分解的養分主要在一個月內釋出，對土壤的有效供應期限約為 1 個月；田鼠排遺提供土壤微生物較易取得的食物資源而使其活性增加，加速土壤有機質的分解，微生物扮演的角色尚須進一步的研究以了解。綜合而言，在土壤有機質含量很高，但是分解緩慢的高山生態系，田鼠公廁不僅提供養分，並能加速原來土壤中的有機質分解，對高山生態系而言，扮演相當重要的角色。

關鍵字：台灣高山田鼠、排遺、公廁、氮循環、分解

Abstract

Herbivore returns nitrogen to soil by defecating high-nitrogen wastes. The fast decomposition rates of feces provide a “fast cycle” for returning plant nitrogen to soil. The temporal production and spatial distribution of feces can affect the dynamics of nutrient availability in soil, and change plant community structures. The effects of feces were well-documented for large herbivores, but not herbivorous small mammals. The Taiwan vole (*Microtus kikuchii*) is the dominant herbivorous rodent in alpine meadow in Taiwan. They deposit large amount of feces at latrine sites. I want to investigate the effects of latrines on soil nitrogen. This thesis is divided into four aspects: (1) Nitrogen output of vole; (2) The temporal dynamics of vole latrines; (3) The effects of latrines on soil nitrogen; (4) The effects of latrines on plant litter decomposition. I conducted vole and latrine survey starting in 2007 at an alpine meadow in He-huan Mountain. Nitrogen output was acquired by rearing voles in the laboratory. I also conducted two field and one laboratory incubation experiments with latrines. The results showed that, annual nitrogen output of voles was 0.33~0.41 kg N ha^{-1} $year^{-1}$. Vole latrines increased the extractable nitrogen in soil, especially inorganic nitrogen. The release of nutrients from liable part of feces to soil occurred within one month. Latrines also provided liable carbon to microbes, increasing microbial activities and decomposition rates of soil organic matters. The spatial patterns of vole and latrine abundances were highly

heterogeneous in alpine meadow. The temporal dynamics of voles and latrines further increased the spatial heterogeneity of soil nitrogen. Alpine meadows had high soil organic matters, yet decomposition rates were low. Vole latrines not only quickly return nutrients back to soil, but also enhance decomposition rates of soil organic matters, thus play a crucial role in alpine ecosystems.

Keyword: decomposition, feces, latrine, *Microtus kikuchii*, nitrogen cycling, Taiwan voles

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Introduction

In many terrestrial ecosystems, plant growth is nitrogen limited (Aerts and Chapin 2000). The main nitrogen used by plants is inorganic nitrogen (NH_4^+ & NO_3^-). Yet, inorganic nitrogen content is usually very low in soil, and must be supplied from the mineralization of organic matters by microbes. Soil organic matter (SOM) is the largest nutrient reserve in grassland ecosystems. It contains more than 60% of C, N, and P within the ecosystem (Dubeux et al. 2007). Decomposition rate of SOM is affect by the quality of organic matters, content of lignin, N, P and C:N ratio, and the weather, temperature and moisture (Taylor et al. 1989, Semmartin et al. 2004). Factors that alter the quantity and quality of SOM can profoundly influence the nitrogen cycles in terrestrial ecosystems (Dubeux et al. 2006a, Dubeux et al. 2007).

Herbivores can have a strong impact on plants and environment in an ecosystem (Gibson 1989, Haynes and Williams 1993, Pastor et al. 1993, Sirotnak and Huntly 2000, Bakker and Olff 2003, Villarreal et al. 2008). Particularly, herbivores largely influence the quantity and quality of SOM, thus change the nitrogen turnover rate (Holland and Detling 1990, Pastor et al. 1993, Bakker et al. 2004, Semmartin et al. 2004, Fornara and Du Toit 2008). Over short term, herbivores remove standing vegetation, and contribute to litter buildup (Canals and Sebastia 2000, Bakker et al. 2004, Semmartin et al. 2004, Dubeux et al. 2006b, Fornara and Du Toit 2008). Herbivores also deposit high-nitrogen waste products (urine and feces). Furthermore, defoliation by herbivore grazing can quickly stimulate nitrogen uptake ability of roots (Seagle et al. 1992, Bardgett et al. 1998, Frost and Hunter 2007). The increased plant N content through herbivore grazing stimulation and animal excreta would decrease the C:N ratio of plants. The increased plant quality, in turn, could increase the quality of plant litter (Day and Detling 1990, Haynes and Williams 1993). High quality SOM has a faster decomposition rate, which

enhances nitrogen turnover (Holland and Detling 1990).

Over long term, soil disturbance (Inouye et al. 1987, Gibson 1989, Questad and Foster 2007, Villarreal et al. 2008) and selective foraging by herbivores could change plant community composition (Pastor et al. 1993, Sirotnak and Huntly 2000, Stark et al. 2002) thus SOM composition. For example, animal burrow can increase soil aeration. Burrows also mix upper and lower layer soil, redistributing nutrients and bringing organic matter into soil, which stimulate microorganism activity (Inouye et al. 1987, Gibson 1989, Canals and Sebastia 2000). Herbivores often selectively forage on high-quality plants (low C:N ratio), decreasing the abundance of those plants (Pastor et al. 1993, Sirotnak and Huntly 2000), increasing the proportion of low quality litter, which in turn decreases mineralization rates (Pastor et al. 1993, Sirotnak and Huntly 2000).

The nitrogen cycle in a grassland ecosystem could be described in a soil-plant-animal system (Fig. 1.) that has two sub-cycles (Haynes and Williams 1993, De Mazancourt et al. 1998, Bakker et al. 2004). In one sub-cycle, plants return nutrients to soil via litter. With slow turnover rates, most of the litter is added to the SOM compartment (Bardgett et al. 1998). Only part of the litter is decomposed, nutrients released and become readily available. It is named the slow cycle (Bardgett et al. 1998, Bakker et al. 2004). In the other sub-cycle, herbivores create a shortcut in the slow cycle by consuming plants. Herbivore wastes have a faster turnover rate, and contain more easily accessible nutrients, i.e., a fertilizer effect (Williams and Haynes 1995, Willott et al. 2000, Dubeux et al. 2007, Moe and Wegge 2008). It is name the fast cycle (Pastor et al. 1993, Bardgett et al. 1998, Bakker et al. 2004). In this research, I focused on investigating the effects of nitrogen return from feces, yet also examined the effects of feces on plant litter decomposition.

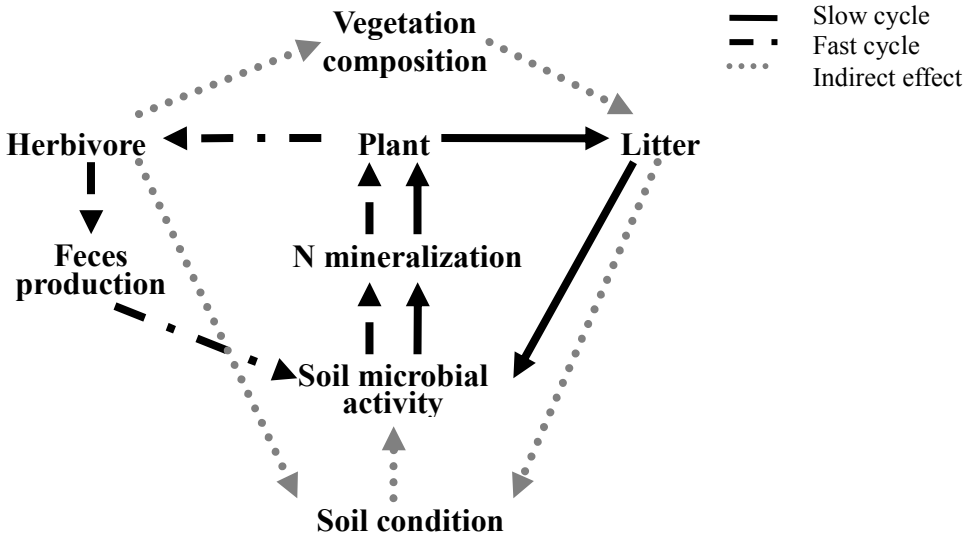


Fig. 1. The soil-plant-animal relationship in grassland ecosystems as adopted from Bakker et al. (2004).

Herbivores can return a large amount of nitrogen to soil via feces, e.g., cattle 1.3~4.7 kg ha^{-1} yr^{-1} (Schimel et al. 1986) and elk 4.9~45.6 kg ha^{-1} $year^{-1}$(Singer and Schoenecker 2003)(Frank et al. 2000). Nitrogen released from feces can create high nutrient concentration localized patches (Afzal and Adams 1992, Haynes and Williams 1993, Williams and Haynes 1995, Lovell and Jarvis 1996). For example, Willott et al. (2000) found that rabbit latrine increased 2-fold organic carbon concentration and 4-fold inorganic nitrogen concentration in soil beneath latrines, and decreased more than 30% root:shoot ratio of barley in laboratory culture experiment with latrine. Feeley (2005) also found that howler monkey latrine form a "fertile island" with increased 2~3-fold nitrogen concentration and 2~4-fold phosphate concentration in the soil.

Plants can quickly pick up nitrogen from feces, as supported by using stable isotope ^{15}N (Cochran et al. 2000, Frost and Hunter 2007). Not only do herbivore's feces have fertilizer effect but also carnivore's. River otter feed on high $\delta^{15}N$ value food (intertidal fish and invertebrates) and then feces had higher $\delta^{15}N$ value than plants (Ben-David et al. 1998). Plants growing in latrine site had higher $\delta^{15}N$ value than no latrine site. River otter transport N & P from water-to-land at latrine site supplying limited elements to plants (Ben-David et al. 1998, Ben-David et al. 2005, Crait and

Ben-David 2007).

Feces also contain a large amount of carbon. The amount of carbon released is often greater than that of nitrogen during decomposition (Pastor et al. 1993, Pastor et al. 1996). When SOM is high in soil, the readily available carbon may be very low (Hatch et al. 2000). Animal feces add fresh labile carbon to the soil, and can promote an increase in the biomass and activity of soil microbes (Afzal and Adams 1992, Williams and Haynes 1995, Cochran et al. 2000, Frank et al. 2000, Hatch et al. 2000). Several studies found that after animal dung were deposited, microbial biomass and activity were increased, but adding fertilizer N could not elicit the same response (Lovell and Jarvis 1996, Hatch et al. 2000). Feces can elicit more nutrient mineralization in soil. Pastor et al. (1993) found soil incubation with intact moose fecal pellet on it could mineralize more carbon and nitrogen than combining the mineralized amount of moose fecal pellet and soil incubation separately.

Most researches of feces fertilization focused on large herbivores, especially ungulates and cattle. Few focused on small herbivores. According to previous research, however, small herbivores could provide similar amount of nitrogen from feces as large herbivores. Clark et al. (2005) estimated annual nitrogen output from fecal, urinary, and total nitrogen by small mammals in Center for Subsurface and Ecological Assessment Research (CSEAR), 1.00 (0.91~1.05), 2.75 (2.55~2.95), and 3.73 (3.46~3.99) kg N ha^{-1} $year^{-1}$, respectively. Bakker et al. (2004) found after excluding large herbivore (cow) small herbivores (vole) return more N to soil through feces than large herbivores (cow) do. Pastor et al. (1996) found the potential mineralizable nitrogen in voles feces of Minnesota was 0.16 kg N ha^{-1} $year^{-1}$. Although the nitrogen from feces was much lower, the value was still higher than the moose on Isle Royale (0.006 kg N ha^{-1} $year^{-1}$). The vole's feces also had a faster decomposition rate than moose (vole, k = 0.69~1.73;

moose, k =0.025~0.191). The great high turnover rates of nutrients in small mammals' feces may affect seasonal availability of nutrients, although the amount was a very small portion of overall annual nutrient budgets of ecosystem.

Taiwan voles (*Microtus kikuchii*) often deposit large amounts of fecal pellets at the same sites, and form "latrines". Latrines are easily observable in the field. At the alpine meadows of the He-huan Mt., large- or medium-sized herbivores are scarce (吳 2004), the Taiwan vole is the most dominant small herbivore (Ho 2009). It provides a good opportunity for studying the effects of small herbivore feces on the N cycling of alpine ecosystems.

Research objectives

I aimed to reveal the effects of Taiwan vole's latrine on soil nitrogen in an alpine meadow. I focus on two questions. First, whether latrine can increase soil nitrogen content? Second, whether latrine can increase the decomposition rate of plant litter?

I approached the questions by measuring feces output of voles and nitrogen content of fecal pellets in the laboratory, and by monitoring the temporal and spatial dynamics of vole latrines. I created artificial latrines to perform field and laboratory incubation experiments.

Materials and Methods

Study Site

The study site was located at the He-huan Mountains (24°08'36.4"N, 121°17'17.4"E), ~ 3000 m in altitude, Nantou County, in central Taiwan. It was near the

western boundary of the Taroko National Park. Mean annual temperature was 7.0 ℃, and mean annual rainfall 3,500 mm based on weather information collected at the High-Altitude Station of the Institute for the Endemic Species Research, 5 km from our study site. The weather could be divided into wet (May ~ October) and dry (November ~ April) seasons, with sporadic snow during January to March.

The study site was an alpine meadow on a 30~45° slope facing east, surrounded by fir forests (composited of *Abies kawakamii* & *Tsuga formosana*). Yushan cane (*Yushania niitakayamensis*), alpine silver grass (*Miscanthus sinensis*), and *Carex spp.* were the three dominant plant species in the meadow. The local plant community was described in details by Ho (2009). The soil was acidic (pH 3.3 in $CaCl_2$), and described as "Typic Haplumbrept, fine, illitic, frigid" (King 1993).

Two Taiwan vole survey plots were set up at upper (plot A, H), middle (plot B, G), and lower (plot C, D) sections of the slope each (Fig. 3A) on October 2005 for a plant-vole interaction experiment (Ho 2009). The six sampling plots were at least 200 meters apart from each other. Each plot had three parallel trap lines ran perpendicular to the tree line jointing meadow and surrounding forest. Each line had seven trap stations, and formed a 7 × 3 trapping grid in each plot (Fig. 3). Trap lines and trap stations within each plot were 10 m apart.

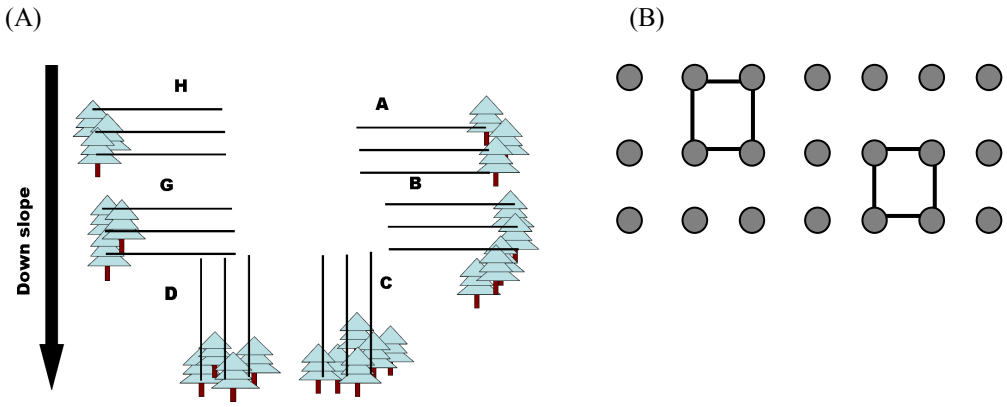


Fig. 2. (A) The six Taiwan vole survey plots on the slope of an alpine meadow. Each plot had three parallel trap lines ran perpendicular to the tree line jointing meadow and forest. The six plots were at least 200 meters from each other. (B) Each trap line had seven trap stations (solid circle). Trap lines and trap stations within each plot were 10 m apart. Two 10 × 10 m quadrats within the trapping grid were randomly selected to conduct latrine survey.

Nitrogen Output by Taiwan Voles

I used the methodology described in Clark et al. (2005) to estimate the nitrogen output of voles. I measured nitrogen contents of fecal pellets, and daily defecation rates of Taiwan voles. I also monitored vole population and latrine population dynamics.

Taiwan vole population survey

I conducted a population survey every two months from July 2007 to May 2009, except during the snowy season (December–February), to estimate the population sizes of Taiwan voles using the capture-mark-recapture method. The survey was a continuation of a plant-vole interaction experiment starting in October 2005 (Ho 2009). An Ugglan special live trap (250 × 78 × 65 mm) was placed at each trapping station. Each trap was baited with rolled oats mixed with peanut butter, provided with a ball of

shredded newspaper for warmth. During each trapping session, traps were checked twice daily at dawn (07:00~10:00) and nightfall (15:30~17:30) for three consecutive nights, with a total of 6 trap checks. When voles were captured, each individual was given a unique toe-clip for future identification. The following information of each individual was recorded: trap station, species, toe-clip ID, sex, reproductive condition (testes scrotal or abdominal for males; vagina perforated or non-perforated, and signs of pregnancy and nursing for females), health condition (occurrence of parasites and scars), and body weight. Animals were then released immediately at the station where they were captured. I added 15 m buffer zone to each side of trapping grid to calculate the effective trapping area which came to be 50×90 m^2. I applied the Pradel model in Program MARK (Cooch and White 2010) to estimate vole population abundance, and divided the value by 4500 m^2 to calculate density.

Daily defecation rates of voles

Five adult Taiwan voles of each sex (weighed $\geqq$ 28 g, pregnant females were excluded) were captured from an alpine meadow near the study site, brought back to the laboratory in the High-Altitude Station in July 2007, March 2008, April 2009, and January 2010. They were housed individually in standard rat cages (L×W×H: 40 × 25 × 12 cm), with 3-inch thick wood shaving bedding, maintained in a light regime similar to

the wild, and provided with fresh Yushan cane (*Y. niitakayamensis*) leaves and water ad libitum. (Ho 2009, Yeh 2010). Voles were maintained in such an environment for at least 12 hours before the fecal pellets collection trials started. Each vole was then weighed, and moved into a clear standard rat cage with 1 cm mesh wire at the bottom (a collecting cage). Water and fresh Yushan cane leaves were provided ad libitum for 24 hours. At the end of trial, voles were removed from cages, and released back to alpine meadow. I collected and weighted all fecal pellets (fresh weights) in the collecting cage. A small portion of each fecal sample was dried at 65℃ for 48 hours. Samples were then finely ground to analyze carbon and nitrogen contents. The rest of fresh fecal samples were stored in a -20 ℃ freezer, and later used in the field and laboratory incubation experiments.

Vole Latrine Survey

I randomly selected two 10 × 10 m quadrats within the trapping grid in each plot to conduct latrine survey (Fig. 3B). The survey period was conducted accompanying vole population survey (bimonthly, except January) during July 2007 to July 2008, except that plot G and H were not surveyed until September 2007. There were total of six surveys (5 in plot G and H). A vole latrine was arbitrarily defined as any vole fecal pile with more than 10 fecal pellets. Each latrine was marked with a flag with a unique

ID. I recorded the ID, location, and number of fecal pellets of each latrine during survey. I also graded the conditions by the % of a latrine that became disintegrated, whiten, or molded. I estimated the abundance, recruitment rate, and persistent rate of latrines at each plot using the Pradel model in Program MARK (Cooch and White 2010).

Natural Latrines

At July and September 07, I searched 5 vole latrines at the field in the same meadow of defecation rate experiment. I collected soil samples (a 10-cm diameter × 15-cm deep) beneath vole latrine and a random 1 m far paired control. I also collected the new leave of Yushan cane near each soil sampling site. Soil samples were divided into two parts: 0–5 cm and 5–15 cm in depth. I took approximately 150 g from each part, and passed them through a sieve (mesh no: 3'/2, 5.66 m/m). 10 g of each sample was treated with 100 ml 2 N KCl solution at 160 rpm/min for 1 hour in an orbital shaker. The extractant was filtered with Whatman No.1 filter paper (11 μm). The filtrate was then analyzed by the Kjeldahl Distillation Apparatus to estimate the amount of extractable inorganic nitrogen, and extractable ammonium. I then calculate extractable nitrate by subtracting extractable ammonium from extractable inorganic nitrogen. The rest of 150 g soil samples were air-dried, finely ground for measuring total Kjeldahl nitrogen. Cane leaves were washed with deionize water, oven-dried at 65℃ for 48 hours,

and cut into fine pieces. The $\delta^{15}N$ of cane leaves was analyzed by DELTA V Isotope Ratio Mass Spectrometer (Thermo Scientific®, USA)

Field Incubation on Natural Soil

In order to investigate the effects of latrines on soil nitrogen, I performed a field incubation experiment on natural soil in the field. I collected vole fecal pellets from traps during vole population survey on March 2007. Fecal pellets were pooled together, dried at 65℃ for 48 hours. In July 2007, I randomly selected two sites (at least 20 m apart) at each plot to conduct the incubation experiment. I made artificial latrines by placing fecal pellets, 0.5 g dried fecal pellets each, in tea bags. At each site, I placed two artificial latrines on the ground level, 1 m apart from each other, and covered each latrine with a 1-cm wire mesh to reduce disturbance. A total of 24 artificial latrines (6 plots × 2 sites × 2 latrines) were installed. I then collected a soil sample (a 10-cm diameter × 15-cm deep) serving as initial soil condition, near (~50 cm) each artificial latrine for soil nitrogen analysis. I retrieved one artificial latrine at each site every two months (September and November). One soil sample was taken from beneath each retrieved artificial latrine. A control soil sample was also taken randomly approximately 1 m away from the latrine.

Upon retrieval, tea bags were oven-dried at 65℃ for 48 hours. After drying, fecal

pellets were removed from tea bags and weighed. The soil samples were treated the same way describe above.

The results of the field incubation on natural soil showed high spatial heterogeneity of natural soils in nitrogen content, and likely rendered the results non-significant (see Results section). In order to avoid the problem of soil heterogeneity, I used sieved and homogenized soil in the following two incubation experiments: one in the field, the other in a climate-controlled chamber.

Field Incubation on Homogenized Soil

Preparing soil

Bulk of soil was taken from the study area in May 2008, and divided into black color A horizon and brown color B horizon. Each horizon was painstakingly passed through a sieve with 2 mm mesh to remove coarse roots and pebbles. The homogenized soil was stored at 4 ℃ before the incubation experiments started. Basic characteristics of the soil were shown in Table 1.

Table 1. The characteristics of soil from the study area used in the field and laboratory incubation experiments.

Soil Horizon	pH		Soil Texture			Organic Mater (%)	Bulk Density (g/cm^3)	Fine soil content (g/cm^3)	Maximum Water Holding Capacity (water weight/dry soil weight)
	Soil : Water	Soil : KCl	Sand (%)	Silt (%)	Clay (%)				
A	4.36	3.33	54.03	35.98	9.99	17.19	0.31	0.35	214.9%
B	4.51	3.61	41.02	41.99	16.99	9.31	0.38	0.67	157.8%

Experimental setup

I used PVC pipes with a 25 cm length, 5 cm inner diameter, 0.5 cm wall thickness to perform the field incubation. According to the fine soil content (Table 1), I filled each pipe with approximately 10 cm (221.33 ± 0.31 g) of B horizon soil (lower horizon), topped with 10 cm (108.58 ± 0.02 g) of A horizon soil (upper horizon), leaving 5 cm on top without soil. The bottom of each pipe was covered with a sheet of nylon cloth holding in place with rubber bands to prevent the loss of soil. Three experimental treatments were installed: Control (C, added no vole fecal pellets), Single (S, added 2.5 g of fresh vole fecal pellets), and Double (D, added 5.0 g of fresh vole fecal pellets). Fecal pellets were added on the top surface of soil. I covered the top of each pipe with a nylon mesh screen (2 mm mesh) to prevent additional organic matter from falling into the pipe. Each treatment had 24 replicates, with a total of 72 pipes. The 72 pipes were divided into six groups (blocks) of 12 pipes, 4 pipes from each treatment that were buried together as a group. I buried the 72 pipes into the ground 20 cm in depth in early September 2007. The tops of pipes protruded 5 cm from the ground surface, which served to prevent water runoff from upper slope into the pipes during storms. The soil surface in the pipes leveled the surrounding soil surface. The six groups were buried approximately 3 m from each other at the study site. The precipitation, and air and soil temperature during the incubation period was shown in Table 2. I retrieved one pipe per

treatment from each group monthly. Retrieved samples were stored at 4 ℃ immediately until analysis.

Table 2. Precipitation and air and soil temperature recorded at the High-Altitude Station of the Institute for the Endemic Species Research Center, 5 km from our study site. Values were daily averages (mean±1se) between two sample retrieving. For example, Sep. – Oct. gave the average value for the period between the first and second retrieving

Incubation period	Sep. – Oct.	Oct. – Nov.	Nov. – Dec.	Dec. – Jan.
Precipitation (mm)	48.0 ± 17.4	3.9 ± 2.0	2.4 ± 1.1	2.5 ± 1.3
Air temperature (℃)	10.3 ± 0.2	8.2 ± 0.4	2.6 ± 0.5	0.2 ± 0.4
Soil temperature (℃)				
10 cm depth	11.6 ± 0.1	10.3 ± 0.2	5.2 ± 0.3	2.5 ± 0.2
20 cm depth	11.1 ± 0.1	9.9 ± 0.2	4.8 ± 0.3	2.1 ± 0.2

Chemical analyses

The soil column was pushed out from each pipe. Fecal pellets, upper horizon soil, lower horizon soil, and upper-lower mixed layer were separated carefully, and weighed. Ten grams of soil samples of upper and lower horizon each were oven-dried at 105 ℃ for 24 hours to measure water content. Another portion of soil was air-dried for several days and finely ground to analyze the nitrogen, carbon, and soil organic matter contents (see below). Soil organic matter content was only analyzed for the first and last retrieved samples (October 2008 & January 2009) firstly. If had a significant different between treatments or times, and then analyzed the second and third retrieved samples. Microbial biomass C & N, extractable nitrogen, and extractable carbon, were measure by the chloroform fumigation-extraction method (CFE, see below). Because I couldn't completely separate soil particles from fecal pellets, I took some finely ground samples

of fecal pellets to analyze ash content by using Nabertherm. I used the ash content to estimate the proportion of soil in each fecal pellet sample.

Laboratory Incubation on Homogenized Soil

Preparing soil, feces, & plant litter

I examined the effects of decomposition of both vole latrine and plant litter on soil nitrogen in this experiment. The soil and vole fecal pellets I used were the same batch as the one prepared for the field incubation described earlier. I used Yushan cane leaves to represent plant litter because Yushan cane was the most dominant ground cover at the study site (Ho 2009), and its leaves was the major component of ground litter. In December 2008, I picked the upper most litter layer of Yushan cane leaf litter from the ground surface. In addition, I also picked entirely brown withering leaves from randomly chosen Yushan canes. I collected a total of 1 kg of leaves, about half from each source. Leaf litter was mixed thoroughly, then oven-dried at 45 ℃ for 48 hours. I picked out intact leaves, and stored them at 4 ℃ before the incubation experiment started.

Experimental setup

The laboratory incubation was conducted in the Soil Chemistry Laboratory of Dr.

Ciao-Ping Wang of the Forest Research Institute in Taipei, Taiwan. The microcosms used in incubation were acrylic tubes with a dimension of 30 cm height, 12 cm inner diameter, and 0.5 cm wall thickness. The tube had airtight lids with silicon seal on both the top and bottom. The top lid had an irrigation hole (2 cm in diameter) in a sleeve stopper as well as two small airflow holes. The bottom lid had a hole for collecting water percolated through the tube. The water was guided to a collecting bottle with a -150 ~ -200 hPa suction force. The microcosms were set up using the following steps. After securing the bottom lid on the tube, a nylon mesh (1 mm mesh) was laid inside the tube at the bottom to provide an even distribution of suction pressure, followed by an ash-free filter paper (Schliecher & Schnell Grade 42: 2.5 μm) and a sheet of nylon cloth (0.1 μm pore size). Both the nylon cloth and filter paper kept soil particles from entering the water collecting bottle. A layer about 1 cm in height (100 g) of purified quartz powder was added as a buffer zone between soil and filter paper. According to the results of field incubation (see Results section), the effects of vole latrines occurred mainly in A horizon soil. Thus, I only used A horizon soil in the laboratory incubation. I put in 787.81 ± 0.04 g (10 cm in height, the A layer is about 10 cm thick in the field) A horizon soil to the tube, leaving a headspace of approximately 1.5 l. Microcosms were kept in dark at 4 ℃ in a walk-in chamber before all incubation setup was ready. A total of 32 microcosms were prepared. Experimental treatments were installed in a 2 × 2

(fecal pellet × leaf litter) factorial design: Control (C, added no vole fecal pellets or Yushan cane leaves), Litter (L, added 2.0 g Yushan cane leaves), Feces (F, added 11.0 g fresh vole fecal pellets), Feces and litter (F+L, added 2.0 g Yushan cane leaves then 11.0 g fresh vole fecal pellets). All the vole fecal pellets and Yushan cane leaves were put on the soil surface. Each treatment had 7 replicates arranged randomly in the chamber. After all treatments were ready (August 2010), the chamber temperature was raised to 12℃, similar to the average air temperature in July at the He-huan Mt. field study site. Four microcosms served as initial condition samples. Water was sprinkled into microcosms daily to simulate rainfalls. The amount of water added was based on precipitation in 2007, 2008, & 2009 at the field site. That was, an average of 10.94 mm per day, or 7.03 mm per day after excluding storms following typhoons. I used the average value, 9 mm per day, to simulate rainfalls because in the field storm rainfalls would runoff and not all the water would percolates through the local soil. Given the 6 cm × 6 cm × π transaction areas of the microcosms, I added 100 ml pure water daily to simulate rainfalls using a full-cone irrigation jet made of polyvinyliden-fluorid to ensure a symmetrical water distribution. The headspace was constantly flushed with outdoor air through the airflow holes at a rate of 33~41 ml/min.

Previous studies indicated that mineralization rate was faster at the initial than later stages. In order to monitor the mineral condition at the initial stage, leachates

(water percolated through soil) were collected at two-day intervals in the first two weeks. Afterwards, the collecting intervals were changed to four days. I analyzed the following parameters: $ammonium_{(water)}$, $nitrate_{(water)}$, (the subscripts are to distinguish them from ammonium and nitrate measured in soil extractant), total water soluble nitrogen, and water soluble carbon in leachate. CO_2 evolution rate was measured (see below) before irrigation at the 2 or 4 days intervals. The incubation ended after 62 days, with a total of 20 collecting days. At the end of incubation, three microcosms were randomly selected from each treatment to measure soil, feces, and leave chemistry. All remaining fecal pellets and leaf litter were carefully retrieved and oven-dried at 45 ℃ for 48 hours. The dried fecal pellets and leaf litter were finely ground, their C and N contents analyzed. Soil columns were divided into upper layer & lower layer by 5 cm. A portion of each soil sample was air-dried several days and finely ground to determine C and N contents. Another portion of each soil sample was oven-dried at 105 ℃ to analyze water content. Microbial biomass C & N, extractable nitrogen, and extractable carbon were analyzed as in the field incubation on homogeneous soil.

I used the values of control as the baseline values of N & C dynamics of other treatments. That is, I would subtract the baseline value from the corresponding observed value, and use the difference to reveal treatment effects. I calculated the relative leaching rate of nitrogen in leachant (relative ammonification rate for $ammonium_{(water)}$

and relative nitrification rate for $nitrate_{(water)}$) as the accumulated values difference between treatment and control then divided by the sampling day, average accumulation rate at each sampling day (Value t+1 minus t, divided by # of days).

As additional references, I also measured the amount of K, Na, Ca, Mg, and P content in leachates, fecal pellets and leaf litter. Since those parameters had less to do with my research goal, I report them in an Appendix, and did not discuss their roles in relation to my current study.

Chemical analysis

Soil microbial biomass, extractable nitrogen, extractable carbon

The CFE method (Brookes et al. 1985) was used to measure the microbial biomass C and N (abbreviated as C_{mic} and N_{mic}). Water content of samples were adjusted to 40~60% of maximal water holding capacity. For each soil sample, 40 g of soil was taken and added to two bottles each, one was control (unfumigation), and the other fumigation. The unfumigation samples were directly extracted with 100 ml 0.5 M K_2SO_4 at 200 rpm/min for 30 min in an orbital shaker. Suspensions were filtered (Schliecher & Schnell 589^3 blueband) and stored in the freezer. The fumigation samples were bathed in chloroform vapor for 24 hours in a desiccator. The desiccator had a cup of approximate 15 ml alcohol-free chloroform and zeolites in it, and was vacuumed to

bring the chloroform to boil for over 30 sec to create chloroform vapor. After fumigation, the samples were moved to a clear desiccator, and then vacuumed several times to remove excessive chloroform. The following extraction steps were the same for both the unfumigation and fumigation samples. The concentrations of carbon and nitrogen in soil extractants of both field and lab incubation and leachates of lab incubation were analyzed by the same method. Carbon concentration was analyzed by a Hochtemperatur-TOC-Analysator liquiTOC (Elementar Analysensysteme GmbH, Hanau, Germany). The concentrations of nitrate, ammonium, total nitrogen and organic nitrogen (total nitrogen – (nitrate + ammonium)) were analyzed by the colorimetric method using a Flow Injection Analyzer (Lachat QuickChem 8000 series, Milwaukee, WI, USA). C_{mic} and N_{mic} were calculated from the following equations:

$$C_{mic} = E_C / k_{EC}$$
$$N_{mic} = E_N / k_{EN}$$

Where:

E_C = (total organic C)$_{fumigated}$ – (total organic C)$_{unfumigation}$
E_N = (total organic N)$_{fumigated}$ – (total organic N)$_{unfumigation}$
k_{EC} = 0.45 (Joergensen 1996)
k_{EN} = 0.54 (Joergensen and Mueller 1996)

C and N content

Some soil was taken from each sample and air-dried for several days. Fecal pellets and leaf litter samples were oven-dried at 45 ℃ for 48 hours. All samples were finely

ground. I used dry combustion method to analyze carbon and nitrogen by an Elemental Analyzer, (EA, Thermo Finnign NA1500, Bremen Germany).

Organic matter content of soil

I took 0.15 g A horizon soils and 0.25 g B horizon soil from the field incubation, and used Walkley-black wet oxidation method to analyze organic C content, then calculated the organic matter content. I mixed soil samples with 0.167 M (1 N) potassium dichromate, added concentrated sulfuric acid (free of chloride) slowly, and swirled the bottle. I waited the solution to cool, and then added 200ml deionize water to halt the reaction. I added conc. phosphoric acid after the samples had cooled, then added several drops of Indicators (o-phenanthroline-ferrouscomplex, diphenylamine) and titrated with ferrous sulfate.

Organic matter content (%) was calculated as following:

$$10 \times (1- (S/B)) \times 1.0 \times (12/4000) \times (1.724/0.77) \times 100/\text{soil weight (g)}$$

Where:

S: Ferrous sulfate titration volume of sample (ml)
B: Ferrous sulfate titration volume of blank (ml).
1.0 is concentration of $K_2Cr_2O_7$ (N)
1.724 is the Van Bemmelen factor (transfer coefficient of organic carbon to organic matter).
0.77 is the recover rate.

CO_2 evolution rate

I shifted the air flowed into microcosms from outdoor airflow to commercial standard air for 40 min. I then shut the airflow valve, and let CO_2 accumulated in microcosms. I took 10 ml gas from the microcosm headspace using syringe twice at the 4th and 120th min each after shut the airflow valve. The sampling sequence of microcosms was random. Gas samples were analyzed by an HP gas chromatograph, with thermal conductivity detector (TCD). The column is an HP-PLOT Q (polystyrene-divinylbenzene (DVB)), used helium as the carrier gas. CO_2 evolution rates were calculated from the following equations:

$$(CO_{2\,(120\ min)} - CO_{2\,(4min)}) \times 1.5\ l / (120 - 4\ min)$$

Concentration headspace volume the time between two samplings

Statistical Analyses

The numbers of male and female voles were pooled among plots, and compared by paired t-test. I used the Kruskal-Wallis tests to examine the effects of month (season) on the daily defecation rates, and the nitrogen & carbon contents of fecal pellets. For natural latrine and field incubation experiment on natural soil, I compared the paired-treatments: with and without latrine using non-parametric Wilcoxon signed rank tests (Shen 2007) on all parameters measured. For the field incubation experiment on homogenized soil, I performed repeated-measure ANOVAs with blocks to test the

effects of fecal pellet quantity over 4 time periods. The six groups buried apart were treated as blocks. I also performed one-way ANOVA to examining the effects of latrine in each month, and used Duncan pairwise comparisons for post hoc comparisons. For the laboratory incubation experiment on homogenized soil, I used the repeated-measure two-way ANOVAs to determine the effects of vole feces, plant litter over time, and their interactions on concentrations of nitrogen and carbon in leachate.

I used chi-square tests to examine main treatment effects on the extractable C & N, C_{mic} & N_{mic}, and C & N content of soil after 62 days of incubation (Shen 2007). I used Kruskal-Wallis tests to examine the interaction between latrine and leaf litter by comparing the concentration of nutrients in fecal pellets between the F and F+L, and in leaves litter between L and F+L.

Results

Taiwan vole population survey

In addition to the Taiwan vole (*M. kikuchii*), several other species of small mammals were caught during 2005~2009 including, Formosan mouse (*Apodemus semotus*), white-bellied rat (*Niviventer culturatus*), Taiwanese long-tailed shrew (*Episoriculus fumidus*), Taiwanese mole shrew (*Anourosorex yamashinai*), and Formosan least weasel (*Mustela nivalis formosanus*). Taiwan vole was the most

dominant small mammal and herbivore at the study site as reported by Ho (2009). The population dynamics of Taiwan voles at the six sampling plots over three and half years were shown in Fig. 3. Taiwan vole populations showed spatial and temporal heterogeneity at the study site. Population densities not only differed among plots, they fluctuated in 2~3 folds magnitude over time at some plots. Generally, there were more females than males (paired t-test, $p < 0.01$, 17.25 ± 1.03 females and 13.28 ± 1.07 males per hectare, Fig. 4).

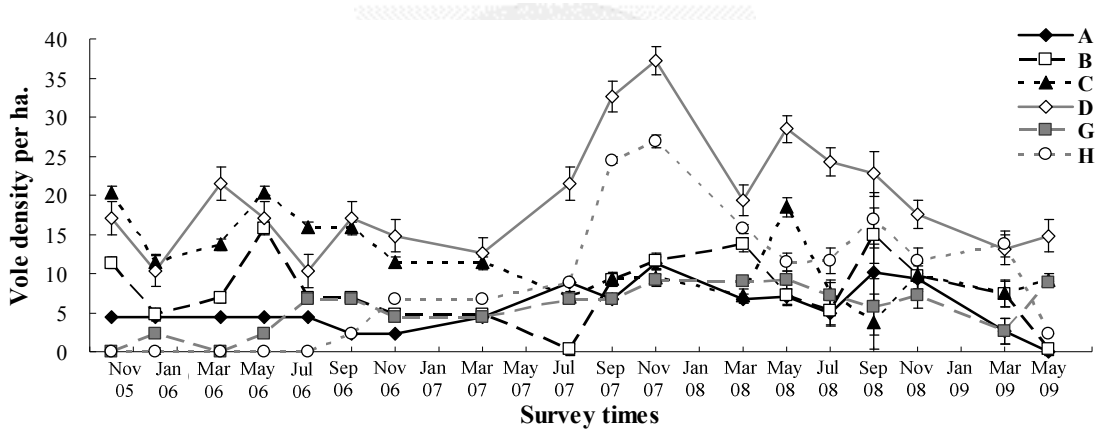


Fig. 3.Vole density (number per hectare) at each sampling plot from Oct.2005 to May 2009, estimated with the Pradel model in Program MARK. Error bars represent ±1se.

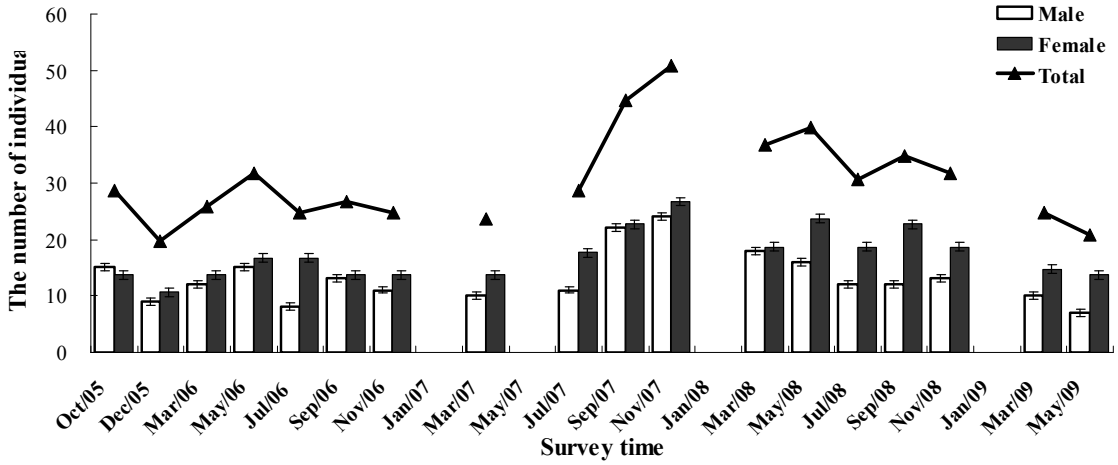


Fig. 4. The number of male and female voles from Oct.2005 to May 2009, estimated with the Pradel model in Program MARK. The error bars represent ± 1se.

Daily defecation rates of voles

Daily defecation rates ranged from 5.16 to 9.41 g per day per vole. There was no difference between male and female voles (Mann-Whitney U test, U = 150, $p = 0.75$) within each month, samples were pooled between sexes. Voles defecated more feces in cold (Jan.-10 and Mar.-08) than warm (Apr.-09 and Jul.-07) months (Kruskal-Wallis test, H = 18.07, *d.f.* = 3, $p < 0.01$; Fig 5). The March-08 sample was lost due to preservation problem before chemical analyses could be performed. Carbon content per gram feces in Apr.-09 was higher than Jul.-07 and Jan.-10, although nitrogen content per gram feces was not different between months (Table 3). The daily total nitrogen output (Kruskal-Wallis test, H = 9.79, *d.f.* = 2, $p = 0.007$; Table 3) and total carbon output (H = 9.30, *d.f.* = 2, $p = 0.01$) per vole were both higher in Jan.-10 than other months caused by differential fecal output.

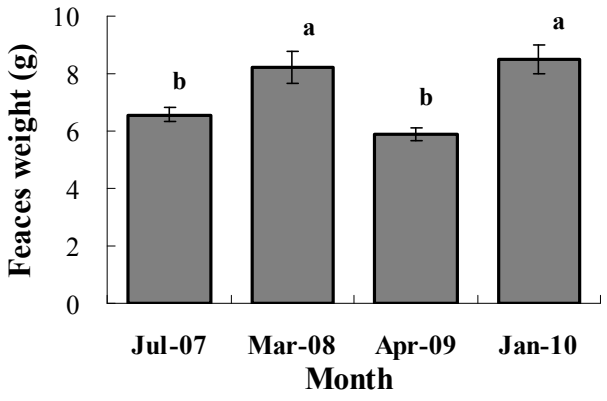


Fig. 5. Daily defecation rates (mean±1se) of voles in different month/year. Different alphabets indicate significant differences based on Kruskal-Wallis test. n = 12, 10, 10, 5, left to right, respectively.

Table 3. The N and C content in vole feces (mg/g), and total N and C output of vole feces per vole produced after 24 hrs in the laboratory. All values give mean±1se.

Month/year	n	N content (mg/g)	C content (mg/g)	C:N	Total N output (mg)	Total C output (mg)
Jun-07	11	11.32± 0.42 [A]	406 ± 2.16[B]	36.2 ± 1.17 [A]	73.4 ± 2.98[B]	2672 ± 108[B]
Mar-08		N/A[b]	N/A	N/A	N/A	N/A
Apr-09	4	13.5 ± 1.02 [A]	427 ± 3.06[A]	32.2 ± 2.28 [A]	74.6 ± 2.90[B]	2505 ± 97.4[B]
Jan-10	7	12.1± 0.81 [A]	403 ± 1.05[B]	34.4 ± 2.31 [A]	101.3 ± 6.07[A]	3417 ± 204[A]
p^a		0.190	0.006	0.349	0.007	0.01

a. Different alphabets in a column indicate significant differences based on Kruskal-Wallis test.
b. The Mar-08 sample was lost due to preservation problem.

Nitrogen output of vole populations

Yeh (2010) observed that Yushan cane at the study site showed little growth and had low quality for voles during November to March. I used the average of feces data (Table 3) from January-10 and March-08 to represent non-growing season; and the average of April-09 and July-07 to represent growing season. I multiplied daily defecation rate by the nitrogen content of feces to obtain daily nitrogen production per individual vole for each season. The values were multiplied by monthly vole density estimates, then by 30 (days) to give monthly nitrogen output by vole feces per hectare (Fig. 6). The values were influenced largely by vole population sizes, ranged from 0 to 114 g ha^{-1} $month^{-1}$. The annual nitrogen output per Taiwan vole came to be 32.45 g $year^{-1}$.

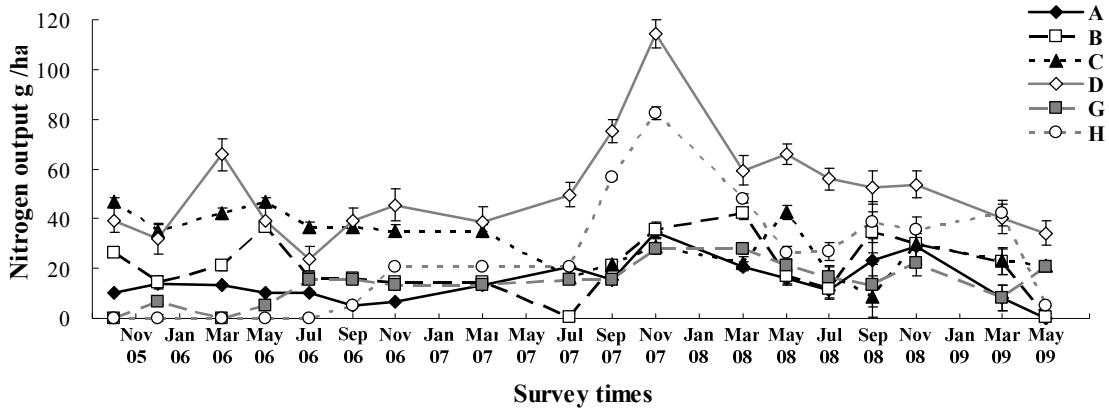


Fig. 6. Nitrogen output of voles in different months. Nitrogen output was calculated by vole density of survey month multiplied by vole daily nitrogen output. Error bars represent ±1se.

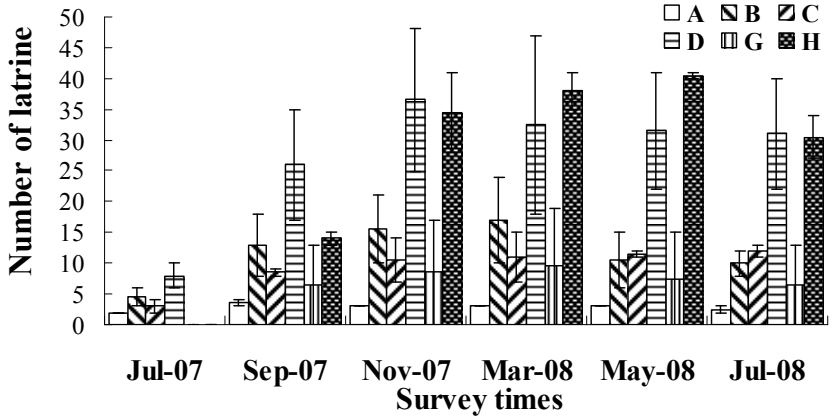


Fig. 7. Number (mean±1se) of vole latrines per 100 m^2 in each plot. The values gave averages of the two quardrats in the same plot. Latrine survey quadrats at plot G and H were not set up until September 2007.

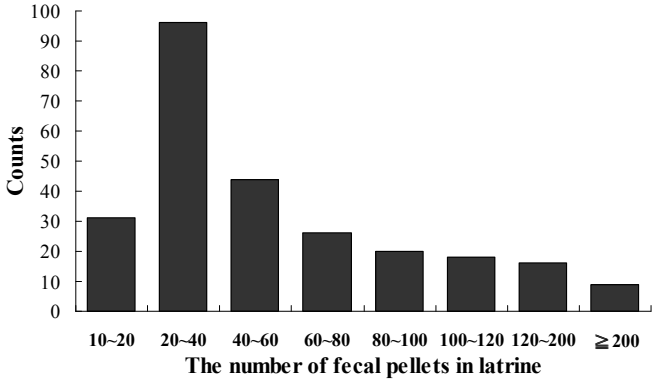


Fig. 8. Frequency distribution of the number of fecal pellets in latrines (n = 263). The number of fecal pellets in latrines ranged from 10 to approximately 700.

Vole latrines survey

I recorded a total of 263 vole latrines during July 2007 to July 2008. The dynamics of latrine numbers showed substantial spatial heterogeneity (Fig. 7). The fecal pellets in latrines ranged from 10 to approximately 700, and mostly between 20~40 (Fig. 8). I defined "active latrines" as newly recorded latrines and those that new pellets had been added to old latrines since last survey (Table 4). The numbers of active latrines ($r = 0.78$, Fig. 9A) and total latrines ($r = 0.69$, Fig. 9B) were both highly positively correlated with the number of voles. The survival rates of latrines, i.e., the percentages of latrines persisted between surveys were generally over 80% (Fig. 10A). After excluding pre-existing latrines, and those persisted beyond the final survey, average persistent time of latrine was 6.82 ± 0.29 months ($n = 89$). It's certainly an underestimation, for example, thirty-one latrines persisted for more than 1 year. The inclusion of those latrines would bring the average persistence time of latrine to 8.36 ± 0.18 months ($n = 120$). The recruitment of new latrine was the highest during July to September; and the lowest during November to March (Fig. 10B & Table 5). The reuse rates of latrines ranged from 11.1~57.1% (Table 5). The dispersion pattern of latrines in each quadrat was all random (based on spatial analyses, results not shown), but the numbers of latrine between quadrats were highly variable.

Table 4. The number of voles and latrines, both active and total, during bimonthly surveys. Active latrines were newly recorded latrines and those that new pellets had been added to old latrines. Total latrines were all latrines recorded, including non-active ones.

Plot	A				B				C				D				G				H			
	Vole	Active +	[a] new	Total	Vole	Active +	new	Total	Vole	Active +	new	Total	Vole	Active +	new	Total	Vole	Active +	new	Total	Vole	Active +	new	Total
Jul/07	4	-	-	4	0	-	-	9	3	-	-	6	10	-	-	16	3	-	-	-	4	-	-	-
Sep/07	3	2	3	7	4	1	17	26	4	1	11	17	15	3	36	52	3	-[b]	-	13	11	-	-	28
Nov/07	5	0	0	6	5	3	5	31	4	4	9	21	17	17	11	73	4	5	4	17	12	4	31	69
Mar/08	3	3	0	6	6	5	3	34	3	4	0	22	9	11	1	65	4	3	2	19	7	16	9	76
May/08	3	1	1	6	3	5	0	21	8	12	3	23	13	27	0	63	4	7	0	15	5	20	11	81
Jul/08	2	2	0	5	2	12	0	20	3	13	6	24	11	36	0	62	3	5	0	13	5	14	3	61

a. "+" indicates the number of active latrines due to new fecal pellets.
b. Latrine survey quadrats at plot G and H were not set up until Sep-07.

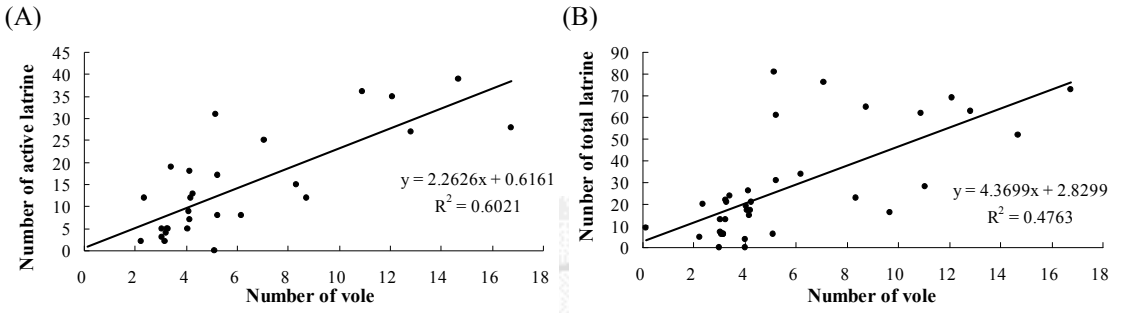


Fig. 9. The relationships between the number of voles and the number of (A) active or (B) total latrines.

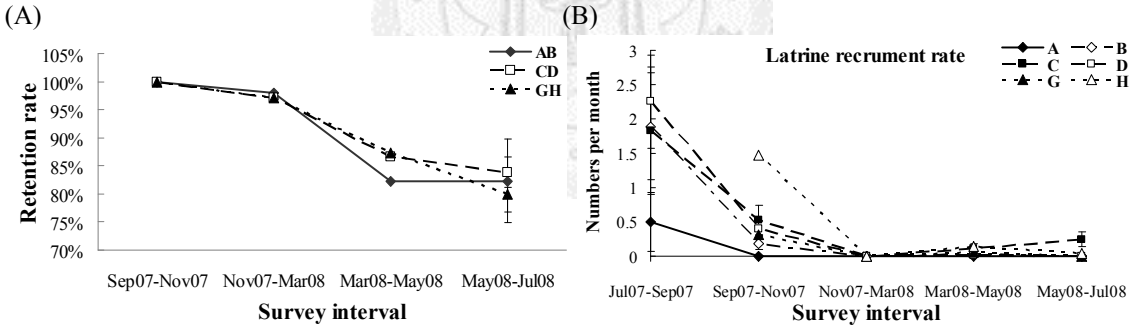


Fig. 10. (A) The percentages of latrines that existed in the beginning of survey that persisted between surveys. (B) Latrine recruitment rates, (the number of new latrines occurred between surveys, in each plot, estimated using Program Mark). Latrine survey quadrats at plot G and H were not set up until September 2007. Error bars represent ±1se.

Field Incubation on Natural Soil

The initial N contents of soil (collected in July 2007) were quite variable spatially (Fig. 11A), ranged from undetectable (concentration under 0.5 μg/g was not detectable by the method used) to 41 μg/g. After two months, the N contents of soil underneath artificial latrines and control soil (collected in September 2007) were not significantly

different (Wilcox signed rank test, NH_4^+, $p = 0.27$; NO_3^-, $p = 0.96$, inorganic N, $p = 0.66$, Fig. 11B). Also, concentrations of ammonium and inorganic N were under the detectable level. In November 2008, almost all samples were under detectable level (data not shown). The weights of fecal pellets in the artificial latrines declined to 65.50 ± 1.18 % and 59.70 ± 1.61 % in September and November 2007, respectively.

Natural Latrine

At the natural latrine site, although there were no values under detectable limit, there was no difference between the soil samples under latrine and control (Paired t-tests, July-07 NH_4-N, $p = 0.35$; inorganic N, $p = 0.66$; TN, $p = 0.35$; Sep.-07 NH_4-N, $p = 0.23$; inorganic N, $p = 0.08$, Fig 12.). The $\delta^{15}N$ of Yushan cane leaves were not different between latrine and control soil (control: -4.45 ± 0.36; latrine: -4.45 ± 0.37; $p = 0.89$). The no-latrine (control) soil sample sometime had a higher N content than the one had latrine. It might be caused by the spatial heterogeneity of alpine soil.

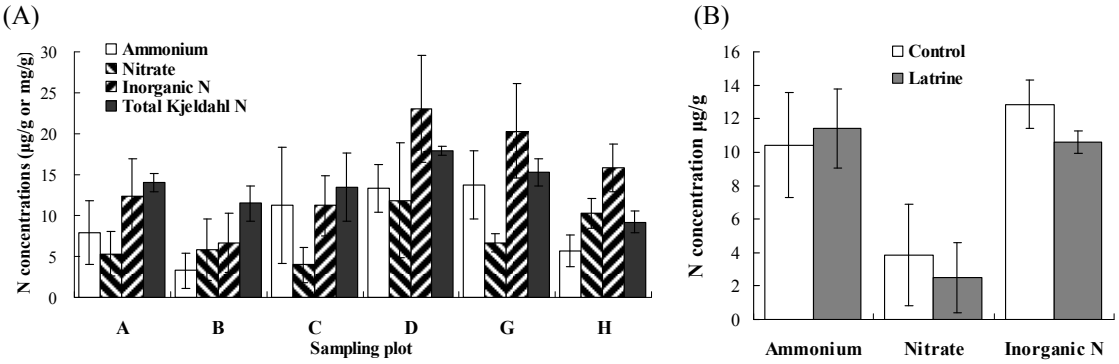


Fig. 11. Nitrogen contents (mean±1se) of soil at the study site. (A) Initial N content measured in July 2007 when the incubation experiment started. n = 4 per plot. (B) Inorganic N concentrations of soil underneath artificial latrines and control measured in September 2007. n= 12. All concentrations were in μg N/g soil, except that total Kjeldahl N was in mg N/g soil.

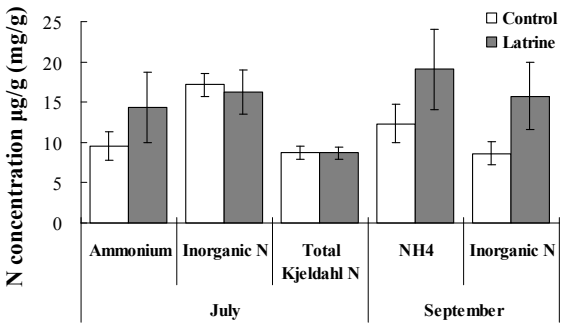


Fig. 12. Nitrogen concentration of soil under natural vole latrine and control. The soil did not analyzed TN at Sep. The error bar represented ± 1 se, n = 5. All concentrations were in µg N/g soil, except that TN was in mg N/g soil.

Field Incubation on Homogenized Soil

There were significant group (block) effects on several measurements (Table 6), and indicated spatial variations in environmental conditions where different groups were buried. All measurement varied with time, except C:N ratio of soil (Table 6). C:N ratio of soil was quiet stable with time. Treatment had different effect at different level (extractant, soil and soil microbes, Table 6, Fig. 13~16). Latrine treatment increased all the N concentration in extractant, but no effect on extractable C. Only microbial biomass N increased after latrine added in, but the microbial biomass C and microbial C:N ratio not changed. Conversely, soil C content was increased with latrine treatment, but N content and C:N ratio of soil were no changed. After one month (September to October) of incubation, latrine significantly increased the entire extractable N in upper horizon, and nitrate and inorganic N in lower horizon soil (Table 6, Fig. 13). Generally, double amount of fecal pellets (treatment D) had greater effects than single amount (treatment S) in elevating soil nitrogen, except organic N of upper horizon. After two months of incubation, extractable inorganic N of both horizons still differed among treatments, likely caused by the increasing nitrate from nitrification (Fig. 13 C, D, E,

and F). No latrine effect was found in the upper horizon after three months, but the nitrate in the lower horizon was still higher than control even after four months. Overall, extractable inorganic N was approximately 1% of soil total nitrogen. Even extractable TN was just 2~3% of soil total nitrogen. Whereas, microbial biomass N was 6~8 % of soil total nitrogen content, more then that of extractable TN (Table 5). Microbial biomass C was also a magnitude higher than extractable carbon (Table 5). Thus, the N and C in microbial biomass were important in alpine soil. There was a sudden decline in microbial biomass C in both horizons in January 2009 (the 4th month of incubation), but not in microbial biomass N (Fig. 15 A, B, C, and D). C:N ratio of microbes remained relative constant in the first 2 months, yet dropped in the later 2 months (Fig. 16 E and F).

The fecal pellets on the soil surface lost little weights between the first and second months. At the end of incubation, fecal pellets maintained > 60% initial weight (Fig. 17). N content had little increase ($F = 6.85$, *d.f.* = 3, $p = 0.013$, Fig. 18A), but C contents did not change significantly during incubation ($F = 3.68$, *d.f.* = 3,$p = 0.06$, Fig. 18B). Fifty-five percent of the initial total nitrogen in fecal pellets remained at the end of incubation, and nearly all the lost nitrogen was gone within the first month (September ~ October, Fig. 19A). The slight increases in TN at the second and third months were likely caused by microbial immobilization. Similarly, most of the loss of total carbon

occurred in the first month (Fig. 19B). The sharp decline occurred between December and January might have been related to the decline of microbial biomass C in soil. The ash content of fecal pellets was 11.05 %.

After one month of incubation, the nitrogen lost from latrines was 7.65 ± 0.26 mg (47.6%) and 15.36 ± 0.27 mg (47.8%) for the single and double fecal pellet treatments, respectively. The increased of extractable TN in upper horizon was 6.94 ± 0.57 mg (62%) and 15.64 ± 0.45 mg (140%) for the single and double treatments than control, respectively. No any significantly increasing was found in addition to extractable N. The total increased amount of N at upper horizon approximates the amount released from fecal pellets.

Table 5. The proportions of extractable N and C to the total N and C in field incubation on homogenized soil. There were 3 treatments: Control (no vole fecal pellets), Single (added 2.5 g of fresh vole fecal pellets), and Double (D, added 5.0 g of fresh vole fecal pellets).

	Treat	$NH4^+$	$NO3^-$	Inorganic N	Extractable organic N	Extractable TN	N_{mic}	Extractable C	C_{mic}
Sep	**Initial**	1.70	0.03	1.72	1.07	2.80	7.70	0.34	3.03
	Control	0.68	0.05	0.73	0.86	1.59	8.80	0.40	3.54
Oct	**Single**	1.20	0.23	1.43	1.07	2.50	8.43	0.39	3.52
	Double	1.90	0.53	2.44	1.20	3.64	7.77	0.36	3.56
	Control	0.49	0.09	0.58	0.82	1.40	7.50	0.41	3.35
Nov	**Single**	0.16	0.66	0.82	0.77	1.59	7.62	0.43	3.17
	Double	0.18	0.96	1.14	0.89	2.03	7.14	0.43	3.05
	Control	0.24	0.18	0.42	0.86	1.28	6.79	0.34	3.00
Dec	**Single**	0.07	0.22	0.30	0.84	1.14	6.65	0.36	3.09
	Double	0.08	0.41	0.50	0.80	1.30	6.50	0.35	3.19
	Control	0.26	0.14	0.41	0.67	1.07	7.74	0.47	2.17
Jan	**Single**	0.07	0.12	0.19	0.66	0.85	8.23	0.47	2.03
	Double	0.09	0.23	0.32	0.68	1.00	7.60	0.50	1.78

Table 6. Results of two-way randomized complete block design ANOVAs of field incubation on homogenized soil. There were 3 treatments: Control (no vole fecal pellets), Single (added 2.5 g of fresh vole fecal pellets), and Double (D, added 5.0 g of fresh vole fecal pellets). Each treatment had 24 replicates, with a total of 72 incubation pipes. The 72 pipes were divided into six groups of 12 pipes, and each group had 4 pipes from a treatment. Effects tested include group, treatment (Txt), and retrieving time

Soil Layer	**Upper (A horizon)**										**Lower (B horizon)**									
Effects	**Group**		**Treatment**		**Time**		**Time × Txt**		**Time × Group**		**Group**		**Treatment**		**Time**		**Time × Txt**		**Time × Group**	
	d.f. =5		*d.f.* =2		*d.f.* =3		*d.f.* =6		*d.f.* =10		*d.f.* =5		*d.f.* =2		*d.f.* =3		*d.f.* =6		*d.f.* =10	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Ammonium	0.94	0.496	10.8	**0.003**	112	**<.0001**	15	**<.0001**	22.49	0.868	2.64	0.09	30.17	**<.0001**	237	**<.0001**	6.39	**0.001**	1.29	0.285
Nitrate	111	**<.0001**	3.77	**0.035**	30.7	**<.0001**	7.91	**<.0001**	1.14	0.379	2.32	0.121	168	**<.0001**	200	**<.0001**	24.3	**<.0001**	2.67	**0.017**
Inorganic N	3.92	**0.031**	79.2	**<.0001**	83.4	**<.0001**	7.32	**0.001**	1.03	0.459	4.52	0.021	210	**<.0001**	60	**<.0001**	21.5	**0.001**	1.62	0.146
Extractable organic N	1.55	0.261	8.4	**0.007**	18	**0.001**	2.4	0.076	0.84	0.627	1.31	0.335	8.67	**0.007**	17.9	**<.0001**	0.86	0.547	0.41	0.959
Extractable TN	2.56	0.097	58.8	**<.0001**	103	**<.0001**	7.6	**0.001**	0.86	0.609	5.06	**0.014**	**136**	**<.0001**	19.5	**<.0001**	6.69	**0.001**	0.81	0.656
Extractable C	0.95	0.489	0.59	0.572	21	**0.0004**	1.68	0.189	0.59	0.572	1.62	0.242	2.06	0.178	171	**<.0001**	0.65	0.69	1.45	0.208
Microbial biomass N	1.96	0.171	8.12	**0.008**	7.53	**0.01**	0.28	0.937	1.06	0.438	1.13	0.407	1.27	0.322	14.8	**0.001**	0.99	0.467	0.91	0.564
Microbial biomass C	6.95	**0.005**	0.38	0.693	33.8	**<.0001**	1.27	0.326	2.57	**0.021**	9.96	**0.001**	2.2	0.161	214	**<.0001**	1.15	0.381	2.43	**0.028**
C:N of microbes	6.21	**0.007**	0.35	0.71	28.9	**0.0001**	1.35	0.293	0.76	0.703	9.61	**0.001**	0.15	0.867	115	**<.0001**	0.63	0.706	1.28	0.289
TN	0.4	0.839	2.32	0.149	4.62	**0.037**	1.18	0.365	0.82	0.652										
TC	1.2	0.375	4.67	**0.037**	9.28	**0.006**	0.78	0.599	1.25	0.309										
C:N	1.99	0.166	0.27	0.772	0.91	0.48	2.02	0.122	1.65	0.138										

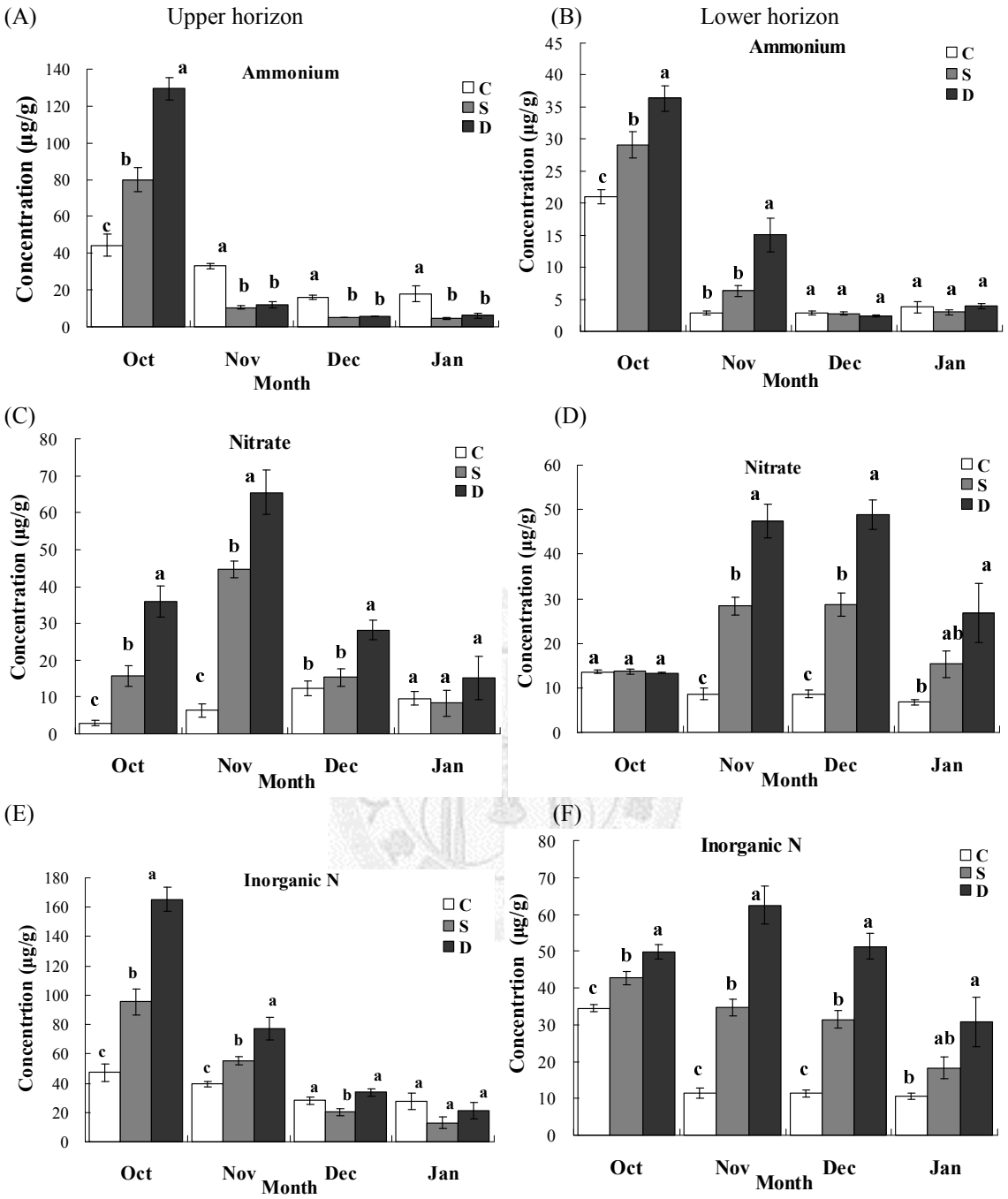


Fig. 13. Concentrations (mean±1se, n = 6) of ammonium, nitrate, and overall inorganic N in the upper (A, C, E), and lower (B, D, F) soil horizons. C, control; S, single fecal pellets; D, double fecal pellets. Different alphabets indicate significant difference between treatments within a given month (Duncan pairwise comparisons).

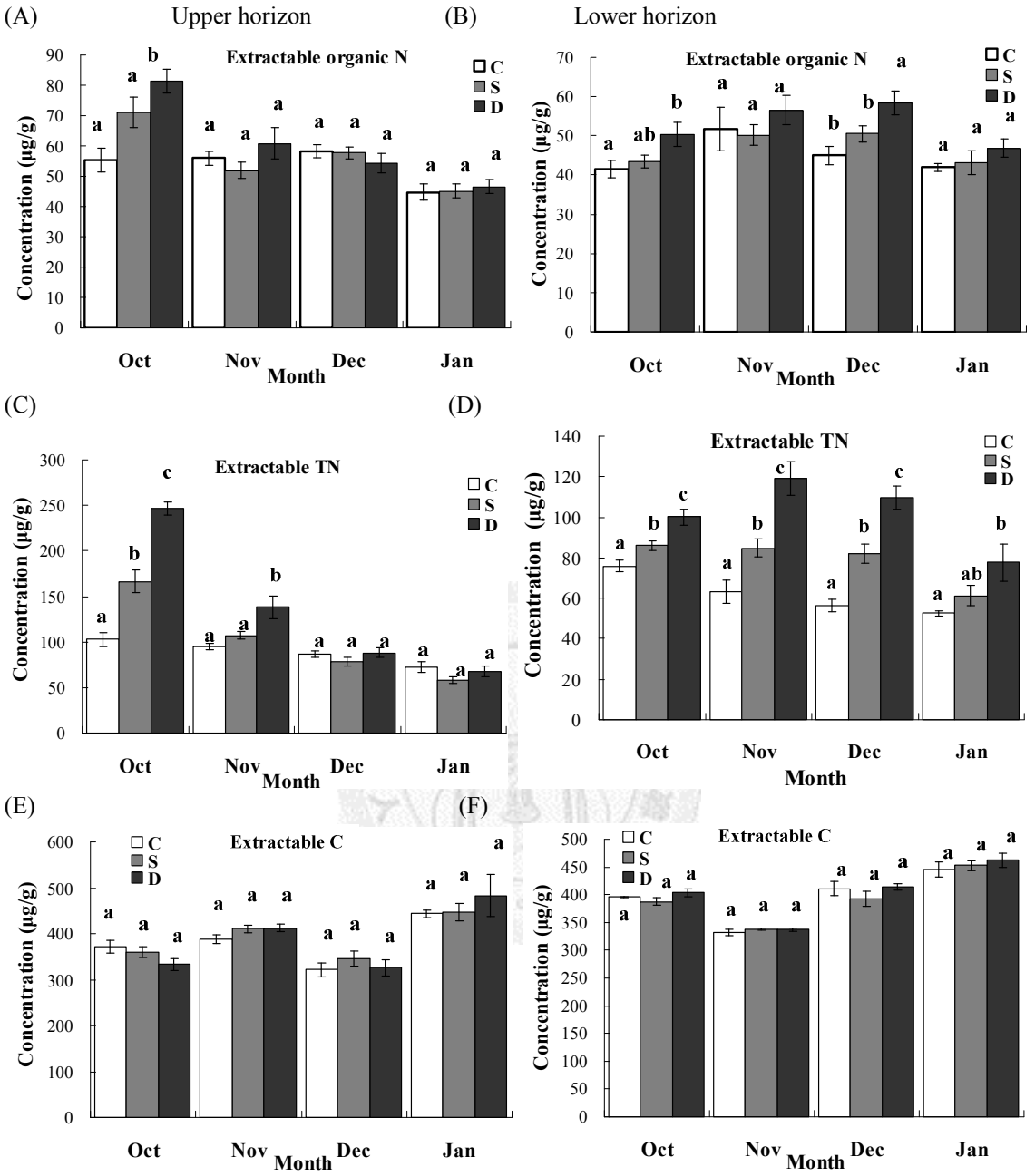


Fig. 14. Concentrations (mean±1se, n = 6) of extractable organic N, extractable total N, and extractable total carbon in the upper (A, C, E) and lower (B, D, F) soil horizons. The treatments are C, control; S, single fecal pellets; D, double fecal pellets. Different alphabets indicate significant difference between treatments within a given month (Duncan pairwise comparisons).

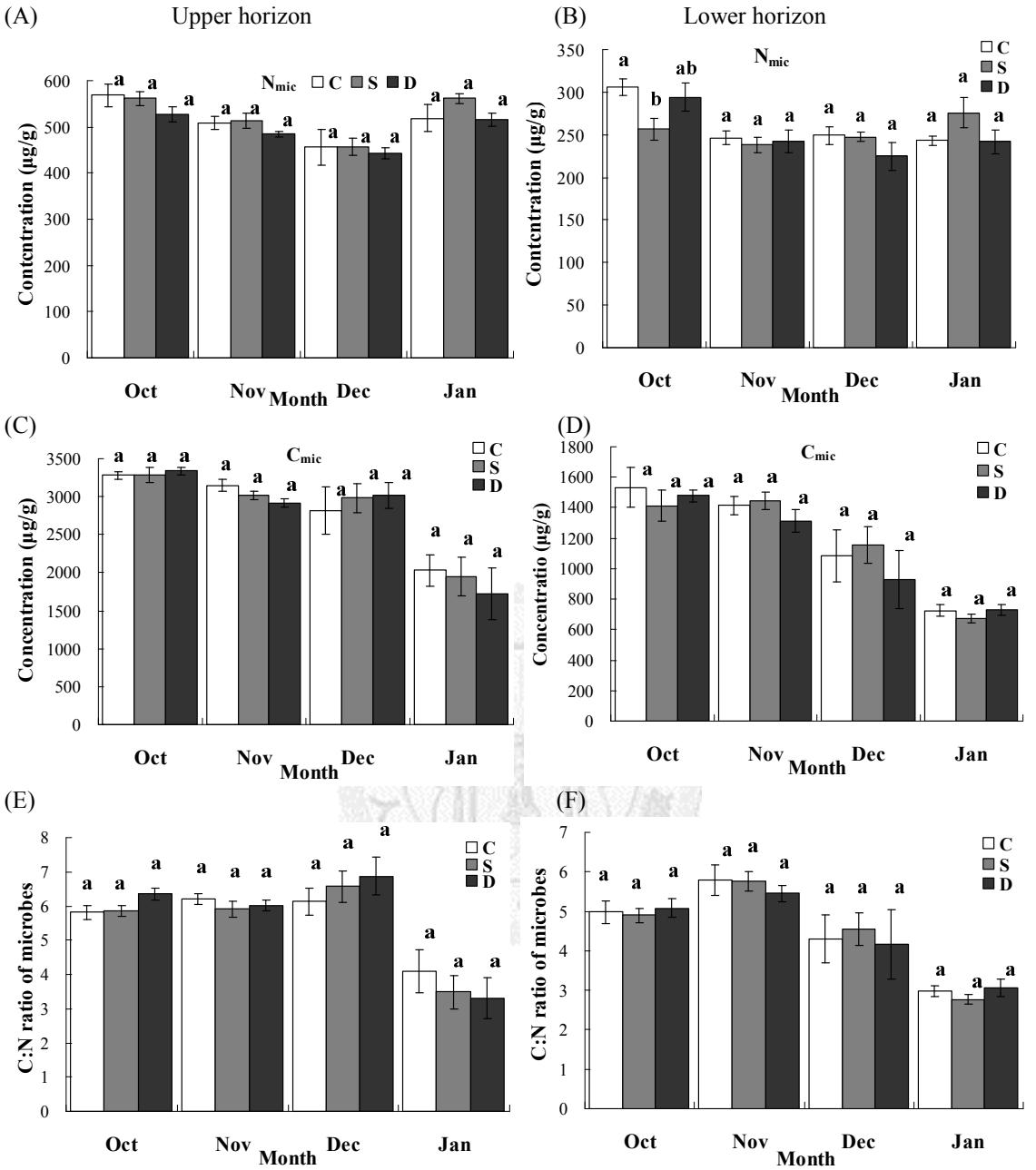


Fig. 15. Concentrations (mean±1se, n = 6) of microbial biomass N and C, and C:N ratio in the upper (A, C, E) and lower (B, D, F) soil horizons. The treatments are C, control; S, single fecal pellets; D, double fecal pellets. Different alphabets indicate significant difference between treatments within a given month (Duncan pairwise comparisons).

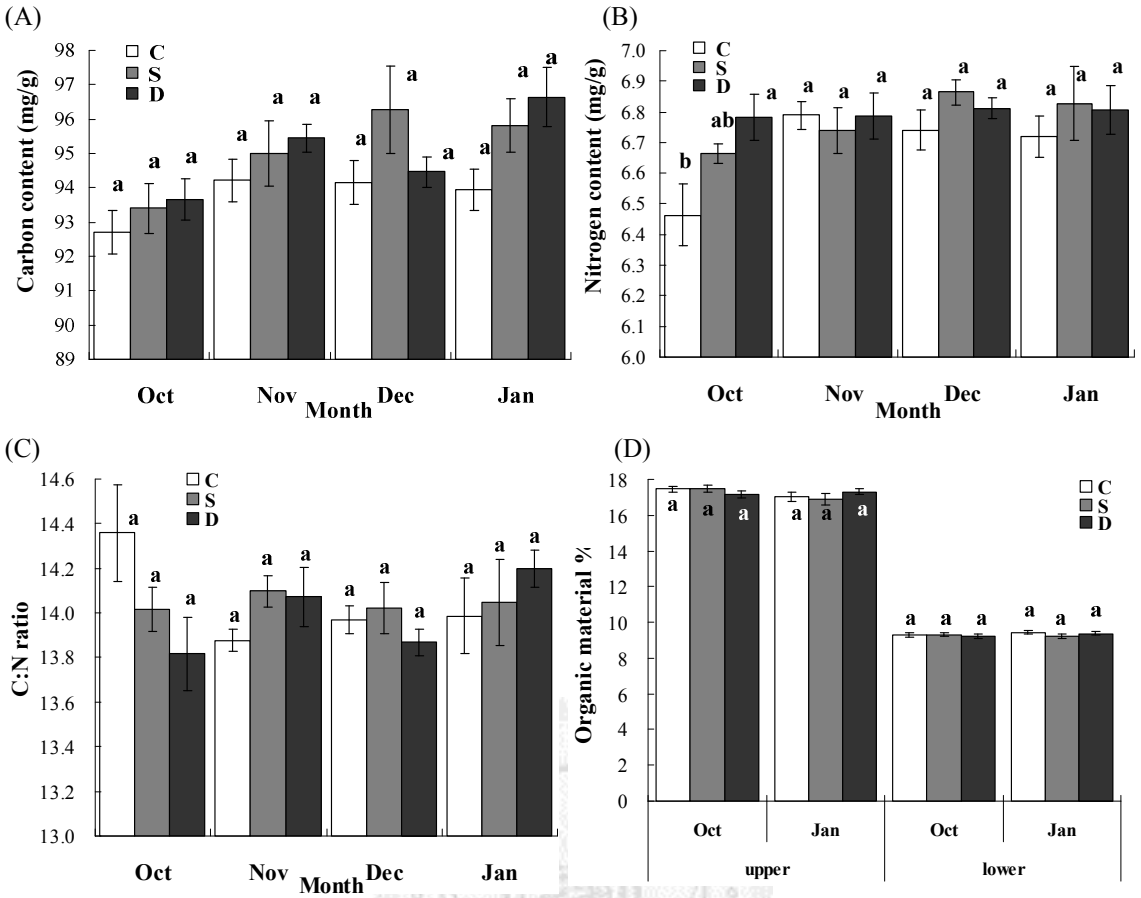


Fig. 16. Concentrations (mean±1se, n = 6) of TN and TC, C:N ratio, and organic material in the upper soil horizons. The treatments are C, control; S, single fecal pellets; D, double fecal pellets. Different alphabets indicate significant difference between treatments within a given month (Duncan pairwise comparisons).

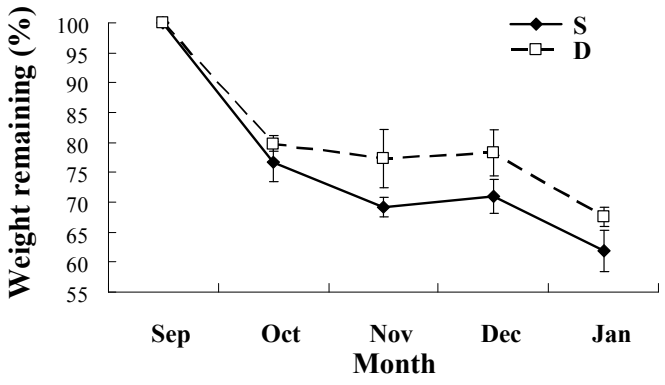


Fig. 17. Percentage (mean±1se, n=6) of initial weight remained in fecal pellets. Treatments were: S, single; D, double fecal pellets

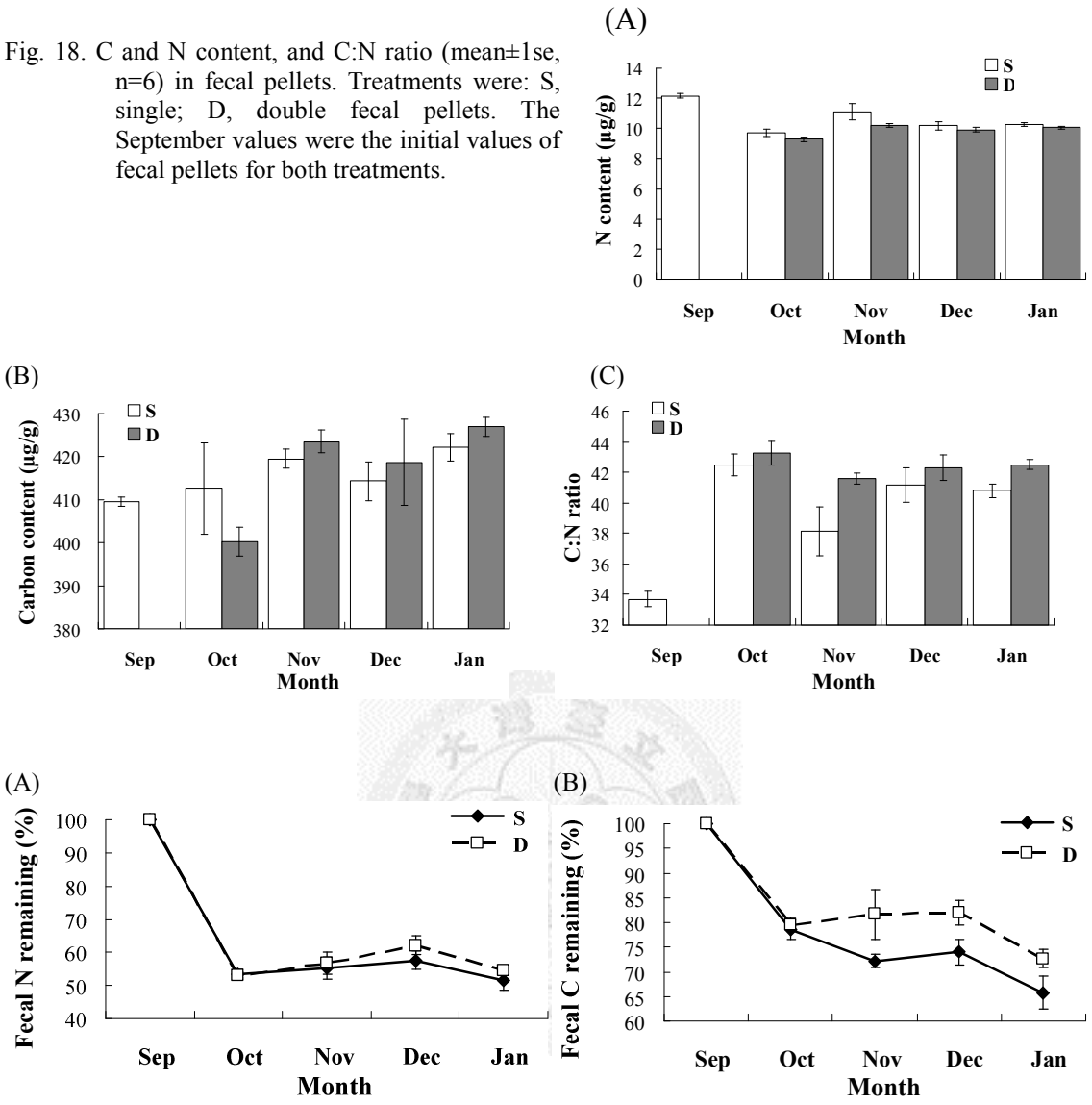


Fig. 18. C and N content, and C:N ratio (mean±1se, n=6) in fecal pellets. Treatments were: S, single; D, double fecal pellets. The September values were the initial values of fecal pellets for both treatments.

Fig. 19. Percentage (mean±1se, n=6) of (A) nitrogen, and (B) carbon remained in fecal pellets. Treatments were: S, single; D, double fecal pellets

Laboratory Incubation on Homogenized Soil

In the beginning of incubation (day 0), the concentrations of all nutrients (Fig. 21) in leachates were not significantly different among treatments (ANOVA, $NH_4^+{}_{(water)}$, $F_{0.05,3,24} = 0.77$, $p = 0.52$; $NO_3^-{}_{(water)}$, $F_{0.05,3,24} = 0.62$, $p = 0.61$; total water soluble N, $F_{0.05,3,24} = 1.04$, $p = 0.39$; water soluble carbon, $F_{0.05,3,24} = 0.50$, $p = 0.68$) indicating that

all microcosms were homogenous. I used the values of control (Fig. 20) as the baseline values of N and C dynamics of other treatments. Vole feces, time, and their interaction all showed significant effects on N concentration, including ammonium$_{(water)}$, nitrate$_{(water)}$, total water soluble N, and water soluble carbon in leachate. In contrary, litter and feces-by-litter interaction had no significant effect on concentrations of N and C in leachant (Table 7, Fig. 21~24). The results showed that dynamic patterns of N and C concentration of leachates from the four treatments could be divided into two groups: with latrine (F and F+L, referred to as latrine group hereafter) and without latrine (C and L, referred to as no latrine group hereafter).

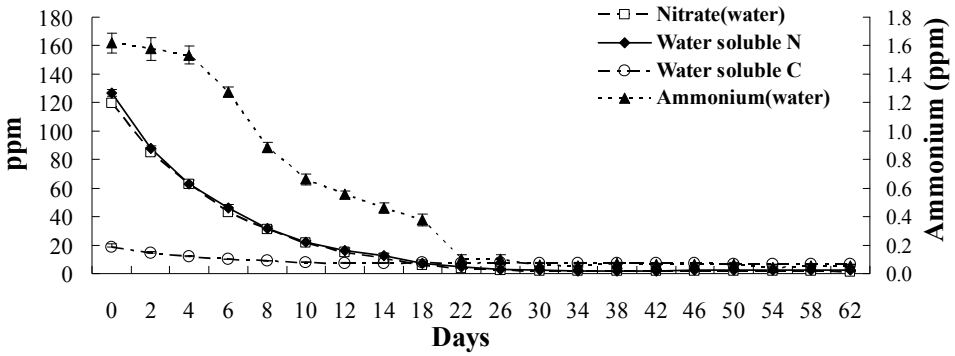


Fig. 20. N and C concentrations of control in leachate. All concentrations were referred to left y axis, except ammonium$_{(water)}$ was referred to right y axis The error bar represented ± 1se, n = 7.

Table 7. Results of repeated-measure two-way ANOVAs that examined the effects of vole feces and plant litter on N and C concentrations of rainfall leachant collected and CO_2 evolution rate during the laboratory incubation experiment.

Factors	Feces		Litter		Feces × Litter		time		time × Feces		time × Litter		time × Feces × Litter	
	d.f.= 1		*d.f.*= 1		*d.f.*= 1		*d.f.*= 19		*d.f.*= 19		*d.f.*= 19		*d.f.*= 19	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Ammonium$_{(water)}$	105	**<.0001**	0.05	0.82	1.18	0.29	40.5	**<.0001**	17.0	**0.001**	0.53	0.87	0.99	0.55
Nitrate$_{(water)}$	25.6	**<.0001**	1.33	0.26	0.08	0.79	292	**<.0001**	21.6	**0.0005**	1.31	0.39	1.71	0.26
Extractable TN	35.0	**<.0001**	1.54	0.23	0.00	0.98	329	**<.0001**	39.1	**<.0001**	14.7	**0.002**	15.4	**0.001**
Extractable C	61.0	**<.0001**	2.16	0.15	2.01	9.58	94.5	**<.0001**	16.8	**0.001**	0.55	0.85	0.53	0.87
CO_2 evolution rate[a]	158	**<.0001**	7.12	**0.01**	1.60	0.22	8.37	**0.0002**	4.99	**0.003**	1.07	0.44	1.37	0.28

a. The degree of freedom of time, time × Feces, time × Litter, and time × Feces × Litter in CO_2 evolution rate were 11.

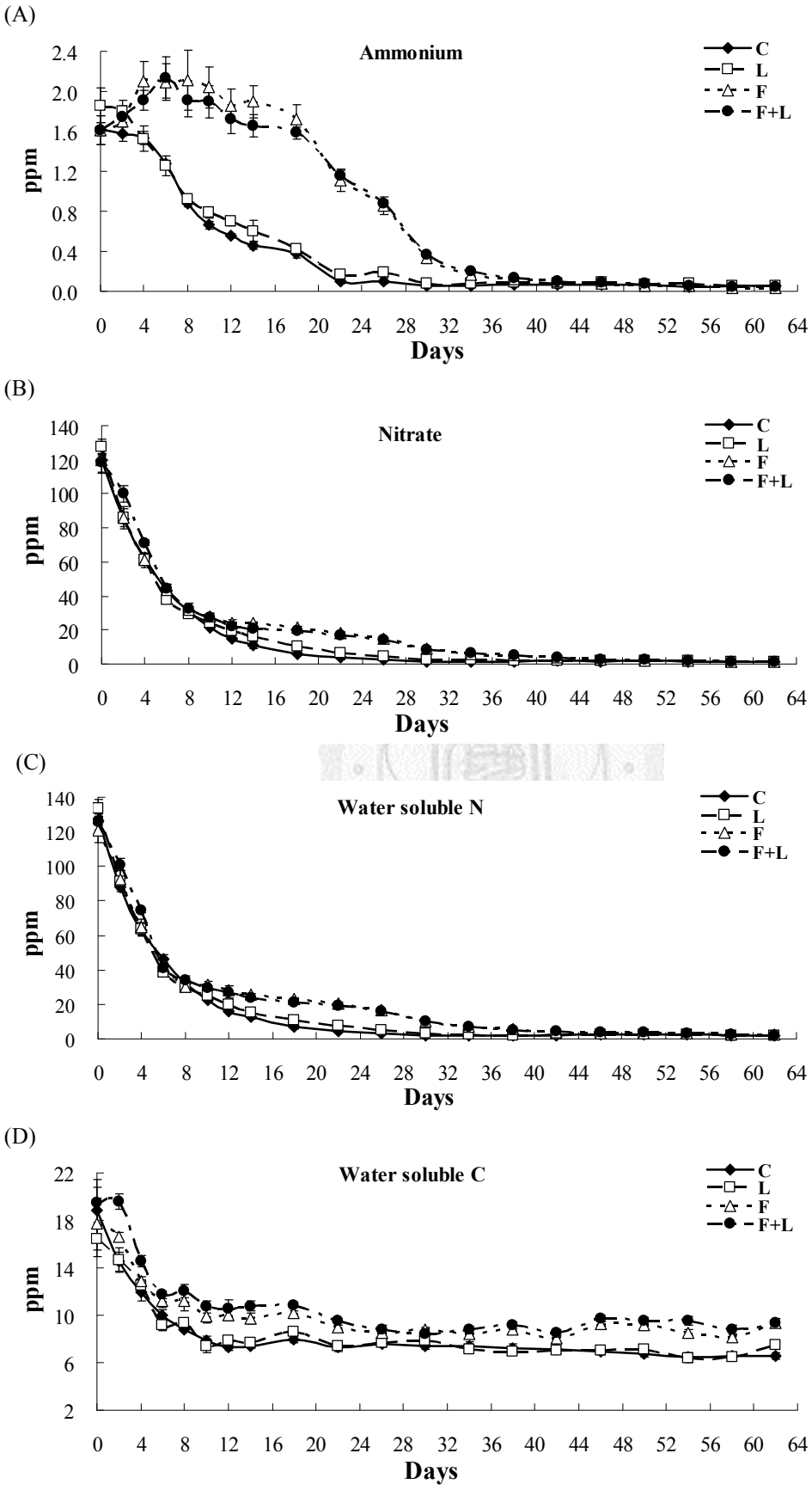


Fig. 21. The concentrations in leachates. (A) Ammonium$_{(water)}$. (B) Nitrate$_{(water)}$. (C) Water soluble N. (D) Water soluble C. The treatments were C, control; L, litter; F, feces; F+L, feces and litter. The error bar represented ± 1se, n = 7.

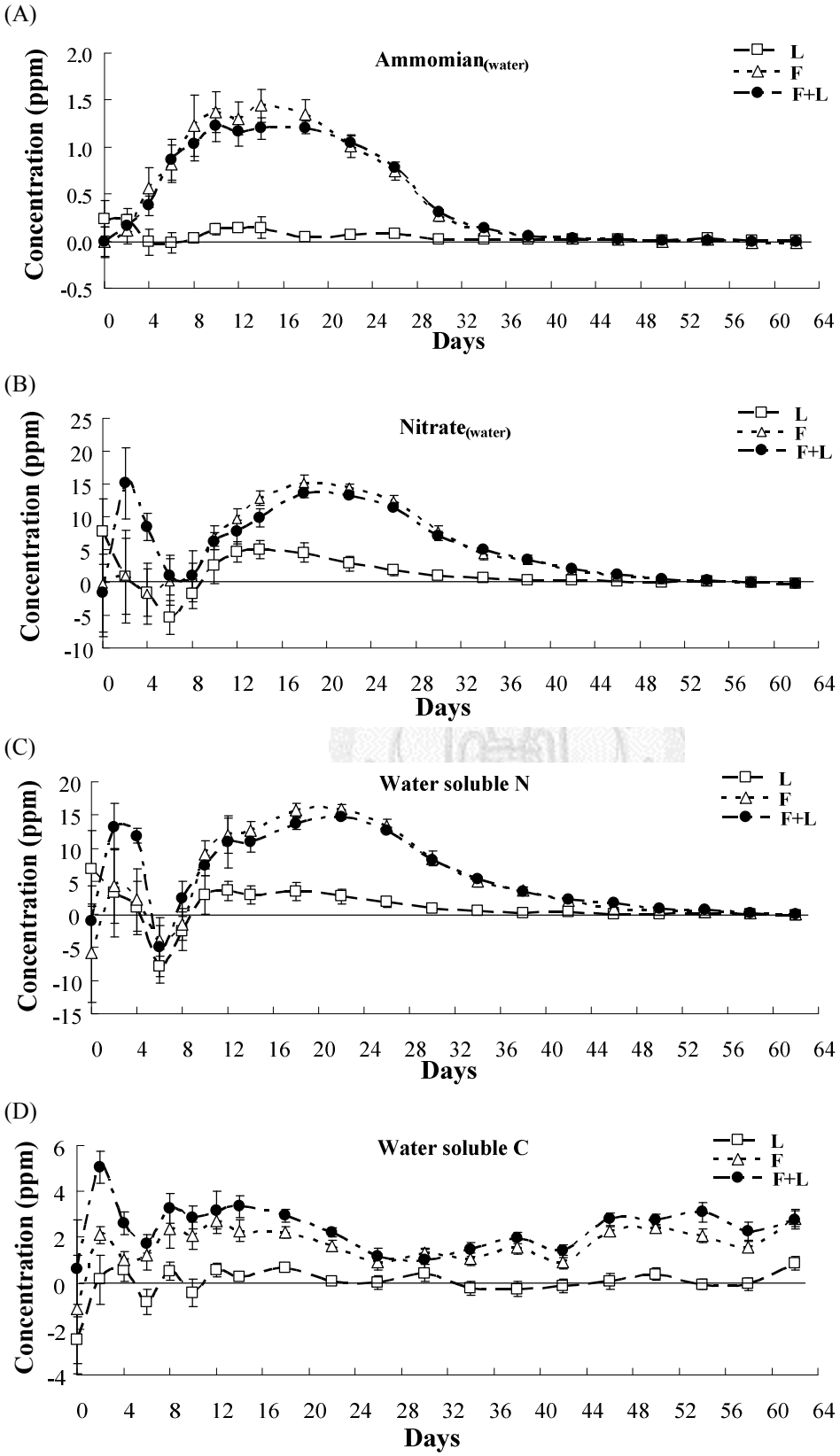
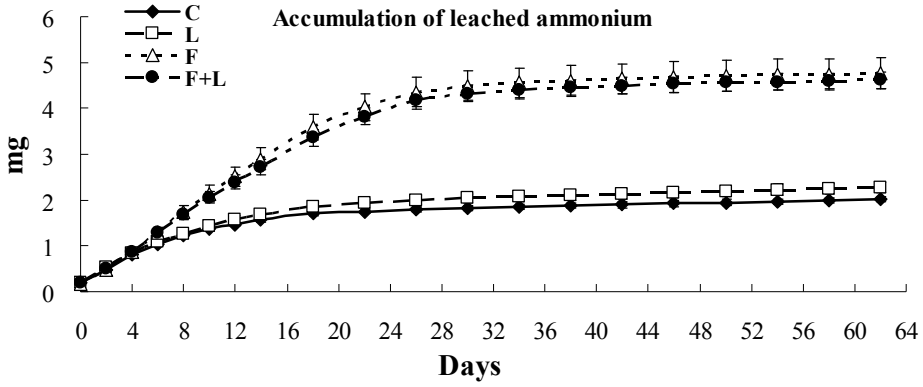
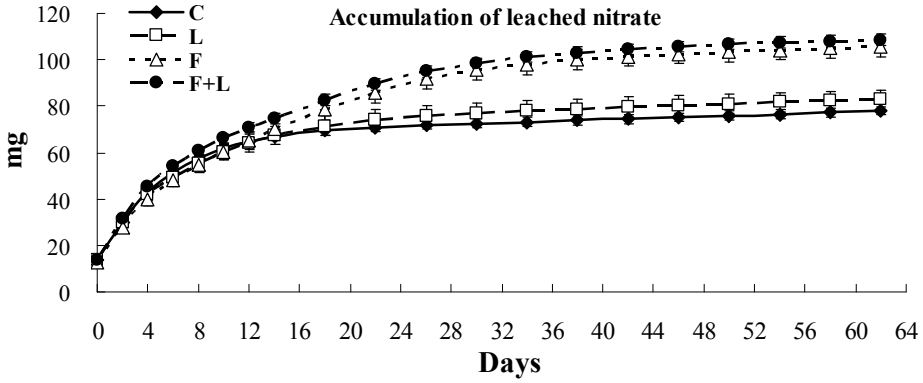


Fig. 22. The concentration difference between treatment and control, obtained by subtracting the average value of control from treatment. (A) Ammonium$_{(water)}$. (B) Notrate$_{(water)}$. (C) Water soluble N. (D) water soluble C. The treatments were L, litter; F, feces; F+L, feces and litter. The error bar represented ± se, n = 7.

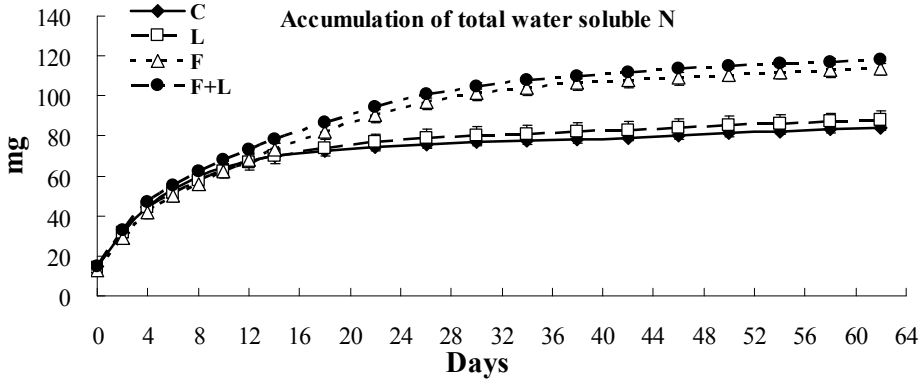
(A)



(B)



(C)



(D)

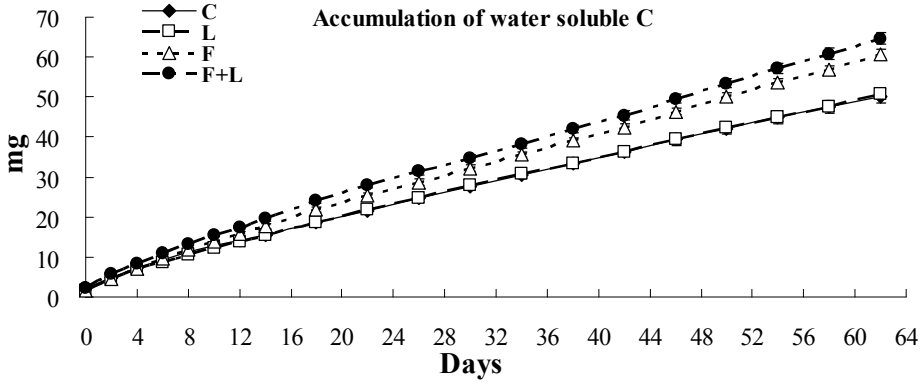


Fig. 23. The total amount of N and C accumulated in leachates. (A) $Ammonium_{(water)}$. (B) $Nitrate_{(water)}$. (C) Water soluble N. (D) Water soluble C. The treatments were C, control; L, litter; F, feces; F+L, feces and litter. The error bar represented ± se, n = 7.

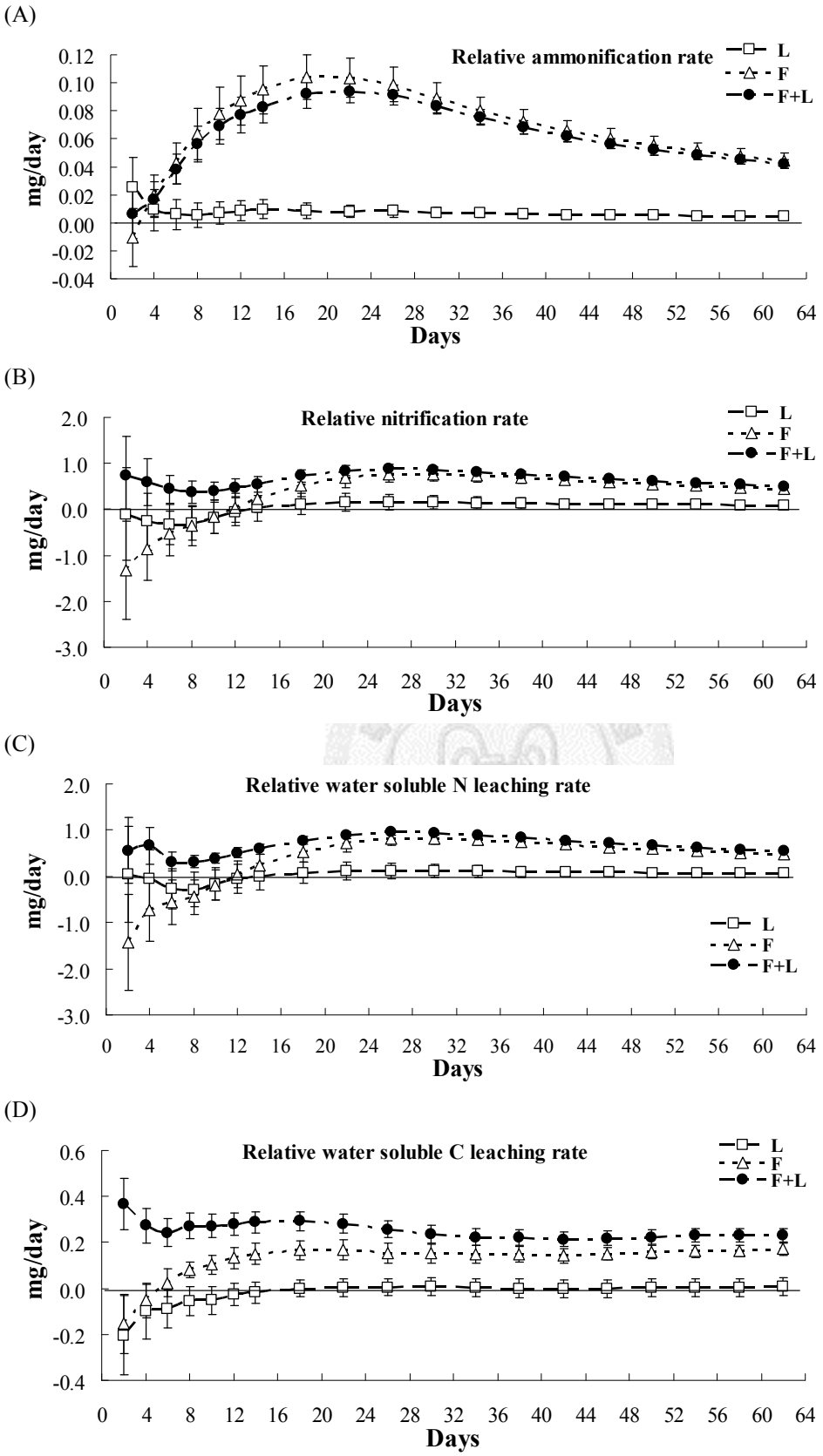


Fig. 24. The relative leaching rate of treatment than control. (A) $Ammonium_{(water)}$. (B) $Nitrate_{(water)}$. (C) Water soluble N. (D) Water soluble C. Relative leaching rate obtained by subtracting the accumulation value of control from treatment, and then divided by the sampling days. The treatments were L, litter; F, feces; F+L, feces and litter. The error bar represented ± se, n = 7.

Ammonium$_{(water)}$ concentration was low in leachate, but its response to treatment was fast, increased almost immediately in latrine group, reached peak on day 10, then declined with time until day 42 when it dropped to the same level as the control (Fig. 22A). The accumulation of leached ammonium$_{(water)}$ between latrine and no latrine group started to show differences on day 6, and at the end the latrine group accumulated nearly 130 % (2.3 mg) more ammonium in leachate than did no latrine group (Fig. 23A). Relative ammonification rates increased dramatically in the latrine group during day 2~18 (Fig. 24A), indicating that ammonification was vigorous at the early incubation stage, and slow down afterward. Plant litter had little effect on ammonium concentration, (Table 7, Fig. 22A and 24A).

Nitrate$_{(water)}$ concentration had a slightly slower response than that of ammonium$_{(water)}$. The increase in concentration started on day 10 in all treatments (L, F, & F+L), about the same time when ammonium$_{(water)}$ concentration peaked (Fig. 22A and B). Nitrate$_{(water)}$ concentration peaked on day 18, and declined with time until day 50 when it reached the same level as the control. Interestingly, nitrate concentration had a much greater response to the litter treatment than ammonium$_{(water)}$ (Fig. 22A and B). The accumulation of nitrate$_{(water)}$ in the latrine group became significantly higher than that in no latrine group on day 22 (Fig. 24B). At the end of incubation, F+L, F, and L treatments had accumulated 30.8 mg (40%), 27.6 mg (35%), and 5.07 mg (7%) higher

concentration of nitrate in the leachant than control, respectively. Relative nitrification rates of L and F+L treatments had the same pattern, decreasing between day 2~8 and increasing between day 8~26. Relative nitrification rate of F treatment increased from day 0 to 26 (Fig. 26A). Relative nitrification rate of all treatments declined after day 26.

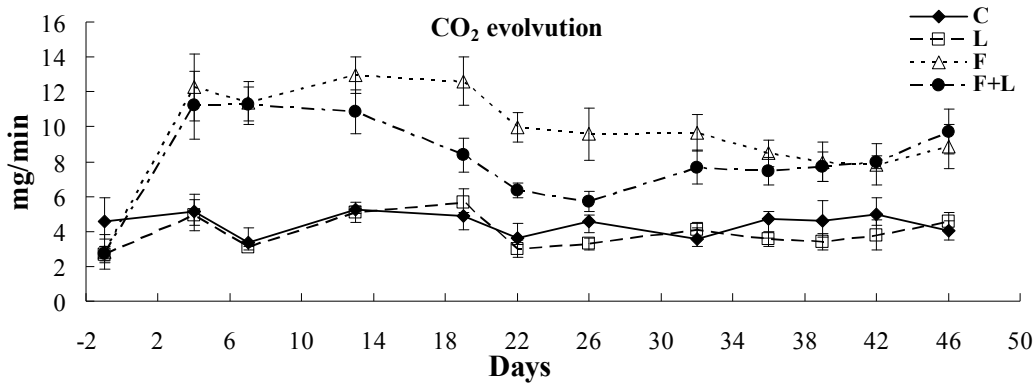
$Nitrate_{(water)}$ made up more than 95% of total soluble nitrogen. Consequently, the response of total soluble nitrogen was very similar to that of $nitrate_{(water)}$ (Fig. 21C, 22C, 23C & 24C). At the end of incubation, the accumulation of total soluble nitrogen in F+L, F, and L treatments had accumulated 33.91 mg (40%), 29.75 mg (35%), and 3.93 mg (5%) higher concentration of total soluble nitrogen than control, respectively (Fig. 23C).

Water soluble carbon responded immediately to F+L treatment, while the response to F treatment did not become clear until day 6. The responses to both treatments peaked on day 8 and remained relatively stable afterward. The response to L treatment did not show difference from the control throughout the incubation (Fig. 21D and 22D). From start till the end, the concentration of water soluble carbon of the latrine group was higher than no latrine group, indicating a constantly high decomposition rate of the latrine group. The accumulation of water soluble organic carbon of latrine group became significantly higher than no latrine group on day 12 (Fig. 23D). Latrine group leached more than 20% (14 mg) carbon than no latrine group at the end. Relative leaching rate of F and L treatments had similar patterns, both were lower than control

before day 4. Afterward, relative leaching rates of F treatment became higher than control, but those of L treatment remained the same as control. Only the F+L treatment had a greater leaching rate than control from start to the end of incubation (Fig. 24D).

CO_2 evolution rates were not different among 4 treatments before incubation started (Fig. 25, ANOVA, F = 0.01, p = 0.998). The rates increased dramatically in latrine group to 2~3 folds of those of no latrine group. The rates of no latrine group remained at approximately 4 mg/min throughout the incubation. Rates of the F+L treatment declined sooner (on day 10), and remained lower than F until day 34. Rates of the F treatment declined on day 20, and joined F+L treatment on day 34. CO_2 evolution rates of latrine group remained 2-fold higher than no latrine group after day 34, indicating that microbial activities were still high toward the end of incubations (Fig. 25).

(A)



(B)

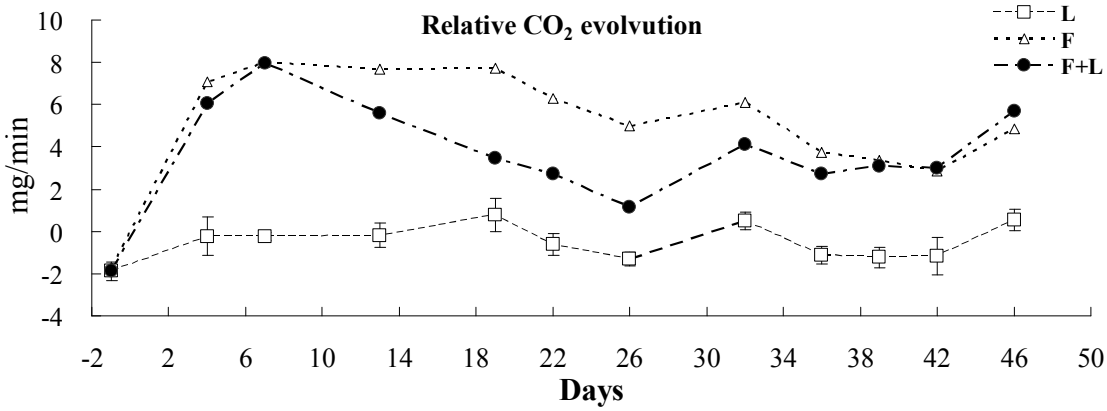


Fig. 25. (A) CO_2 evolution rates. (B) Difference between each treatment and control. The treatments were C, control; L, litter; F, feces; F+L, feces and litter. All treatments were at a steady state after 36 days. The error bar represented ± 1se, n = 7.

The initial chemical constituents of soil were shown in Table 8. At the end of 62 days incubation, there was no difference among treatments in nitrate, extractable TN, and N & C content (Fig. 26 & 27). Feces had effects on upper layer soil in ammonium, inorganic N, and extractable C (Table 9). The effects on lower layer were ammonium, extractable organic N, and microbial biomass C & N. But the concentrations of ammonium and inorganic N were lower in latrine than no latrine group (Fig. 26A & C).

Table 8. The initial chemical constituents (mg/g) of soil used in laboratory incubation. (Mean±1 se, n=4).

Ammonium	Nitrate	Inorganic N	Extractable organic N	Extractable TN	Extractable C
13.8± 3.15	138± 4.18	151± 6.12	25.4± 2.52	177± 7.73	267± 20.9
Microbial biomass N	Microbial biomass C	C:N of microbes	TN (mg/g)	TC (mg/g)	C:N
260± 4.52	993± 68.9	3.84± 0.29	6.18± 0.03	90.0± 0.28	14.5± 0.05

Table 9. Results of non-parametric factorial test that examined the effects of vole feces and plant litter on the N and C concentration of soil, fecal pellets, and leaf litter after 62 days of laboratory incubation. n= 3.

		NH_4^+	NO_3^-	Inorganic N	Extractable organic N	Extractable TN	Extractable C	N_{mic}	C_{mic}	C:N of microbes	TN	TC	C:N
Upper													
Feces	χ^2	5.77	3.69	5.03	3.69	0.92	4.33	1.64	3.69	1.64	1.26	0.10	2.08
	p	**0.016**	0.055	**0.025**	0.055	0.337	**0.037**	0.200	0.055	0.200	0.262	0.749	0.150
Litter	χ^2	0.92	1.64	2.08	0.00	0.23	0.64	3.69	0.23	0.78	0.52	0.31	0.01
	p	0.337	0.200	0.150	1.000	0.631	0.423	0.055	0.631	0.378	0.471	0.575	0.936
Feces × Litter	χ^2	0.10	1.26	0.41	0.23	0.92	0.64	1.26	2.56	2.31	0.52	1.85	0.31
	p	0.749	0.262	0.522	0.631	0.337	0.423	0.262	0.109	0.128	0.471	0.173	0.575
Lower													
Feces	χ^2	5.77	0.31	2.56	7.41	0.03	3.10	6.56	6.56	0.00	1.85	0.00	2.56
	p	**0.016**	0.575	0.109	**0.006**	0.873	0.078	**0.010**	**0.010**	1.000	0.173	1.000	0.109
Litter	χ^2	1.64	0.16	2.08	0.23	1.26	0.64	0.41	0.10	0.00	0.41	0.23	0.16
	p	0.200	0.689	0.150	0.631	0.262	0.423	0.522	0.749	1.000	0.522	0.631	0.689
Feces × Litter	χ^2	0.10	2.56	0.92	0.64	2.08	0.64	0.03	0.23	0.00	0.52	0.41	0.31
	p	0.749	0.109	0.337	0.423	0.150	0.423	0.873	0.631	1.000	0.471	0.522	0.575

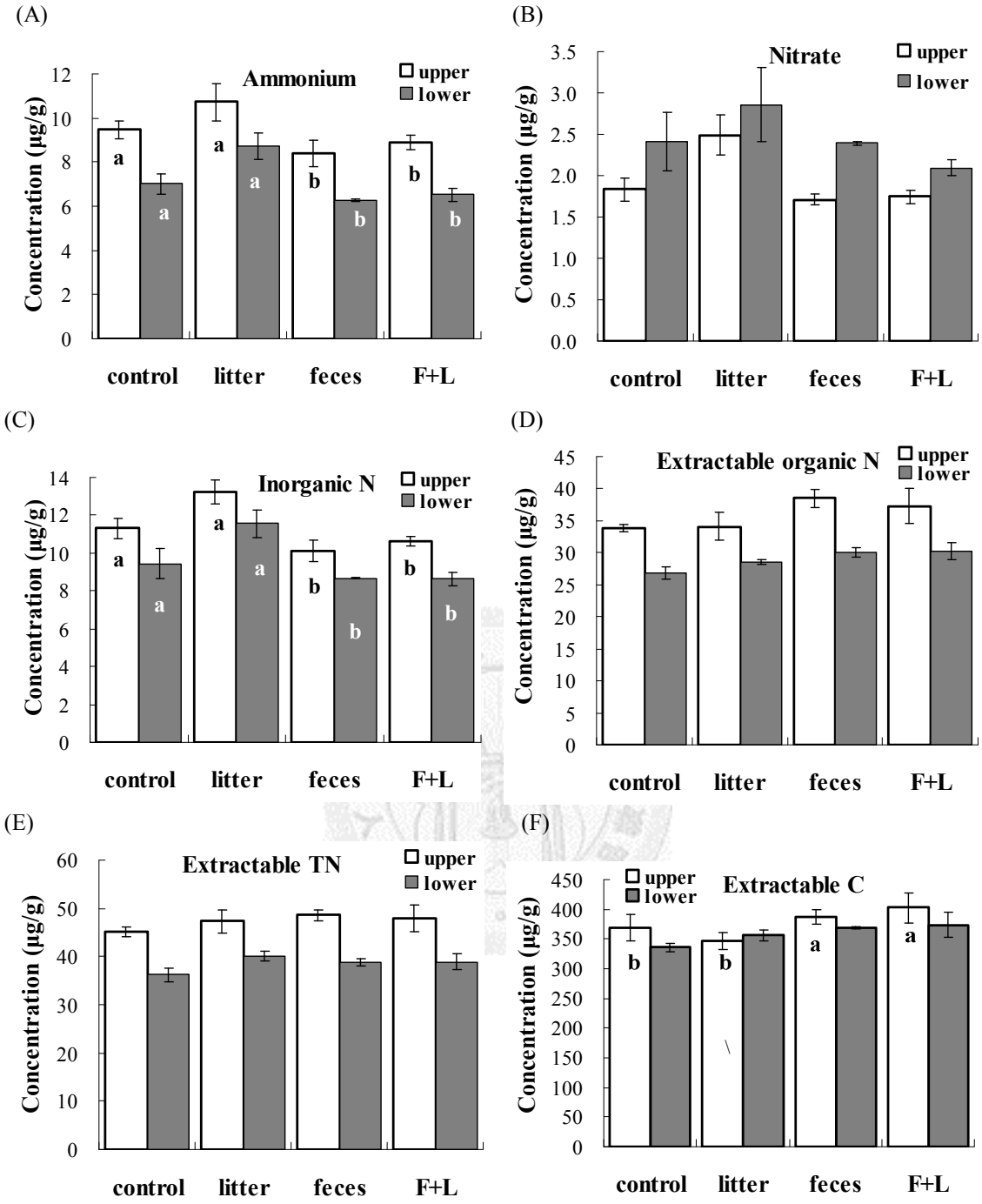


Fig. 26. Concentration (mean±1se, n=3) of extractable C and N in soil at the end of incubation. Different alphabets indicate significant difference among treatments within a given soil layer (non-parametric factorial test (χ^2). No difference in those figures without alphabets

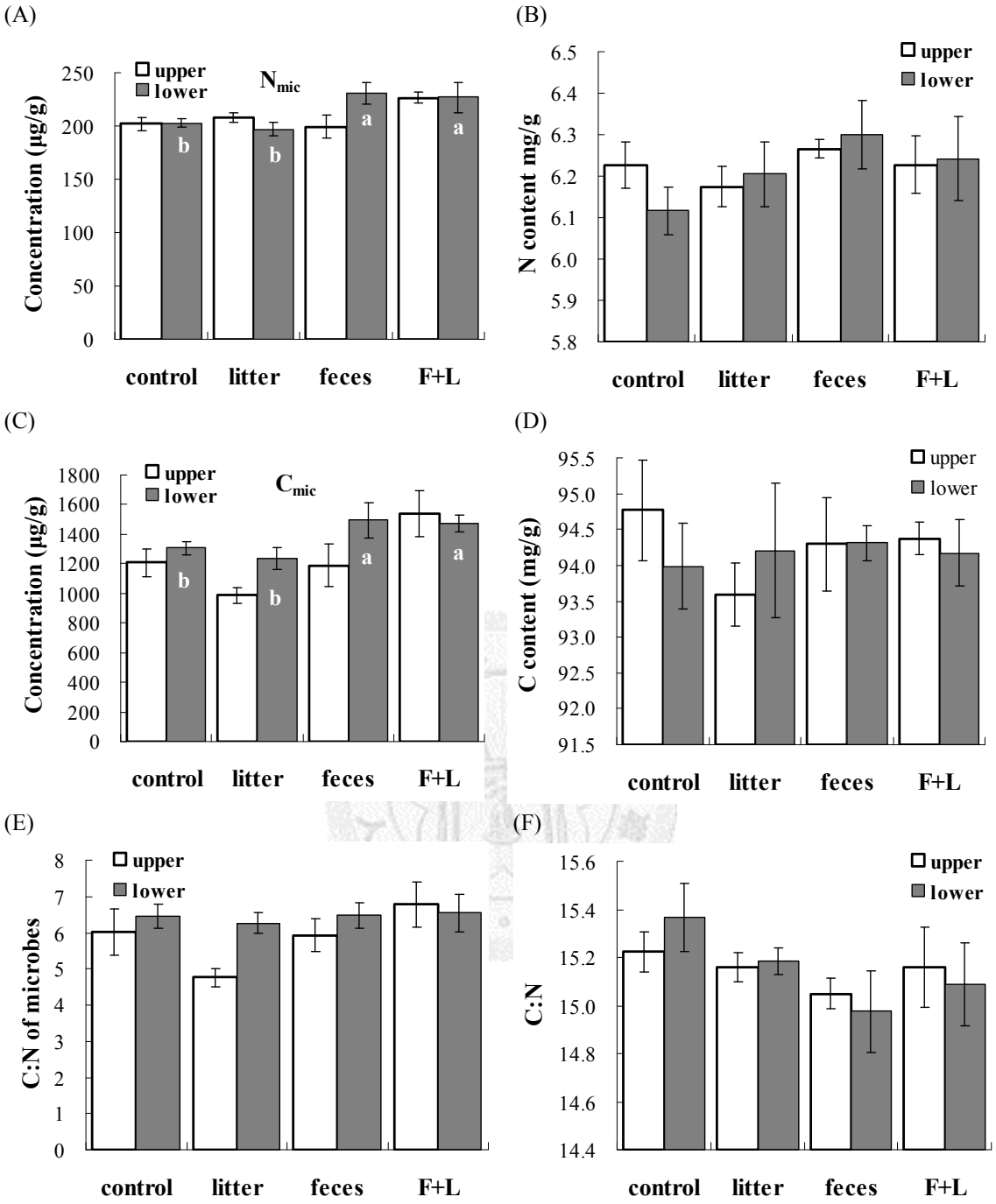


Fig. 27. C and N of soil microbes and soil. (A) Microbial biomass nitrogen. (B) Nitrogen content of soil. (C) Microbial biomass carbon. (D) Carbon content of soil. (E) C:N ratio of microbial biomass. (F) C:N ratio of soil. Values were mean±1se, n = 3. Different alphabets indicate significant difference among treatments within a given soil layer (non-parametric factorial test (χ^2)

The weights of fecal pellets remained at the end were not different between F and F+L treatments (Mann-Whitney U Test, U = 5.5, n = 3, p = 0.64), both 77% of initial weights (Fig 28A). Leaf litters of L and F+L remained at the end (89.56 ± 0.53 % and

84.11 ± 1.02 %, respectively) were significantly different (Mann-Whitney U Test, U = 9.0, n = 3, p = 0.046). Leaf litter decomposed faster when vole fecal pellets were present. At the end of incubation, N and C contents of fecal pellets were still higher than those of leaf litter (Table 10, Fig. 28B & C). Fecal pellets still had 56% nitrogen remained, and the amount reduced was not different between F and F+L treatments (Mann-Whitney U Test, U = 6.50, n = 3, p = 0.38; loss 30.21 ± 0.93 mg N and 33.10 ± 2.59 mg N, respectively). Leaf litter still had 70% nitrogen remained, only 0.30 ± 0.01mg and 0.24 ± 0.03 mg were reduced in L and F+L treatments, respectively, no significant between L and F+L treatment (Mann-Whitney U Test, U = 2.00, n = 3, p = 0.468). The amount and the percentage of nitrogen reduced were much higher for fecal pellets than leaf litter (Table 10). On the other hand, fecal pellets still had 77% carbon remained, and the amount reduced was not different between F and F+L treatments (Mann-Whitney U Test, U = 5.00, n = 3, p = 0.82; loss 616.4 mg and 625.6 mg, respectively). Whereas, leaf litter lost more carbon in F+L than L treatment (Mann-Whitney U Test, U = 9.00, n = 3, p = 0.046).

Table 10. Weight, N, and C content of fecal pellets and leaf litter before and after incubation. Values are mean±1se. n=3. *p* value were calculated from Mann-Whitney U Test. The treatments were L, litter; F, feces; F+L, feces and litter.

Organic matter		Fecal pellets			Leaf litter		
Treatment		Initial	F	F+L	Initial	L	F+L
Weight	g	6.52±0.001	5.07± 0.05	4.99± 0.09	2.00± 0.001	1.80± 0.01	1.68± 0.02
	Remain (%)		77.7 ± 0.84	76.52 ± 1.44		89.6± 0.53	84.1± 1.02
	p		0.637			**0.046**	
Total N amount	mg	71.4± 0.62	41.2± 0.93	38.3 ± 2.59	11.8±0.13	8.75± 3.12	9.32± 0.32
	Remain (%)		58.3 ± 1.32	54.2 ± 3.66		74.9± 1.09	79.8 ± 2.71
	p	0.376			0.258		
Total C amount	mg	2685 ± 5.51	2068± 23.9	2059± 36.8	810± 2.70	715± 3.12	673 ± 8.36
	Remain (%)		77.4 ± 0.89	77.0 ± 1.38		88.5± 0.39	83.3± 1.03
	p		0.822			**0.046**	

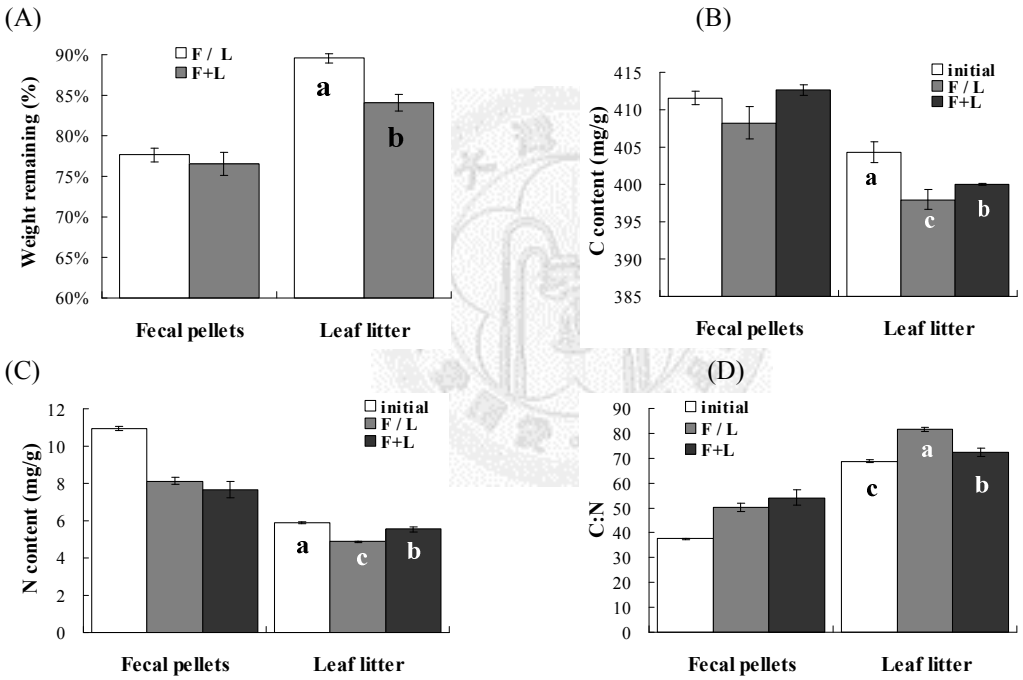


Fig. 28. Fecal pellets and leaf litter in single or mixed treatment after 62 days incubation. (A) Weight remaining. (B) Carbon content. (C) Nitrogen content. (D) C:N ratio. F / L, F was feces treatment for fecal pellets; L was litter treatment for leaf litter. The error bar represented ± 1se, n = 3. Different alphabets indicate significant difference between treatments (Kruskal-Wallis test and post hoc comparisons by Dunn test)

Discussions

Taiwan vole population survey

Taiwan vole populations showed substantial spatial and temporal heterogeneity at

the study site from October 2005 to May 2009 (Fig. 3). There wasn't any clear spatial or temporal pattern. For example, population size at D plot sitting at the bottom of the slope was consistently high though fluctuated between 10 to 35 individuals. Population size at H plot sitting on the top of the slope also fluctuated greatly from 0 to 25 individuals. Sizes of both populations peaked in late 2007 during the three and half year survey. Comparing with cyclic vole populations in North America, Europe, or Japan, the magnitudes of fluctuations of Taiwan vole populations were relatively small. Wu (2007) reported Taiwan vole population densities at a nearby alpine meadow at 10.3~12.5 voles per hectare over 2 years. Wu's sampling area (4 hectare) was larger than mine, and he used a 20 m spacing grid as well as a different type of trap (Sherman single-capture live traps), those factors may have contributed to the lower density estimates than mine in his vole population.

Daily defecation rate

Daily defecation rates of Taiwan voles ranged from 5 to 10 g per day per vole (Fig. 5), which equal to 15~30% of body weight. Voles defecated more feces in cold (Jan.-10 and Mar.-08) than warm (Apr.-09 and Jul.-07) months. Christian (cited in (Pastor et al. 1996)) found that arvicoline rodents deposited 2~4 g feces on high-quality, low-fiber food and > 10 g on poorer-quality, high-fiber food. The defecation rate of Taiwan vole

suggested the quality of Yushan cane leaf was medium. Ho (2009) found that Yushan cane was high in both crude protein and fiber contents during most of the year, and cane leaf was ranked the most or the 2nd most palatable forage among 13 common alpine meadow plants to Taiwan voles. Several studies pointed out protein and fiber contents were major determinants, with positive and negative effects, respectively, of diet choice in herbivores. For example, Yeh (2010) reported that when Taiwan voles were offered different parts of Yushan cane in cafeteria trials, the choice of parts and how much voles ate were mainly determined by fiber content of Yushan cane parts. Crude protein content in Yushan cane leaf was highest and fiber content lowest among all parts almost all year round except in May ~ June when shoots become high in protein and low in fiber. In deed, Taiwan voles prefer leaves over other parts almost all year round except in May ~ June (Yeh 2010). In the cold months (e.g., Jan. and March) when Yushan cane was at its lowest in quality, Taiwan voles might need to consume more leaves in order to meet nitrogen demands, and lead to greater defecation rates in cold months.

The nitrogen content in fecal pellets is a different matter. Clark et al. (2005) found fecal nitrogen of small mammals had a positive relationship with the dietary nitrogen. Although the crude protein contents in cane leaves, ranged between 11.95~19.47 % in different months (Yeh 2010), the range was rather small. Because of that, the nitrogen contents of fecal pellets, at about 12 mg per gram feces, were not different among

months (Table 3). Voles don't just feed on Yushan cane leaves in the field, they feed on all sorts of plants (Ho 2009) as well as invertebrates (W-T. Yeh personal communication). The nitrogen contents of fecal pellets in the field could be lower or higher than what I observed in the laboratory. Nevertheless, high daily output of nitrogen by Taiwan voles in cold months (100 mg per vole per 24 hrs, Table 3) could provide an important nitrogen reservation for plant growth as the suitable growing conditions came (Fornara and Du Toit 2008).

Nitrogen output of vole

Although there were more female than male voles (Fig. 4), there was no sexual difference in daily defecation rates. I calculated annual output of nitrogen by Taiwan voles by multiplying daily defecation rate by the season-specific nitrogen content of feces to obtain season-specific daily nitrogen production per individual vole. The values were multiplied by monthly vole density estimates, then by 30 (days) to give monthly nitrogen output by vole feces per hectare. The annual output of nitrogen through fecal defecation by Taiwan voles at the study site came to be 0.33~0.41 kg N ha^{-1} $year^{-1}$, which was lower than those reported for three small mammal species by Clark et al. (2005). Because Taiwan vole populations had substantial spatial and temporal heterogeneity at the study site, nitrogen output of vole, thus soil nutrient contents, also

formed spatial and temporal heterogeneity at the meadow.

Fecal nitrogen content of Taiwan vole was lower (9.81~15.9 mg/g) than several other herbivores reported: geese 63 mg/g (Cochran et al. 2000), rabbit 20.9 ± 1.0 mg/g (Willott et al. 2000), cow 21.95 ± 0.73 mg/g, rabbit 32.42 ± 1.73 mg/g, common vole 25.00 ± 1.65 mg/g (Bakker et al. 2004), and cattle 27 mg/g (Williams and Haynes 1995). Taiwan vole had similar fecal nitrogen content with sheep and deer: sheep 15 mg/g, deer 12 mg/g (Williams and Haynes 1995), and deer 17 ± 1 mg/g (Moe and Wegge 2008). Fecal nitrogen is not all released in a short period of time. The potential mineralizable pool of feces of red-back vole and meadow vole were 6.43 mg/g and 2.47 mg/g (Pastor et al. 1996), which were much lower than total fecal nitrogen content. Fecal nitrogen released most mineralizable nitrogen in the first week, and released almost all mineralizable nitrogen within three weeks (Pastor et al. 1996). A large portion of fecal nitrogen is not immediately mineralizable. Nevertheless, the instantaneous decay rate *k* of fecal nitrogen for small mammals such as red-back vole and meadow vole were 1.73 and 0.69 wk^{-1}, respectively, faster than that of moose fecal pellets (0.025~0.191 wk^{-1} (Pastor et al. 1996). Haynes and Williams (1993) found the amount of N mineralized from cattle dung is closely related to the total N content. Similarly, I found almost all reduced weight and N and C contents in Taiwan vole feces occurred within the first month in field incubation. The remaining nitrogen of fecal pellets was little more than

50% after four month incubation (Fig. 19A). The mineralizable nitrogen pool of Taiwan vole feces was nearly 50% similar to that of red-back vole. Although the decomposition rate of fecal pellets over long term was not clear, it was clear half of fecal nitrogen output of Taiwan vole returned to soil within one month.

Vole latrine survey

Retention rate of latrines decreased after March. It might be caused by the frequent freeze-thaw process during winter at the He-huan Mt.. Water content of feces pellets in the field was more than 200% that of fecal dry weight. Freeze-thaw process destroyed the structure of pellets, which became more decomposable as soon as temperature rose. When latrine density increased, the reused sign of old latrines also increased (Table 4). It was hard to distinguish new pellets from old ones in the field. I considered old latrine as reused latrine only when the number of pellets increased. It did not include old latrines that added new pellets equal or fewer than decomposed portion. Thus, reuse rate of latrine was underestimated. Although I underestimated the reuse rate of latrine, it was clear active latrines increased with vole number. Field observation indicated that the distribution of latrine was related to plant cover. Vole latrines were more likely found at openings under the canopies of plants. Few latrines were found at bare area. It could be due to the fact that latrines at bare areas were easily bleached,

fragmented and vanished. The distribution of latrine could reveal the active areas of voles. The latrine numbers were not equal between two quadrats within the same plot indicating the distribution of vole was no homogeneous in each plot. The low decomposition rate of feces and the high reusing rate of latrines contributed to the long existing times of latrine.

Field incubation on natural soil

The chemical composition of soil in alpine ecosystems might vary extensively within a very short distance (Körner 2003). At He-huan Mt., the depth of soil can be less than 3 cm at one site, but reach more than 30 cm at the immediate vicinity. The plant species composition was also highly heterogeneous at the study area. The spatial heterogeneities rendered the treatment effect of a small sample size experiment nonsignificant. The sampling interval (two month) also was unsuitable to reveal the effect of latrine. Little latrine effect could be detected in soil at the second month in the field incubation on homogeneity soil. Inorganic nitrogen was a limiting factor of plants at cold seasons in alpine meadows, more samples were under detective level in November.

Field incubation on homogeneity soil

Although the SOM was high in alpine meadow, the inorganic nitrogen was less than 2% of total nitrogen in soil (Table 6). Vole latrine increased the extractable nitrogen, especially the inorganic nitrogen in soil (Fig. 13~16). Ammonium was the first product of mineralization. However, after a month (September) of incubation, most mineralized N was still in ammonium form. It was probably because ammonium had a low mobility with water, and that incubation was conducted in September when the temperature was low, and decomposition rate was reduced. Nitrate increased in the second month, when all other forms of nitrogen decreased, the amount was more than that in the first month. In the lower horizon, there was more nitrate increased than the increased ammonium from the first month, which indicated that nitrate leached down from the upper horizon. The daily precipitation of He-huan could be more than 300 mm on typhoon days. No typhoon occurred during the field incubation, but heavy rains happened in September (Table 2), when the decomposition of latrine had just started and the main form of nitrogen was ammonium. It seemed little nitrogen from latrine leached out in the first month of incubation, since the increased amount of nitrogen approximated the loss nitrogen from feces. Nevertheless, If latrines were located at bare sites, it might increase nitrogen loss by leach (Haynes and Williams 1993, Frank and Evans 1997), especially during typhoon at the nitrification period.

Latrine did not affect N_{mic}, and C_{mic} in field incubation. C_{mic}, but not N_{mic}, declined with time in lower layer soil, and a sudden decline occurred in Jan.-09. The decline of microbial biomass C, but not N, during December ~ March was also reported in Tatachia grassland soil (Cho et al. 2008). Soil freezing decreased microbial biomass, mainly the biomass of fungi (Matzner and Borken 2008). Although freeze-thaw may not affect microbial biomass in alpine ecosystems (Matzner and Borken 2008), the freeze-thaw frequency might be high in He-huan Mt.. The C:N ratio of soil microbes in He-huan Mt. was 6. Brady and Weil (2004) reported that the C:N ratio of bacterial was 5, actinomycete and nematode was 6, fungi was 10. Thus, the soil microbial fauna might be dominated by actinomycetes and bacteria in He-huan alpine meadow. The C:N ratio of microbes dropped to 4 in Jan.-09, and became similar to that of the initial soil of lab incubation, which was stored at 4℃ for a year. The decline of the C:N ratio might be caused by changing microbial fauna in soil, such as the decrease of fungi (Matzner and Borken 2008). The causation of microbial biomass decline needs further research. The microbial biomass N and C were much higher in He-huan Mt. than Tatachia (C_{mic}: 443~931 μg/g dry soil, N_{mic} 41~134 μg/g dry soil; (Cho et al. 2008). The proportion of microbial biomass N and C of soil total N and C were much higher than the extractable portion in the soil (Table 6). The size of active pool of nitrogen in SOM is important for nitrogen availability (Sirotnak and Huntly 2000), and the nutrients in microbes were an

active pool in nutrient cycling (Brookes et al. 1985). The microbial biomass is a large active organic pool. The activity and biomass fluctuation of microbes are very important to the nutrient cycling in alpine meadow.

Laboratory incubation on homogeneity soil

Ammonium quickly responded to the addition of feces. Although ammonium concentration was very low in leachates, the increased ammonium in soil then was largely nitrified to nitrate. Nitrate was more mobile with water, and made up more than 95 % total water soluble N in leachates. The increase of nitrate would increase the risk of nitrogen leaching, if not uptook by plants or microbes. The N concentration of all four treatments met after 44 days (Fig. 21). It indicated the decomposition has already reached a new stable condition after organic matter was added. Although the soil was in stable condition, the concentration of water soluble carbon of latrine group was still higher than no latrine group (Fig. 21D). The higher water soluble carbon indicated the decomposition rate was still higher than no latrine group

Latrine did not affect N_{mic}, and C_{mic} in both field and lab incubation (Table 5 & 9, Fig. 27C & D), except in the lower layer soil of lab incubation. Most of water soluble C in water was water soluble organic carbon, which was easily accessible to the microbial community (Ma et al. 2010). Increasing water soluble C in latrine group, the labile

carbon was washed to lower layer, and supplied food for microbial growth. Lovell and Jarvis (1996) reported that microbial biomass increased in incubation with cattle dung. The amount of added organic matter was much higher in Lovell and Jarvis (1996, 4.5 g dry cattle dung mixed with 125 g moist soil) than in my study. The large amount of labile carbon might have greatly increased the microbial biomass. But the increase of microbial biomass did not always occur with feces deposited. Lovell and Jarvis (1996) also found no microbial biomass increasing in 0~10 cm soil under cattle dung pat in flied experiment, the effect might be masked by the large amount of soil. The microbial biomass of my study was also analyzed in 0~10 cm soil in the field. Although analyzed 0~5 cm in lab incubation, but the mass of soil was more than field incubation. Hatch et al. (2000) found long-term fertilizer input appeared to increase the active component of soil microbial biomass, changed the ratio of active to inactive biomass without increase in biomass. The alpine meadow might have been fertilized by voles for a long time; thus, fecal fertilizer might not increase microbial biomass. Lovell and Jarvis (1996) found although soil under cattle dung did not change microbial biomass in the field, mineral nitrogen was still higher under dung than control. The nitrogen released from feces could increase nitrogen amount and retention in soil for several days.

I found the activity of microbes was stimulated by latrine. The CO_2 evolution rate increased greatly in treatments with added fecal pellets (Fig. 25). Although microbe

density in feces was higher than soil, the total biomass was too low to compare between them. Treatment litter (L) had little effect on CO_2 evolution rate, which was constant through time, similar to the straw-soil mixture reported by Cochran et al (2000). The pattern of CO_2 evolution rate of F and F+L treatments I observed were lower than that observed for geese feces (Cochran et al. 2000) and cattle dung (Lovell and Jarvis 1996). It was likely both previous studies mixed feces with soil, which I did not do. Mixing feces with soil could have a maximum effect on microbial biomass (Lovell and Jarvis 1996). The CO_2 evolution rate in my study reached a stable state after 32 days (Fig. 25) and latrine group remained higher than no latrine group. The higher value in water soluble carbon and CO_2 evolution indicated higher microbial activity and decomposition rate in latrine group. The high microbial activity in soil might deplete soil ammonium. The ammonium of latrine group was lower than no latrine group, and the ammonium was lower than control after the second month in field incubation (Fig. 13A & 26A). Although no significant increase in microbial biomass N, the soil ammonium might be immobilized by microbes or transformed to nitrate due to high nitrification rate.

The decomposition of leaf litter was nearly negligible (Fig 28A). Only 10% of litter mass was lost after 62 days of incubation under 12℃, the high temperature of He-huan Mt. The low decomposition rate of litter had no significant effect on soil C and

N (Table 8 & 10) and only increased nitrate for a short period (Fig. 23A). Yushan cane leaves made up a large portion of ground litter in alpine meadows. The low decomposition rate of Yushan cane leaves indicated low mineralization rate of litter in alpine meadow. The litter production rate of Yushan cane was 9400 kg ha^{-1} $year^{-1}$ (Yeh 2010), yet only decomposed 940 kg yearly. Mixing with fecal pellets increased the decomposition rate of leaf litter. Voles not only provided the labile nutrient through fast cycle, but also enhanced the turnover rate of slow cycle. Increasing the litter decomposed to 1410 kg per year. Organic matter decomposed does not always accompany nitrogen loss. Leaf litter lost more weight and carbon in F+L than L (Fig 28A and B), but not in nitrogen (Table 10). The faster decomposition rate might have been caused by latrine providing suitable environment for microbes. Latrine had better moisture holding ability than leaf litter. Leaf litter became dry faster than fecal pellets in the microcosm. Fecal pellets led microbes had much longer activity time with moisture. Vole fecal pellets also had many fungal spores (Pastor et al. 1996). At lab incubation, little fungi grew on leaf litter in L, yet, all the latrines in F and F+L had fungal fruiting bodies emerged within 35 days of incubation. The fungi also appeared on the leaf litter in F+L. Vole fecal pellets might have inoculated fungi spores to litters. Hatch et al. (2000) found cattle dung could change the ratio of bacteria to fungi in soil. After the labile C from feces was depleted, the large amount of C remained was accessible to

fungi. The increase of fungi could fasten turnover rate of plant residues. The moisture holding ability of latrine also benefited moss growth. Moss could use latrines as substrates. Moss growing on vole latrines was commonly found in the study area.

Vole latrine provided nitrogen through the fast cycle, increasing the inorganic nitrogen at alpine meadow. The existing time of latrines could be long, new fecal pellets added sustained latrines as nutrient “hot spots”. In compared to the slow decomposition rate of leaf litter, the stable amount of easily mineralizable nitrogen returned from latrines plays an important role in the nitrogen cycle process of the ecosystem. Although the nitrogen output from feces I observed was lower than those of other herbivores, I did not include urine in this study. According to Clark et al. (2005), the urinal nitrogen could be more than 2-fold of fecal nitrogen. The single urination by vole was not in large amount like those of cattle and ungulate, thus would not lose nitrogen through leach or ammonia volatilization (Haynes and Williams 1993, Frank and Evans 1997). The distribution of voles was heterogeneous, and the population size fluctuated considerably. Thus, the voles provide a highly spatial-temporally heterogeneous distribution of soil nitrogen, which would influence the plant composition. Although vole latrine could have above-mentioned effect in alpine meadows, the overall influence of vole is not clears. Voles could also alter plant composition through selective herbivory. The relative importance of different effects of herbivore is site-specific

(Holland and Detling 1990, Stark et al. 2002). Furthermore, the interactions and mechanisms are complicated in soil (Bardgett et al. 1998). Pastor et al (1993) found that moose browsing in a boreal forest at Isle Royale National Park promoted less palatable and less decomposable tree species. Alter the plant composition decreased the quantity and quality of litter. Even though the fecal pellets of moose have a co-fertilizing effect with soil, but couldn't compensate the browsing-induced depression. Over long term, the annual N mineralization rate could decrease. Similarly, ungulate promote microbes' activity at sub-oceanic but not at sub-continental tundra heaths (Stark et al. 2002). Voles increased the mineralizable nitrogen in one study site but changed plant community composition in another site decelerating nitrogen cycling in riparian areas in Yellowstone National Park (Sirotnak and Huntly 2000). Further research on the interactions of different effects of voles is needed to understanding the role of vole latrines in alpine ecosystem.

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Appendix

田鼠排遺及箭竹枯葉樣品經過烘乾、細磨後，以灰化法測量鉀鈉鈣鎂磷的含量，每個樣品稱取 0.5g 於坩堝中，經 105℃烘乾 24 小時後，以微程式電腦控制高溫爐 (Nabertherm, Nr82628, Germany) 在 490℃下灰化，再將灰化後的灰燼溶解於 2N HCl 中，並定量至 50 ml，溶解後的水樣與 Lab incubation 所收集的土壤水樣經 0.45 μm 濾膜 (cellulose acetate, Millipore, USA) 過濾後，以感應耦合電漿原子放射光譜儀 (Inductively coupled plasma optical emission spectroscopy, ICP-OES, Jobin-Yvon Horiba group, JY2000, Edison USA) 測定溶液中各鉀鈉鈣鎂磷的濃度。由於 62 天孵育後的排遺跟箭竹枯葉的樣本數只有 3，以無母數來做檢定

從鉀鈉鈣鎂磷的濃度結果，依然可以把 lab incubation 的四種處理 (C: control, L: litter, F: feces, and F+L: feces and litter) 分成有加入田鼠排遺的公廁組 (F and F+L) 與沒加入田鼠排遺的非公廁組 (C and L) 兩組來討論，鉀跟鈉在濃度上與硝態氮有類似的變化（圖 1 跟 2），公廁組有較明顯的濃度上升；從累積洗出量來看，公廁組累積量明顯高出許多；鈣與鎂在濃度上加入田鼠排遺僅有些許的增加（圖 3 跟 4），僅鈣在 F+L 洗出的量多餘 F (表 2、圖 3B)。磷所呈現的趨勢與有機碳的濃度變化趨勢較為相似（圖 5），濃度的變化不大，

排遺鉀鈉鎂磷的含量皆高於箭竹枯葉，僅鈣含量低於箭竹枯葉（表 2），被淋洗出量沒有明顯的下降，排遺的釋放量也都比箭竹枯葉多，排遺經過 62 天孵育後，排遺鉀鈉鎂磷的含量都有明顯的下降（圖 6A and B），但因為樣本數太小，無母數統計上不顯著。箭竹枯葉在 L 組中，鉀鎂磷的含量有明顯的下降（圖 6C and D），鈉則沒有顯著差異，而鈣含量反而比起始值還高；在與排遺混合的 F+L 組中，僅在鉀的含量是比起始值還低，鈉鈣鎂磷的濃度都比起始值還高，尤其鈣增加量相當高。從總量上來看，排遺的鉀鈉磷含量在孵育後皆剩餘不到 30%（表 2），而且

在 F 跟 F+L 間沒太大差異；鎂則在 F 與 F+L 個別剩下約 30%與 40%，鈣剩餘量最多，在 F 與 F+L 個別剩下 40 % 與 50% 左右。箭竹枯葉在單獨孵育下 (L)，只有鈣的總量是比孵育前來的多，其他養分都有所損失，以鉀所剩餘的量最少；與排遺共同處理 (F+L)，鉀大部分仍然是釋出土壤中，但剩餘量明顯比枯葉單獨處理時高，鎂雖然也有所流失，但剩餘量高達 97%，而鈉鈣磷的總量皆比孵育前來的多，尤其是鈉，因為有一個樣本孵育後鈉含量增加為孵育前的 3 倍，由於樣本數太少，無法去除極端值。

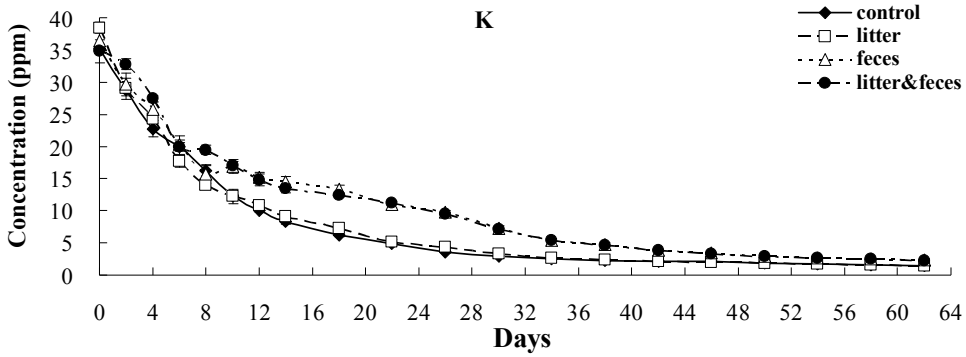
表 1.各處理 62 天總累積洗出量減去 control 累積量的差值 (mg)

Treatment	K	Na	Ca	Mg	P
L	0.88	0.12	-1.58	0.28	0.000
F	17.77	1.79	4.79	1.59	0.056
F+L	18.54	1.89	7.01	1.87	0.056

表 2.田鼠排遺與箭竹枯葉鉀鈉鈣鎂磷的總量，F 與 L 為排遺跟枯葉單獨加入的處理，F+L 為排遺與枯葉共同處理，孵育時間為 62 天，n = 3。

Organic matter		Fecal pellets						Leaf litter					
Treatment		Initial	se	F	se	F+L	se	Initial	se	L	se	F+L	se
Weight	g	6.52	0.00	5.07	0.05	4.99	0.09	2.00	0.00	1.80	0.01	1.68	0.02
	Remain (%)			77.68	0.84	76.52	1.44			89.56	0.53	84.11	1.02
K	mg	36.86	0.62	2.68	0.32	2.16	0.39	2.40	0.22	0.16	0.03	0.71	0.09
	Remain (%)			7.27	0.88	5.85	1.05			6.49%	1.36	29.55	3.77
Na	mg	1.63	0.09	0.22	0.06	0.23	0.06	0.07	0.02	0.05	0.01	0.14	0.06
	Remain (%)			13.55	3.56	13.99	3.75			68.92	17.71	198.46	80.22
Ca	mg	11.58	0.19	4.40	0.37	5.80	0.19	5.23	0.07	6.53	1.27	7.51	1.47
	Remain (%)			38.01	3.19	50.12	1.67			124.98	24.23	143.68	28.08
Mg	mg	3.06	0.06	0.88	0.04	1.14	0.05	0.70	0.03	0.37	0.08	0.69	0.12
	Remain (%)			28.84	1.34	37.32	1.78			53.06	11.92	97.33	17.24
P	mg	14.48	0.13	3.14	0.11	2.95	0.25	0.49	0.01	0.21	0.04	0.70	0.14
	Remain (%)			21.70	0.73	20.40	1.75			42.13	8.85	141.59	28.35

(A)



(B)

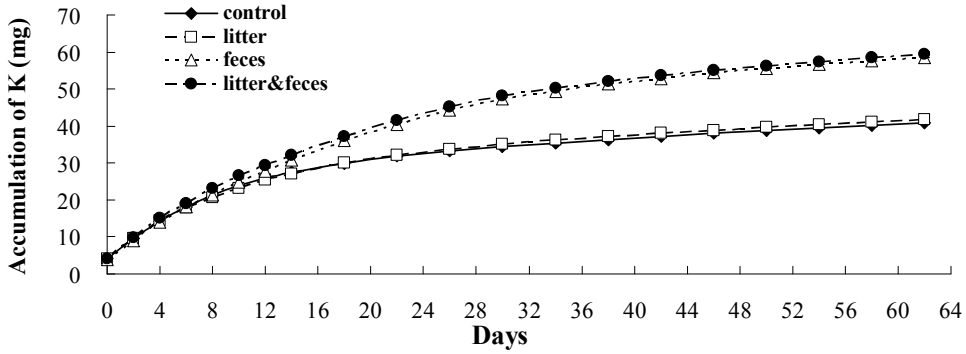
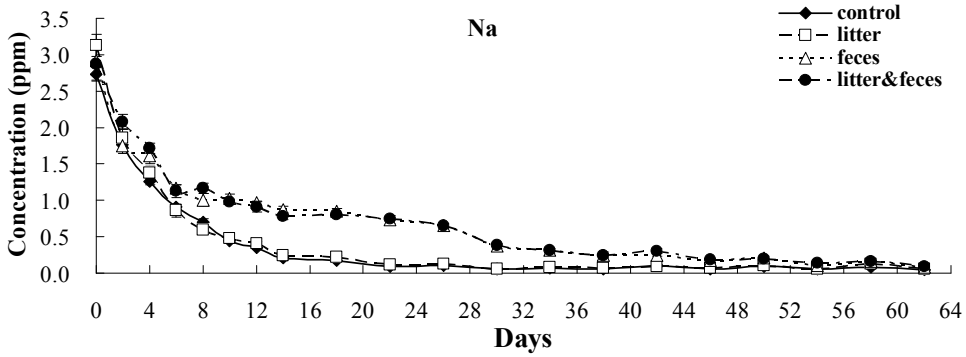


圖 1.鉀在淋洗水中的含量；(A)鉀在水樣中的濃度變化；(B)鉀在水樣中的累積量

(A)



(B)

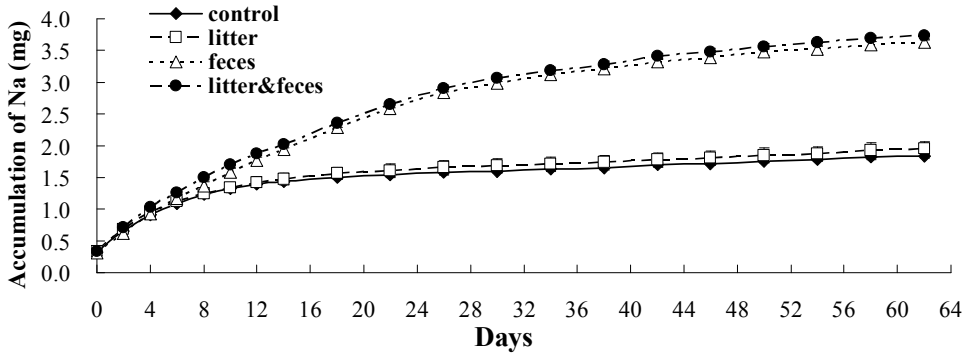
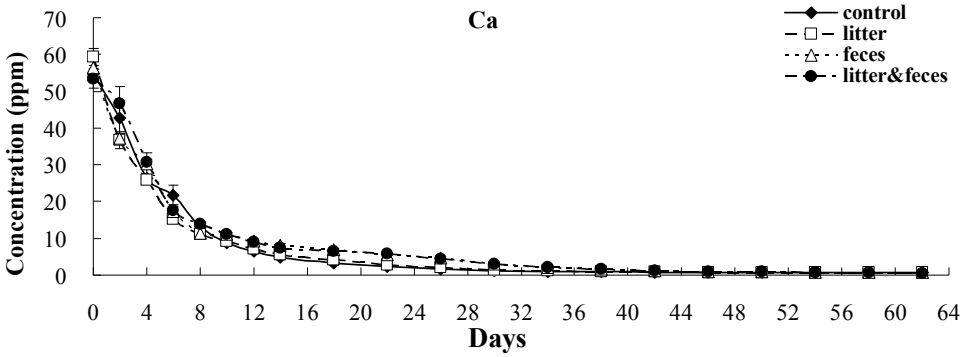


圖 2.鈉在淋洗水中的含量；(A)鈉在水樣中的濃度變化；(B)鈉在水樣中的累積量

(A)



(B)

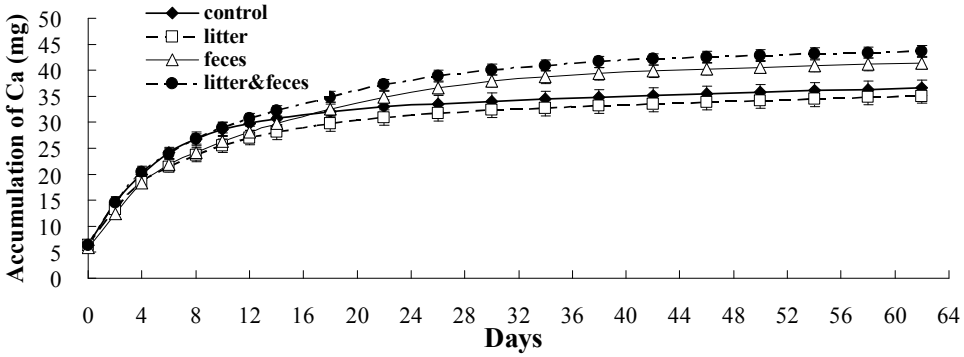
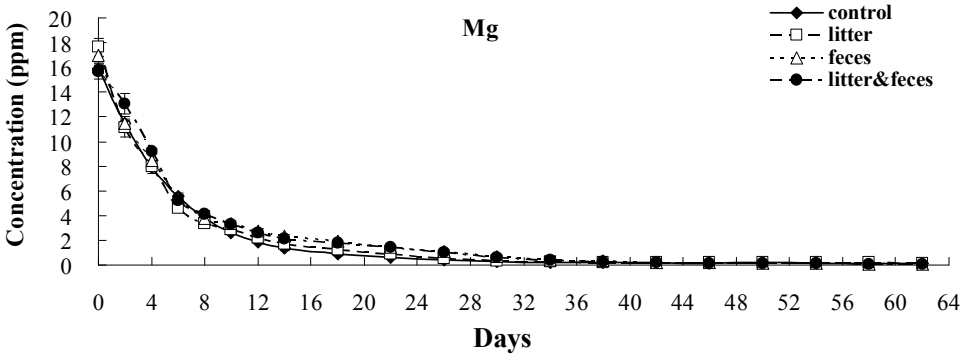


圖 3.鈣在淋洗水中的含量；(A)鈣在水樣中的濃度變化；(B)鈣在水樣中的累積量

(A)



(B)

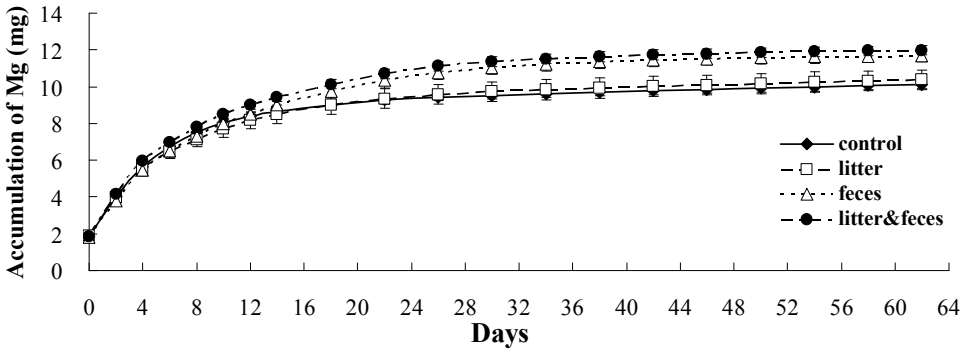


圖 4.鎂在淋洗水中的含量；(A)鎂在水樣中的濃度變化；(B)鎂在水樣中的累積量

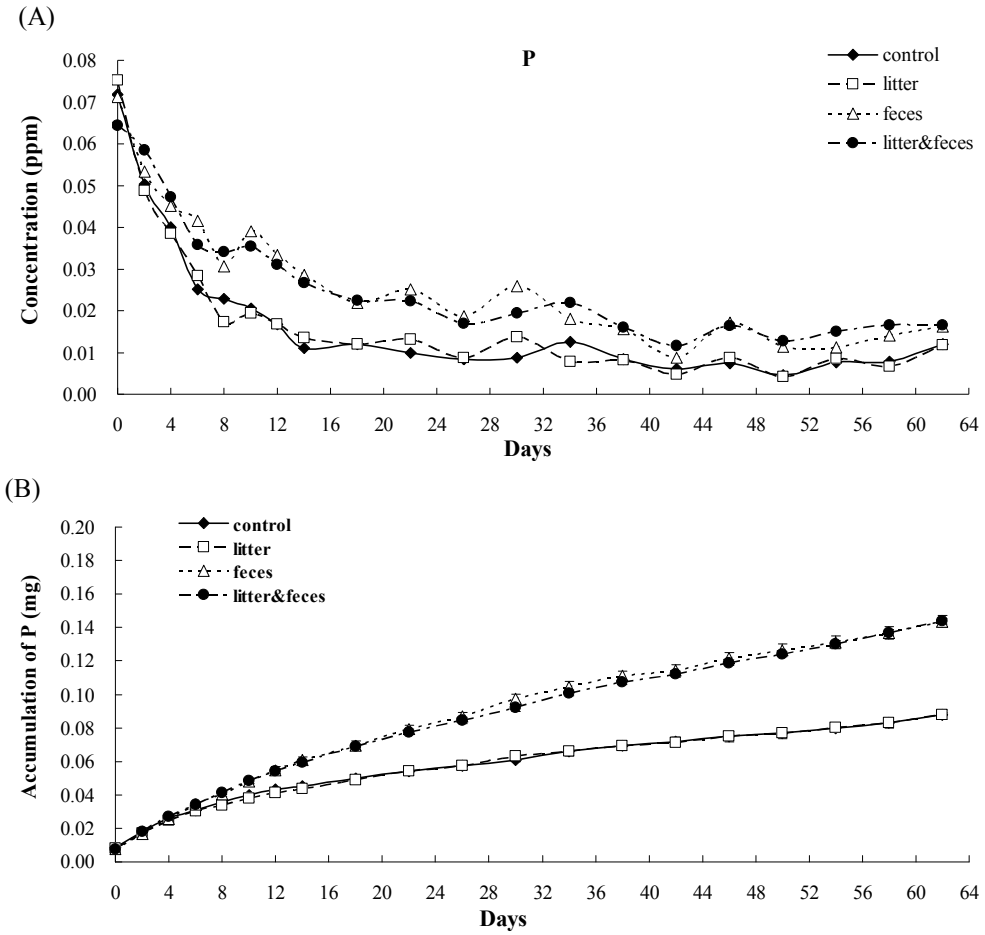


圖 5. 磷在淋洗水中的含量(A)水中淋的濃度；(B)磷在水樣中的累積量

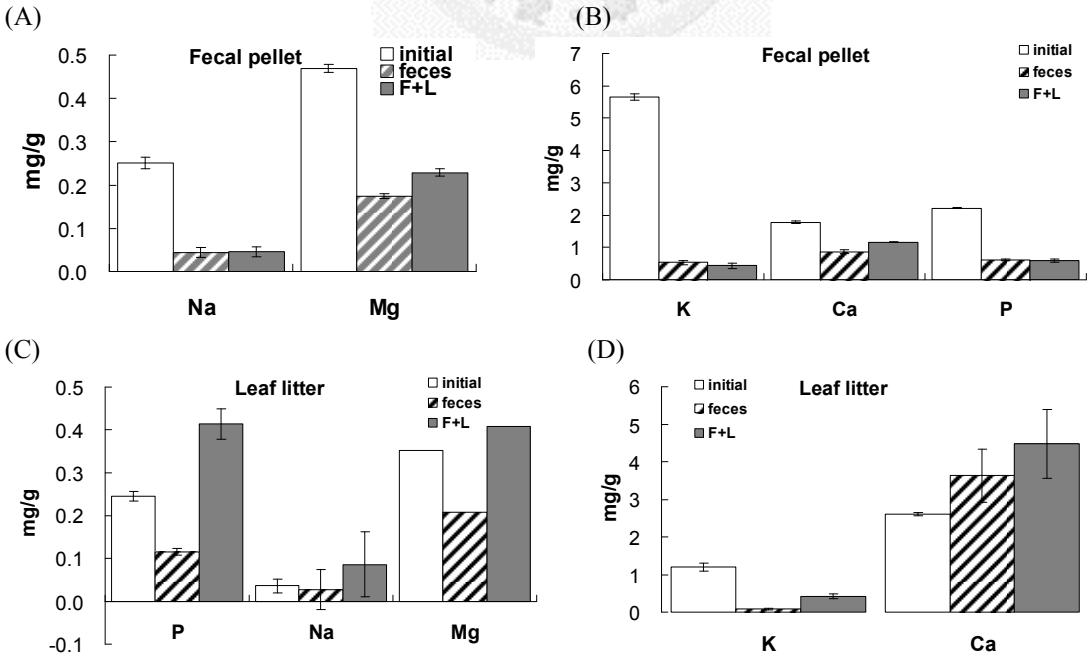


圖 6.田鼠排遺與箭竹枯葉養分濃度 (A)田鼠排遺中的鈉與鎂。(B)田鼠排遺中的鉀、鈣與磷。(C)箭竹枯葉中的鈉與鎂。(D)箭竹枯葉中的鉀、鈣與磷。

排遺中鉀鈉鎂磷的含量不僅比箭竹枯葉多，而且釋出的比例相當高，排遺相較於箭竹枯葉而言，較具有養分提供的價值，箭竹枯葉與排遺共同處理的結果，可能有受到排遺的污染，而使得箭竹枯葉的養分含量上升，甚至比孵育前的枯葉含量還高，養分經過大量水的淋洗，仍然有部份被保留在枯葉層中，雖然被保留下來的量最多也僅排遺所釋出的三分之一（表 2），這些保留在枯葉表面的養分，也許對微生物的生長或是活性有很大的幫助；從土壤淋洗水來看，雖然箭竹枯葉會固定部份養分，不過各養分在 F+L 所洗出的累積總量與 F 沒有差異（表 1），F+L 甚至還比 F 洗出更多的鈣，這些養分在土壤中流轉與運作機制，在此論文中並沒有探究，不過微生物一定在其中扮演了重要的角色；箭竹枯葉會把養分固定下來可以減少流失，雖然在本實驗所被固定的養份量相當的少，但合歡山高山草原土壤上的枯落物量相當多，在 0.3 × 0.3 m^2 的樣方的調查，有機值含量 OL 78.67 g/m^2、OF 634.67 g/m^2 與 OH 154.22 g/m^2，本實驗僅加入 OL 的部份，雖然孵育實驗加入 2 g 的箭竹枯葉大於上述的測量值，不過高山草原地面上的枯落物量在空間上也是分佈不均的，在枯落物多的地方，可以減緩養分從公廁中流失。